I. WELCOME ADDRESS

Fish is an essential part of human diet since ancient times. From a global viewpoint food fish remains a very important source in human nutrition providing about 16 % of total protein supplies. People greatly appreciate and enjoy the variety of aquatic food delivered by nature, and sea food is more popular than ever. The health benefits of fish and shellfish consumption are numerous, and therefore every effort has to be made to guarantee an environmental friendly and sustainable fishery and to avoid losses of valuable food due to spoilage of products.

As highly perishable commodity, safety and quality of fish and fishery products must be ensured by permanent efforts of industry, traders, and also governments. Scientific research is an integrative part in the network of fish quality assurance from producer to consumer. Scientifically based knowledge of raw material, processes and product composition is essential to keep and improve the high standard fish as food has reached world wide.

The topics of the WEFTA meeting 2004 in Lübeck address not only the current requirements related to quality, benefits and risks for human health, but are also dealing with aspects of animal welfare. Organic fish production, and processing of fish avoiding as far as possible painful and stressing burden of animals, are important issues for future work.

I hope that this conference will further support the dialogue between European and overseas fishery technologists and the various beneficiaries of the results of fisheries product research, namely consumers, industry, trade, as well as governmental and political decision makers.

Renate Künast,
Federal Minister of Consumer Protection, Food and Agriculture

II. ADDRESS FROM THE ASSOCIATION FOR THE PROMOTION OF FISH QUALITY RESEARCH (FORSCHUNGSGEMEINSCHAFT FISCHWIRTSCHAFT E.V.)

It is really a great pleasure and honour for the Association for the Promotion of Fish Quality Research to assist and help the Federal Research Centre for Nutrition and Food, Department for Fish Quality, in organising the WEFTA meeting in Lübeck in 2004.

The Association for the Promotion of Fish Quality Research was founded more than 50 years ago by fish processing companies, suppliers to the fish industry and private people who were interested in supporting research in quality of fishery products and fish processing technology.

Our Association is also holding a (much smaller) annual meeting where information about the ongoing research projects in the Department for fish Quality is presented and where actual questions and interesting developments in our field are freely discussed. Also research projects which are of interest for the fish industry are directly addressed to the Department, however, it is their decision to take them up or not. We are informed continuously about the work of the Department by receiving all reprints of publications and can get advice by asking the respective scientist in the Department. The fee for membership which is dependent on the size of the company can be used by the Department for material supporting research e.g. books, literature, smaller equipment and other purposes.

I as the executive secretary and on behalf of the President of the Association Mr. Rebhan, who is on a business trip abroad, wish the WEFTA meeting 2004 all success, fruitful discussions and any progress in making fishery products healthier, safer, convenient and more tasty.

Katrin Oetjen, executive secretary FF
III. ADDRESS FROM THE FEDERAL RESEARCH CENTRE FOR NUTRITION AND FOOD, DEPARTMENT FOR FISH QUALITY

It is a great honour and pleasure to welcome all of you to the 34th WEFTA Annual Meeting 2004 in Lübeck, Germany. After the meetings in Hamburg in 1973 and 1985 this is the third organised by the permanent German member institute of WEFTA. During the 31 years the name of our institute has changed from “Institute of Biochemistry and Technology” to “Institute of Fishery Technology and Fish Quality” to “Department for Fish Quality”. Recently we have left the “Federal Research Centre for Fisheries”, we are now affiliated with the “Federal Research Centre for Nutrition and Food”, but the department is still located in Hamburg, Palmaille 9, and the research is still covering all aspects of fish quality.

The contents of the more than 70 full and short contributions which will be presented at the meeting demonstrate that one of the original aims of WEFTA, namely improving seafood quality through research, is still of great importance, but has been completed and amended with new objectives. Firstly, the term quality does not only mean sensory, chemical and microbial quality, but is also including all aspects of improving human health and well being (SEAFOODplus!). Secondly, the consumers awareness to eat nutritious, healthy and safe seafood, with low content of pollutants, toxins and other undesirable compounds is steadily increasing. Thirdly, authenticity, traceability and sustainability of the resource are broad subjects of current research. In the future, however, the results of all these subjects have to be communicated directly to consumers in an appropriate and understandable language to increase consumer’s confidence in seafood in many parts of the world.

At this year’s meeting you may miss the poster presentation, which had become a permanent part of the previous meetings. There were three reasons for replacing posters by short oral presentations. The first reason is based on the observation that it is very difficult for participants to read all the posters presented in separate locations at times, when you actually need time to refresh yourself after the sessions. The second reason is that at the last meetings only a small percentage of participants took actively part in poster sessions (e.g. discussion with authors). The third reason is that a short oral presentation given by younger (or older) scientists may be a very useful experience and experiment to learn to KISS, Keep It Short and Simple! All who are interested in more details have the proceedings at hand, which are available at the beginning of the meeting and are free to contact the authors any time between the sessions or later.

The editors wish to express their gratitude to all participants involved in making the annual WEFTA Meeting a success and to all authors and people behind the scene in helping to make the present publication of the Proceedings in due time possible.

Finally, we would like to take the occasion to thank the Association of the Promotion of Fish Quality (Forschungsgemeinschaft Fischwirtschaft), a charitable association of German fish industry, suppliers, trade, laboratories and private persons very much for supporting us in organising this annual WEFTA meeting 2004 in Lübeck.

_Hartmut Rehbein, research director FRCFN, Hamburg_
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# TABLE OF CONTENTS

WELCOME ADDRESS .................................................................................................................. I

ADDRESS FROM THE ASSOCIATION FOR THE PROMOTION OF FISH QUALITY RESEARCH ......... II

ADDRESS FROM THE FEDERAL RESEARCH CENTRE FOR NUTRITION AND FOOD, DEPARTMENT OF FISH QUALITY ......................................................................................... III

ORGANISATION ........................................................................................................................ IV

## 1. Desirable nutritive components in seafood

1.1. NUTRITIVE AND UNDESIRABLE COMPONENTS IN FAROESE COD-FILLET COMPARED WITH CORRESPONDING FINDINGS IN THE OTHER NORDIC COUNTRIES ........................................... 1
Hóraldur Joensen and Heidi Gregersen

1.2. STUDY OF THE POSSIBLE INTERACTIONS BETWEEN $\alpha$-TOCOPHEROL AND AROMATIC/ALIPHATIC AMINES .................................................................................................................... 7
Narcisa M. Bandarra, Ana Rodrigues and Irineu Batista

1.3. ESSENTIAL AND TOXIC METALS IN SEAFOOD COMMERCIALISED IN PORTUGAL ............... 13
H. M. Lourenço, J. Assunção, C. Raimundo, C. Afonso, M. F. Martins and M. L. Nunes

1.4. THE EFFECT OF FISH HERRING MUSCLE PRESS JUICE ON THE GENERATION OF REACTIVE OXYGEN SPECIES FROM HUMAN MONOCYTES STIMULATED WITH PHORBOL MYRISTATE ACETATE .......... 16
Guðjóhn Gunnarsson, Ingrid Undeland, Ann Lindgård, Ann-Sofie Sandberg and Bassam Soussi

1.5. NOVEL NATURAL COMPOUNDS FROM GRAPE BYPRODUCTS AS INHIBITORS OF RANCIDITY IN FISH LIPIDS AND IN FROZEN FATTY FISH ............................................................................................................... 18
M. Pazos, M.J. González, J.L. Torres, J.M. Gallardo and I. Medina

1.6. PREVENTING SEAFOOD LIPID OXIDATION AND TEXTURE SOFTENING TO MAINTAIN HEALTHY COMPONENTS AND QUALITY OF SEAFOOD (LIPIDTEXT A SEAFOODPLUS PROJECT) ................................................. 22

1.7. OCCURRENCE OF PEPTIDES IN TROUT MUSCLE DURING POST MORTEM STORAGE AND COOKING .............................................................................................................................. 25
Caroline Bauchart, Didier Rémond, Christophe Chambon and Martine Morzel

1.8. INFLUENCE OF HERRING (CLUPEA HARENGUS) ON BIOMARKERS FOR CARDIOVASCULAR DISEASE ................................................................................................................................. 30
Helen Allenström, Anna-Maria Langkilde, Ingrid Undeland and Ann Sofie Sandberg

## 2. Fish farming and processing

2.1. PRE-RIGOR FILLETING AND QUALITY OF FED ATLANTIC COD (GADUS MORHUA L.) .................. 32
Stilje Kristoffersen, Torbjørn Tobiassen, Lars A. Godvik, Margrethe Esaiassen and Ragnar L. Olsen

2.2. A STUDY ABOUT FLESCH QUALITY OF WILD AND CULTERED COMMON DENTEX (DENTEX DENTEX LINNAEUS 1758) ........................................................................................................... 33
Şükran Cahtı, Tolga Dincer, Aslı Cadun, Kürşat Firat and Şahin Saka

2.3. FROM POND TO TABLE – TRANSPARENCY IN AQUACULTURE PRODUCTION WITH REGARD TO FISH HEALTH, ANIMAL WELFARE AND FARM MANAGEMENT .................................................................... 39
Dirk Willem Kleingeld, Reinhard Kruse and Frerk Feldhusen

2.4. THE USE OF ANTI-MOUSE TYROSINASE ANTIBODY TO INVESTIGATE MECHANISMS OF MELANISATION IN FARMED COD ........................................................................................................ 44
Marie Cooper and K. Midling

2.5. MINIMAL PROCESSING OF NEW FARMED FISH SPECIES ................................................................ 45
J.T. Rosnes, G.H. Kleiberg, B.T. Lunestad and G. Lorentzen
2.6. EFFECTS OF VEGETABLE DIETARY LIPID SOURCES ON FAT CONTENT AND FATTY ACID PROFILE IN TURBOT (*PSETTA MAXIMA*) ........................................................................................................................................50  
S. Lois, E. Silva, S. Cabaleiro, M. V. Ruiz Osenda, A. Teijido and I. Medina

2.7. RELATIONSHIP BETWEEN SENSORY AND INSTRUMENTAL TEXTURE ANALYSIS OF FARMED COD ........................................................................................................................................54  
Turid Mørkøre, Hanne Morkemo and Trine Galloway

2.8. VARIATION IN COPPER LEVEL, TEXTURE AND GAPING OF FARMED SALMON. SAMPLING TIME SHOWED HIGHER IMPACT THAN FEED COMPOSITION ........................................................................................................................................55  
Turid Mørkøre

2.9. CONTRACTION OF PRE-RIGOR SALMON FILLETS. EFFECT OF FEEDING AND STRESS ........................................................................................................................................56  
Turid Mørkøre, Pablo Mazo, Reidun Lilleholt, Vildana Tahirovic and Olai Einen

2.10. COMPARISON OF PERCUSIVE STUNNING AND ASPHYXIATION OF FARMED SOLE (*SOLEA SOLEA*) WITH RESPECT TO DEVELOPMENT OF RIGOR MORTIS AND PRODUCT QUALITY ........................................................................................................................................57  
Hans van de Vis, Karin Kloosterboer, Martine Veldman and Bert Lambooij

2.11. TRADITIONAL AND INNOVATIVE STUNNING/SLAUGHTERING METHODS FOR EUROPEAN SEA BASS COMPARED BY THE COMPLEX OF THE ASSESSED BEHAVIOURAL, PLASMATIC AND TISSUE STRESS AND QUALITY INDEXES AT DEATH AND DURING SHELF LIFE ........................................................................................................................................58  
B. M. Poli, F. Scappini, G. Parisi, G. Zampacavallo, M. Meccati, P. Lupi, G. Mosconi, G. Giorgi and V. Vigiani

2.12. TAILORING THE FATTY ACID COMPOSITION OF TROUT FILLETS FOR HEALTH PURPOSES: PRELIMINARY RESULTS ........................................................................................................................................64  
Pier Paola Gatta, Silvia Testi, Marina Silvi, Giampiero Pagliuca, Alessio Bonaldo, Arjen Roem and Anna Badiani

2.13. SELECTED FATTY ACID CONTENTS OF COOKED N-3 PUFA-ENRICHED TROUT FILLETS ........................................................................................................................................69  
Anna Badiani, Silvia Testi, Marina Silvi, Elisa Zironi, Alessio Bonaldo, Alessio Pecchini and Pier Paolo Gatta

2.14. NUTRITIONAL TRAITS OF DORSAL AND VENTRAL FILLETS FROM FARMED EUROPEAN SEA BASS, GILTHEAD SEA BREAM AND RAINBOW TROUT ........................................................................................................................................73  
Silvia Testi, Alessio Bonaldo, Anna Badiani and Pier Paolo Gatta

3. Undesirable components in aquatic food products

**Miscellaneous**

3.1. COMPARISON OF HISTAMINE CONTENTS OF SARDINE (*SARDINA PILCHARDUS*) CAUGHT IN DIFFERENT SEASON DURING REFRIGERATED STORAGE ........................................................................................................................................77  
Nalan Gokoglu and Pinar Yerlikaya

3.2. INCIDENCE OF LISTERIA SPP. IN FISH AND ENVIRONMENT OF FISH MARKETS IN NORTHERN GREECE ........................................................................................................................................80  
Nikolaos Soultos, Amin Abrahim, Konstantinos Papageorgiou and Vasilios Steris

3.3. AEROMONAS SPECIES ISOLATED IN FISH AND ENVIRONMENT OF FISH MARKETS IN NORTHERN GREECE ........................................................................................................................................83  
Amin Abrahim, Nikolaos Soultos, Vasilios Steris and Konstantinos Papageorgiou

3.4. DISTRIBUTION OF MERCURY AND CADMIUM IN SWORDFISH (*XIPHIAS GLADIUS*) ........................................................................................................................................86  
Erwin Schuirmann

3.5. RELATION BETWEEN TOTAL BODY LENGTH AND MERCURY LEVELS IN SOME FISH SPECIES ........................................................................................................................................90  
H. M. Lourenço, C. Afonso, M. F. Martins, A. R. Lino and M. L. Nunes

3.6. INTERLABORATORY STUDY: DETERMINATION OF CHLORAMPHENICOL (CAP) RESIDUES IN SHRIMPS ........................................................................................................................................91  
Ute Schröder

3.7. DIFFERENCES IN STRUCTURAL DECOMPOSITION OF CONNECTIVE TISSUE IN COD (*GADUS MORHUA*) AND SPOTTED WOLFFISH (*ANARHICHAS LUPUS*) ........................................................................................................................................94  
3.8. COMPOSITIONAL CHARACTERISTICS OF CLAM (*Ruditapes decussatus*, L.) AND WARTY VENUS (*Venus verrucosa*, L.) ..................................................................................................................95 
Şikran Cakli, Aslı Cadun, Tolga Dincer, Emre Caglak and Latif Taskaya

3.9. DESALTED COD PRODUCTS PRESERVATION: EFFECT OF DIFFERENT MICROBIAL LOADS IN RAW MATERIALS ..........................................................................................................................99 
Sónia Pedro, Carla Pestana, Irineu Batista and Maria Leonor Nunes

3.10. CHANGES IN LIPIDS AND PROTEINS DURING STORAGE OF MINCED MACKEREL (*Scomber scombrus*) AT –2°C AND -10°C TEMPERATURE ..................................................................................102 
Revilija Mozuraityte, Ivar Storrø and Turid Rustad

3.11. SOUS VIDE TECHNOLOGY FOR UNDERUTILISED FISH SPECIES .................................................................................................................106 
J.D. Fagan and T.R. Gormley

3.12. TECHNOLOGICAL IMPLICATIONS OF ADDITION OF GRAPE FIBRE TO RESTRUCTURED FISHERY PRODUCTS ..................................................................................................................110 
Isabel Sánchez-Alonso and A. Javier Borderías

3.13. EVALUATION OF THE QUALITY OF HAKE PRODUCTS DURING FROZEN STORAGE .................................................................................................................114 
A. Martins, M. R. Bronze, I. Batista and M. L. Nunes

3.14. EFFECT OF MODIFIED ATMOSPHERE ON THE SHELF LIFE OF COMMON OCTOPUS (*Octopus vulgaris*) .................................................................................................................................................117 
Amparo Gonçalves and Maria Leonor Nunes

3.15. EFFECT OF DIFFERENT PREVIOUS ICING CONDITIONS ON SENSORY, PHYSICAL AND CHEMICAL QUALITY OF CANNED HORSE MACKEREL (*Trachurus trachurus*) ...........................................................................119 
Vanesa Losada, Ines Lehmann, Reinhard Schubring and Santiago P. Aubourg

3.16. INCREASE IN FILLETING YIELD AND BY-PRODUCTS FROM COD IN FACTORY TRAWLERS ..........122 
Helgi Nolsøe

3.17. PROCESSING FORECAST OF COD .............................................................................................................................................125 
Sveinn Margeirsson, Gudmundur R. Jonsson, Sigurjon Arason and Gudjon Thorkelsson

3.18. EFFECTS OF STORAGE IN OZONISED SLURRY ICE ON THE SENSORY AND MICROBIAL QUALITY OF SARDINE (*Sardina pilchardus*) ........................................................................................................128 
Carmen A. Campos, Óscar Rodríguez, Vanesa Losada, Santiago P. Aubourg and Jorge Barros-Velázquez

3.19. LIPID CHANGES RELATED TO QUALITY DURING SARDINE (*Sardina pilchardus*) CHILLED STORAGE: EFFECT OF OZONISED SLURRY ICE ..................................................................................................132 
Vanesa Losada, Carmen Piñeiro, Marcos Trigo, José M. Antonio, Jorge Barros-Velázquez and Santiago P. Aubourg

3.20. LIPID DAMAGE ASSESSMENT DURING COHO SALMON (*Oncorhynchus kisutch*) CHILLED STORAGE .................................................................................................................................................136 
Vanesa Losada, Julio Gómez, Liliana Maier, Mª Elisa Marin, Julia Vinagre, Mª Angélica Larrain, Vilma Quitral, Alicia Rodriguez and Santiago P. Aubourg

3.21. INFLUENCE OF STORAGE METHOD AND FRESHNESS MASS TRANSFER PHENOMENA DURING SALMON (*Salmo salar* L.) SALTING ........................................................................................................139 
Lorena Gallart Jornet, Turid Rustad, Isabel Escriche, José Manuel Barat and Pedro Fito

4. Authenticity of aquatic food

4.1. COMPOSITIONAL ANALYSES OF COD (*Gadus morhua*) AND ATLANTIC SALMON (*Salmo salar*) BY HIGH RESOLUTION 1H MR: APPLICATION TO AUTHENTICATION ANALYSES .....................................143 
I. Martínez, T. Bathen, I. B. Standal, J. Halvorsen, M. Aursand and I. S. Gribbestad

4.2. RELATIVE QUANTITATIVE TAQMAN™ REAL TIME POLYMERASE CHAIN REACTION SYSTEM FOR THE IDENTIFICATION AND QUANTIFICATION OF THE MOST VALUABLE CANNED TUNA FISH SAMPLES ...........................................................................................................148 
Miguel Angel Pardo
4.3. NEW ISSUES ABOUT AN OLD STORY: AUTHENTICATION OF TUNA CANS .................................................152
M.J. Chapela Garrido, C. G Sotelo, R. I. Pérez-Martín, M. A. Pardo, B. Pérez-Villarreal and P. Gilardi

4.4. TREATMENT OF TUNA PRODUCTS WITH CARBON MONOXIDE; PRINCIPLES OF ASSESSMENT AND ACTUAL ANALYTICAL ASPECTS .....................................................................................................................153
Frerk Feldhusen, Reinhard Schubring, Hartmut Rehbein and Reinhard Kruse

4.5. CONFIRMATION OF THE ORIGIN OF SALMON – FAT ANALYSES REFLECT THE FISH DIET ...............158

4.6. FISHTRACE: A DNA DATABASE FOR EUROPEAN MARINE FISH – GENETIC CATALOGUE, BIOLOGICAL REFERENCE COLLECTIONS AND ONLINE DATABASE OF EUROPEAN MARINE FISHES (EC PROJECT QLRI-CT-2002-02755) .................................................................161
Veronique Verrez-Bagnis

4.7. IDENTIFICATION OF COMMERCIAL GADOID SPECIES BY PCR-RFLP ...........................................................164
Miguel Angel Pardo

4.8. THE FISH-TRACE PROJECT: A STRATEGIC RESOURCE OF INFORMATION ABOUT TRACEABILITY OF FISH PRODUCTS ......................................................................................................................168
Maria Pérez

4.9. DIFFERENTIATION OF WILD SALMON, CONVENTIONALLY AND ORGANICALLY FARmed SALMON ................................................................................................................ ......................................171
Ute Ostermeyer

5.  Novel analytical methods

5.1. HEADSPACE ANALYSIS OF VOLATILES COMPOUNDS IN CANNED WILD ALASKA PINK SALMON HAVING VARIOUS DEGREES OF WATERMARKING .................................................................172
Alexandra C.M. Oliveira, Charles Crapo, Brian Himelbloom, Jennifer Hoffert and Carey Vorholt

5.2. DEVELOPMENT OF A COLORIMETRIC SENSOR FOR FISH SPOILAGE MONITORING BASED ON TOTAL VOLATILE BASIC NITROGEN (TVB-N) MEASUREMENT ..............................................................177
Alexis Pacquit, King Tong Lau, June Frisby, Danny Diamond and Dermot Diamond

5.3. EXPLORATIVE ANALYSES OF 16S RDNA MICROBIAL COMMUNITY IN FARMED SALMON FILLETS PACKED IN MODIFIED ATMOSPHERE (MAP) WITH A CO2-EMITTER .........................................................182
Anlaug Ádland Hansen, Thomas Eie, Maria Pilar Concoles Tamarit and Knut Rudi

5.4. OXIDATION OF PROTEINS IN RAINBOW TROUT MUSCLE ............................................................................ ...186
Inger V.H. Kjersgård and Flemming Jessen

Sabine Mierke-Klemeyer, Jörg Oehlenschläger, Reinhard Schubring and Meike von Klinkowström

5.6. STRUCTURAL CHARACTERIZATION OF FISH MUSCLE TISSUE BY IMAGE PROCESSING ......................192
Michael Kroeger

5.7. EVOLUTION OF K VALUE IN FARMED GILTHEAD SEABREAM, SEABASS AND SENEGALESE SOLE .................................................................196
Margarita Tejada, Almudena Huidobro and Gamal Mohamed

5.8. EVALUATION OF SEAFOOD PROTEOLYSIS BY IMMUNOLOGICAL TECHNIQUES: α-ACTININ AS A BIOMARKER OF SHELF-LIFE IN CHILLED PRODUCTS .................................................................200
Mónica Carrera, Vanesa Losada, Carmen Piñeiro, Lorena Barros, José Manuel Gallardo, Jorge Barros-Velázquez and Santiago P. Aubourg

5.9. TWO-DIMENSIONAL GEL ELECTROPHORESIS ANALYSIS OF FISH MEAL PRODUCTION ...................204
Morten Ruud, Harald B. Jensen and Eyolf Langmyhr

5.10. DEVELOPMENT OF AN ENZYME IMMUNOASSAY FOR TYPE-I BREVETOXIN DETECTION IN SHELFISH .................................................................................................................................205
N. Argarate, B. Pérez Villarreal and B. Alfaro Redondo
5.11. EVALUATION OF THE EUROPEAN FOUR-PLATE TEST FOR SCREENING DIFFERENT ANTIBIOTIC RESIDUES IN TROU TS ................................................................. 208
Berna Kilinc, Şükran Cakli and Carsten Meyer

5.12. LOSS OF REDNESS (A*) AS A METHOD TO FOLLOW HEMOGLOBIN-MEDIATED LIPID OXIDATION IN FISH MINCE ............................................................. 211
Daniel Wetterskog and Ingrid Undeland

5.13. QUANTITATIVE DETERMINATION OF POLYPHOSPHATES ADDED TO FRESH AND DEEP FROZEN FISH BY MEANS OF THERMO DIFFERENTIAL PHOTOMETRY ........................................... 214
Reinhard Kruse

5.14. QUALITY CHANGES IN FISH BY-PRODUCTS EVALUATED BY HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY .............................................................. 216
E. Falch, T. Storseth and M. Aursand

5.15. SPECTRAL CHARACTERISATION OF COD MUSCLE AND NEMATODES .............................................. 220
Heidi Nilsen, Karsten Heia, Agnar H. Sivertsen, Svein K. Stormo and Edel Elvevoll

5.16. NEMATODE DETECTION IN COD FILLETS BASED ON IMAGING SPECTROSCOPY .................................. 221
Karsten Heia, Heidi Nilsen, Agnar H. Sivertsen and Jens Petter Wold

5.17. ALGAL TOXIN TESTING IN MUSSELS BY USING CHEMICAL AND BIOLOGICAL METHODS: TWO EQUIVALENT APPROACHES ? ..................................................... 222
Stefan Effkemann, Ernst Jütting, Ingo Nauch, Reinhard Tiebach and Frerk Feldhusen

6. Entire utilisation of the catch

6.1. CHARACTERIZATION OF LIVERS LIPIDS FROM FISH SPECIES HARVESTED IN ALASKA ................................. 226
Alexandra C.M. Oliveira and Peter J. Bechtel

6.2. UTILISATION OF BY-PRODUCTS FROM FARMED ATLANTIC SALMON (SALMO SALAR ) ........................................ 231
Hege Michelsen, Eva Falch and Turid Rustad

6.3. PREPARATION AND CHARACTERISTICS OF PROTEASES FROM ATLANTIC COD AND THEIR APPLICATIONS IN INDUSTRY AND MEDICINE (ENZYPRO, QLK1-CT-2002-70871) ......................................................... 232
Linda Helgadottir, Sigridur Olaflsdottir and Jon Bragi Bjarnason

6.4. PREVENTION OF HB CATALYZED OXIDATION IN WASHED COD MUSCLE BY AN AQUEOUS FRACTION OF HERRING (CLUPEA HARENGUS) ............................................................ 236
Thippeswamy Sannaveerappa, Ingrid Undeland and Ann-Sofie Sandberg

6.5. NEW WAYS TO A BETTER UTILIZATION OF THE RAW FISH: FILLET-LIKE RESTRUCTURATES FROM MINCED FISH .............................................................. 238
Christoph Schneider

6.6. THE POSSIBILITY FOR INDUSTRIAL PRODUCTION OF DRIED FISH HEADS IN THE NORTHERN PART OF NORWAY ................................................................. 242
Hilde Herland, Morten Heide and Even Tidemann

6.7. CHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF A FINFISH SPECIES REJECTED TO SEA: ROCK COD ................................................................. 243
M. J. González, E. Silva, C. Núñez, C. Piñeiro, J.M. Gallardo and I. Medina

6.8. GELATIN EXTRACTION FROM CAPE HAKE AND BLUE SHARK SKIN ............................................................. 247
Irineu Batista, Patrícia Fradinho and Célia Silvestre

6.9. IMPROVING PRODUCTION OF MINCED FISH PRODUCTS ..................................................................................... 251
J.T. Rosnes, G.H. Kleiberg, T. Bekkeheien, S. Øines and D. Skipnes
1.1 NUTRITIVE AND UNDESIRABLE COMPONENTS IN FAROESE COD-FILLET COMPARED WITH CORRESPONDING FINDINGS IN THE OTHER NORDIC COUNTRIES.

Hóraldur Joensen and Heidi Gregersen

Twenty-four cod (Gadus morhua) from the Faroe Plateau were sampled in the autumn 2000 and five pooled samples were thereafter analysed for protein, ashes, water content, lipids, fatty acids, amino acids, vitamins, bulk minerals, trace elements, heavy metals, PCB, pesticides and toxaphenes. The analyses were conducted at the Food, Environmental & Veterinary Agency, Faroe Islands (protein, ashes, water), The Icelandic Fisheries Laboratories, Iceland (fat, fatty acids, iodine), Le Centre de Toxicologie du Quebec, Canada (minerals, heavy metals and organochlorines) and The University of Quelph, Canada (vitamins and amino acids). The results were compared with corresponding findings in national food tables from Sweden, Norway, Denmark and Iceland. It was not stated in these food tables where and when the cod was caught. Comparisons should therefore be taken with a grain of salt. The investigations demonstrated that cod from the Faroe Islands contained more proteins, amino acids and less water than cod from Denmark, Sweden and Iceland. The content of fatty acids was comparable. Cod from Faroe Islands contained higher values of potassium, magnesium and selenium than cod from Denmark, Norway, Sweden and Iceland. On the other hand, the manganese content was lowest in the Faroe-cod. The quantity of phosphor, calcium, iron, copper and iodine is at the same level as in the other Nordic Countries. Except for pyridoxin and folic acid, the level of vitamins was similar to the corresponding values in the other Nordic Countries. The content of pyridoxine and folic acid was 4-8 times lower and 5-8 times higher, respectively, in the Faroe-cod than in cod from the other Nordic Countries. The burden of mercury in the Faroese cod was low and proportional to the body-length. The lead and cadmium proportions were low and below the detection limit, respectively. The amount of PCB, DDT, pesticides, toxaphene was under the limit of detection. The high concentrations of folic acid in Faroese cod-fillet and the fact that deficiency of folic acid during the periconceptual period leads to neural tube defects such as spina bifida in the foetus prompted a subsequent folic acid project, encompassing three-year old cod from Norway, Denmark Faroe Islands and Iceland caught in the autumn 2003. The analyses were made in England (Eclipse Scientific Group, Chatteris, England). The results demonstrated that cod-fillet from Iceland was richest in folic acid. Though, the proportions in Icelandic cod were only a fifth of the aforementioned high quantities in cod from Faroe Islands in 2000. The discrepancy between the findings in the autumn 2000 and 2003 raises the question whether the folic acid content in cod-fillet fluctuates monthly or annually, or whether the reproducibility of the applied methods is unsatisfactory?

Introduction

Quantitative analysis of foods plays an important role in obtaining the necessary information for the purpose of nutritional labelling as well as surveillance of environmental pollutants. The purpose of this project is to analyse Faroese cod-fillet for some nutrients and contaminants and to compare the results with corresponding findings in the other Nordic Countries.
Material

Results

Organochlorines

Five pooled samples (2000) were analysed for Arochlor 1260, PCB (28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187), organochlorinated pesticides (β-BHC, α-Chlordane, γ-Chlordane, cis-Nonachlor, Hexachlorobenzene, Mirex, Oxychlordane, trans-Nonachlor, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDT, o,p'-DDT) and Toxaphenes (Parlar no 26 (T2), Parlar no 32, Parlar no 50 (T12), Parlar no 62 (T20), Parlar no 69, total toxaphenes). The results were below the limit of detection. These were: 61.6 µg/kg lipid (β-BHC, p,p'-DDT o,p'-DDE, p,p'-DDE, o,p'-DDD), 30.8 µg/kg lipid (PCBs and the other organochlorinated pesticides), 31 µg/kg lipid (Parlar no 26, 32, 50), 124 µg/kg lipid (Parlar no 62, 69), 513 µg/kg lipid (total toxaphene).

Nutritional value

The findings demonstrate that Faroese cod-fillet (2000) has a higher content of proteins and lower amount of water and fat compared with cod from Denmark, Sweden and Iceland (Table 2). The cod from Sweden has the lowest and highest quantity of protein and water, respectively, and consequently also the lowest energy content (Table 2).

Table 1. Sampling data. The gutted Danish cod was bought on the fish market in Hirtshals, therefore no sex-ratio.

<table>
<thead>
<tr>
<th>Fishing zone</th>
<th>N</th>
<th>Vessel</th>
<th>Fishing date</th>
<th>Position</th>
<th>Depth (m)</th>
<th>Ungutted weight ± sd (g)</th>
<th>Total length ± sd (cm)</th>
<th>Age ± sd (year)</th>
<th>f (%)</th>
<th>m (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iceland</td>
<td>25</td>
<td>Bjarni Sæmundsson</td>
<td>05.10.03</td>
<td>65°46'N 23°42'W</td>
<td>91</td>
<td>825 ± 125</td>
<td>66 ± 6</td>
<td>2.7 ± 0.6</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Faroe Bank</td>
<td>25</td>
<td>Mascot</td>
<td>20-22.10.03</td>
<td>61°00'N 08°5-7'W</td>
<td>107-132</td>
<td>3163 ± 798</td>
<td>66 ± 6</td>
<td>2.7 ± 0.6</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Faroe Plateau</td>
<td>25</td>
<td>Magnus Heinasen</td>
<td>17.10.03</td>
<td>62°23'N 07°27'W</td>
<td>94</td>
<td>1983 ± 214</td>
<td>58 ± 2</td>
<td>3.9 ± 0.5</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>Norway</td>
<td>23</td>
<td>Johan Hjort</td>
<td>13.09.03</td>
<td>72°58'N 31°12'E</td>
<td>279</td>
<td>454 ± 172</td>
<td>38 ± 5</td>
<td>3.2 ± 0.4</td>
<td>41</td>
<td>59</td>
</tr>
<tr>
<td>Denmark</td>
<td>25</td>
<td>Anon. vessel</td>
<td>xx.10.03</td>
<td>Kattegat</td>
<td>—</td>
<td>2578 ± 596</td>
<td>65 ± 5</td>
<td>3.9 ± 0.6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Faroe Plateau 24 Magnus Heinason 07-08.10.2000 62°21'N 07°38'W 99 3274 ± 1065 64 ± 7 4 ± 2 46 54

Table 2

<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>Protein (%)</th>
<th>Ashes (%)</th>
<th>Water content (%)</th>
<th>Fat content (%)</th>
<th>Energy content KJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faro Islands</td>
<td>24</td>
<td>20.2 ± 0.3</td>
<td>1.24 ± 0.04</td>
<td>78.6 ± 0.3</td>
<td>0.15 ± 0.09</td>
<td>350</td>
</tr>
<tr>
<td>Denmark</td>
<td>44</td>
<td>19.2</td>
<td>1.2</td>
<td>80.6</td>
<td>0.6</td>
<td>349</td>
</tr>
<tr>
<td>Sweden</td>
<td>44</td>
<td>17.0</td>
<td>1.1</td>
<td>82.0</td>
<td>0.7</td>
<td>315</td>
</tr>
<tr>
<td>Iceland</td>
<td>37</td>
<td>18.1</td>
<td>1.0</td>
<td>81.2</td>
<td>1.1</td>
<td>350</td>
</tr>
</tbody>
</table>
Fatty acids

The most conspicuous fatty acids in Faroese cod-fillet (2000) are the 16:0, 20:5n3 and 22:6n3 (Table 3). Apart from Danish cod, the total content of saturated fatty acids is comparable in Faroese, Norwegian and Icelandic cod. The amount of monounsaturated fatty acids in Faroese cod is lower than in Icelandic and Norwegian but higher than in Danish cod. Even if the cod-fillet from Faroe Islands is the leanest it has the highest proportions of the healthy polyunsaturated fatty acids compared with cod from Norway, Iceland and Denmark (Table 4). The total of the beneficial n3-fatty acids is the same in Faorese as in Norwegian cod (Table 4).

<table>
<thead>
<tr>
<th>Fatty acid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
</tr>
<tr>
<td>15:0</td>
</tr>
<tr>
<td>16:0</td>
</tr>
<tr>
<td>16:1n9</td>
</tr>
<tr>
<td>16:1n7</td>
</tr>
<tr>
<td>16:2n4</td>
</tr>
<tr>
<td>16:3n4</td>
</tr>
<tr>
<td>16:4n1</td>
</tr>
<tr>
<td>18:0</td>
</tr>
<tr>
<td>18:1n9</td>
</tr>
<tr>
<td>18:1n7</td>
</tr>
<tr>
<td>18:1n5</td>
</tr>
<tr>
<td>18:2n6</td>
</tr>
<tr>
<td>18:3n3</td>
</tr>
<tr>
<td>18:4n3</td>
</tr>
<tr>
<td>20:1</td>
</tr>
<tr>
<td>20:2n6</td>
</tr>
<tr>
<td>20:4n6</td>
</tr>
<tr>
<td>20:4n3</td>
</tr>
<tr>
<td>20:5n3</td>
</tr>
<tr>
<td>22:1</td>
</tr>
<tr>
<td>21:5</td>
</tr>
<tr>
<td>22:5n3</td>
</tr>
<tr>
<td>22:6n3</td>
</tr>
<tr>
<td>24:1</td>
</tr>
<tr>
<td>Unidentified</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Country</th>
<th>Total fat (%)</th>
<th>ΣSFA (%)</th>
<th>ΣMUFA (%)</th>
<th>ΣPUFA (%)</th>
<th>Σn3 (%)</th>
<th>Σn6 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faroe Islands</td>
<td>0.18</td>
<td>24.2</td>
<td>13.5</td>
<td>58.2</td>
<td>54.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Norway1</td>
<td>0.37</td>
<td>22.2</td>
<td>17.8</td>
<td>57.4</td>
<td>54.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Iceland2</td>
<td>1.1</td>
<td>24.1</td>
<td>22.8</td>
<td>49.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark2</td>
<td>0.6</td>
<td>16.7</td>
<td>6.7</td>
<td>41.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Fatty acid content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>15:0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>16:0</td>
<td>19.2 ± 0.5</td>
</tr>
<tr>
<td>16:1n9</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>16:1n7</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>16:2n4</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>16:3n4</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>16:4n1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>18:0</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td>18:1n9</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>18:1n7</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>18:1n5</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>18:2n6</td>
<td>0.4 ± 0.0</td>
</tr>
<tr>
<td>18:3n3</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>18:4n3</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>20:1</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>20:2n6</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>20:4n6</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>20:4n3</td>
<td>0.6 ± 0.0</td>
</tr>
<tr>
<td>20:5n3</td>
<td>17.4 ± 0.7</td>
</tr>
<tr>
<td>22:1</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>21:5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>22:5n3</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>22:6n3</td>
<td>33.9 ± 0.7</td>
</tr>
<tr>
<td>24:1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Unidentified</td>
<td>3.7 ± 0.3</td>
</tr>
</tbody>
</table>
Bulk and trace minerals

Faroese cod-fillet (2000) has a relatively low and high level of sodium and potassium, respectively. The ratio is approximately 8 and is the highest among the northern countries (Table 5). The quantity of phosphor is higher in Faroese and Norwegian cod than in Danish, Swedish and Icelandic cod. The content of magnesium is lower than in Norway, Denmark and Sweden but at roughly the same level as in Iceland. The amount of magnesium is highest in Faroese cod-fillet (Table 5). The ratio of calcium to magnesium is 0.3; the same is found in Iceland. This ratio is 1.3 in Norway and 0.6 in Denmark and Sweden (Table 5). The concentration of manganese and selenium is lowest and highest, respectively, in Faroese cod-fillet. The iron-content is at the same level as in Norway, Denmark and Sweden. The Icelandic cod has an exceptionally high amount of iron (Table 5). The zinc-concentrations are similar in the Nordic Countries. The quantity of copper is relatively high in Faroese cod and is slightly lower and than higher than Swedish and Norwegian cod, respectively. However, the copper-quantity is more than twice as high as in Danish and Icelandic cod (Table 5). The amount of iodine is a bit lower in Faroese cod than in Icelandic and Danish cod, but is almost three and four times higher than in Swedish and Norwegian cod, respectively (Table 5).

Vitamins

The content of thiamine (B1) in Faroese (2000) and Icelandic cod is approximately at the same level, which is one third lower than in Norwegian and Danish cod (Table 6). Cod from Norway has roughly three times higher quantities of riboflavin (B2) than cod from Faroe Islands, Denmark and Iceland. Faroese cod contains a concentration of niacin (B3), which is eleven times lower than Norwegian and Danish cod and seven times lower than Icelandic cod. The Faroese and Danish cod have similar amounts of pantothenic (B5) acid. The corresponding concentrations in Norwegian and Icelandic are about 30% and 40% higher. The concentration of pyridoxine (B6) in Faroese cod is lower than in Norwegian, Danish and Icelandic cod by a factor of 4, 6 and 9, respectively. However, Faroese cod exceeds Icelandic, Danish and Swedish cod in folic acid (B7) content by a factor of 5, 6 and 8, respectively. The level of cobalamin (B12) in Faroese and Norwegian cod is inferior to Icelandic and Danish cod by circa 40% (Table 6). The extraordinary high concentrations of folic acid in 2000 prompted a subsequent folic acid project in 2003 encompassing cod from Norway, Denmark, Faroe Islands and Iceland. These results demonstrated that cod-fillet from Iceland was richest in folic acid (Table 7). The concentrations of folic acid were significantly different in most cases (Table 7).

Table 5

<table>
<thead>
<tr>
<th>Country</th>
<th>Na (mg/100 g)</th>
<th>K (mg/100g)</th>
<th>P (mg/100g)</th>
<th>Ca (mg/100g)</th>
<th>Mg (mg/100g)</th>
<th>Mn (µg/100g)</th>
<th>Fe (µg/100g)</th>
<th>Se (µg/100g)</th>
<th>Zn (µg/100g)</th>
<th>Cu (µg/100g)</th>
<th>I (µg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faroe Islands</td>
<td>54 ± 10</td>
<td>428 ± 7</td>
<td>220 ± 10</td>
<td>9 ± 2</td>
<td>33 ± 1</td>
<td>0.010 ± 0.002</td>
<td>0.21 ± 0.02</td>
<td>0.039 ± 0.003</td>
<td>0.449 ± 0.027</td>
<td>0.042 ± 0.012</td>
<td>0.151 ± 0.044</td>
</tr>
<tr>
<td>Norway</td>
<td>58</td>
<td>340</td>
<td>220</td>
<td>28</td>
<td>21</td>
<td>0.03</td>
<td>0.16</td>
<td>0.027</td>
<td>0.42</td>
<td>0.033</td>
<td>0.04</td>
</tr>
<tr>
<td>Denmark²</td>
<td>76</td>
<td>338</td>
<td>200</td>
<td>15</td>
<td>25</td>
<td>0.02</td>
<td>0.2</td>
<td>0.028</td>
<td>0.38</td>
<td>0.019</td>
<td>0.172</td>
</tr>
<tr>
<td>Sweden³</td>
<td>50</td>
<td>355</td>
<td>190</td>
<td>16</td>
<td>28</td>
<td>0.03</td>
<td>0.25</td>
<td>0.027</td>
<td>0.39</td>
<td>0.06</td>
<td>0.055</td>
</tr>
<tr>
<td>Iceland⁴</td>
<td>118</td>
<td>332</td>
<td>177</td>
<td>6.7</td>
<td>25</td>
<td>0.02</td>
<td>0.97</td>
<td>0.028</td>
<td>0.38</td>
<td>0.019</td>
<td>0.204</td>
</tr>
</tbody>
</table>

Amino acids

The content of all the measured amino acids in Faroese cod (2000) transcends the corresponding values in Norwegian, Danish and Swedish cod (Table 8). These findings are also consistent with the relatively high protein-content in Faroese cod (Table 2).

Table 8

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Faroe Islands</th>
<th>Norway</th>
<th>Denmark</th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>2159 ± 110</td>
<td>1400</td>
<td>1870</td>
<td>1660</td>
</tr>
<tr>
<td>Serine</td>
<td>841 ± 25</td>
<td>700</td>
<td>770</td>
<td>680</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>3189 ± 62</td>
<td>2400</td>
<td>2580</td>
<td>2280</td>
</tr>
<tr>
<td>Glycine</td>
<td>841 ± 42</td>
<td>600</td>
<td>890</td>
<td>790</td>
</tr>
<tr>
<td>Histidine</td>
<td>484 ± 14</td>
<td>400</td>
<td>370</td>
<td>330</td>
</tr>
<tr>
<td>Taurine</td>
<td>96 ± 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>1193 ± 46</td>
<td>1000</td>
<td>1110</td>
<td>980</td>
</tr>
<tr>
<td>Threonine</td>
<td>936 ± 38</td>
<td>700</td>
<td>770</td>
<td>680</td>
</tr>
<tr>
<td>Alanine</td>
<td>1193 ± 43</td>
<td>1000</td>
<td>1110</td>
<td>980</td>
</tr>
<tr>
<td>Proline</td>
<td>621 ± 17</td>
<td>500</td>
<td>580</td>
<td>520</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>704 ± 33</td>
<td>600</td>
<td>680</td>
<td>600</td>
</tr>
<tr>
<td>Cystine</td>
<td>191 ± 8</td>
<td>130</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>1012 ± 26</td>
<td>700</td>
<td>980</td>
<td>870</td>
</tr>
<tr>
<td>Methionine</td>
<td>686 ± 25</td>
<td>500</td>
<td>580</td>
<td>640</td>
</tr>
<tr>
<td>Lysine</td>
<td>1923 ± 77</td>
<td>1600</td>
<td>1690</td>
<td>1450</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>948 ± 29</td>
<td>700</td>
<td>890</td>
<td>790</td>
</tr>
<tr>
<td>Leucine</td>
<td>1607 ± 53</td>
<td>1300</td>
<td>1380</td>
<td>1220</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>777 ± 30</td>
<td>700</td>
<td>710</td>
<td>630</td>
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<td>Σalpha</td>
<td>19401</td>
<td>14800</td>
<td>17090</td>
<td>15220</td>
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</table>

Heavy metals

The findings reflect a correlation between length and concentration of mercury in Faroese cod (Larsen & Dam 1999: “AMAP phase 1 The Faroe Islands. Stange, Maage, Klungsøy 1996: “Contaminants in fish and sediments in the North Atlantic Ocean, TemaNord 1996:522”). Bioaccumulation in cod-filet is evident (Table 9). Cod from Norway contain up to 11 times more mercury than Faroese cod. Cadmium and lead concentrations in Faroese cod are lower and higher, respectively, than in Norwegian cod. Bioaccumulation of these undesirable heavy metals is not as obvious as in the case of mercury (Table 9).
Table 9: n.a.: not analyzed

<table>
<thead>
<tr>
<th>Country</th>
<th>Position</th>
<th>Date</th>
<th>N</th>
<th>Age (year)</th>
<th>Length (cm)</th>
<th>Hg (mg/kg wetweight)</th>
<th>Cd (mg/kg)</th>
<th>Pb (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faroe Islands</td>
<td>62°21’N 7°38’W</td>
<td>10.2000</td>
<td>24</td>
<td>4 ± 2</td>
<td>64</td>
<td>0.043 ± 0.008</td>
<td>&lt;0.0002</td>
<td>0.0107 ± 0.0001</td>
</tr>
<tr>
<td>Faroe Islands</td>
<td>62°23’N 7°30’W</td>
<td>10.1997</td>
<td>44</td>
<td>n.a.</td>
<td>59</td>
<td>0.028</td>
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</table>

Authors

Hóraldur Joensen\(^a\) (corresponding author) and Heidi Gregersen\(^b\)

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1.2 STUDY OF THE POSSIBLE INTERACTIONS BETWEEN $\alpha$-TOCOPHEROL AND AROMATIC/ALIPHATIC AMINES

Narcisa M. Bandarra, Ana Rodrigues and Irineu Batista

Introduction

The high level of $\omega_3$ polyunsaturated fatty acids in fish oils is important in terms of nutritional benefits. However, this characteristic is responsible for the susceptibility to oxidation of these lipids. Thus, in order to protect these fatty acids against oxidation is important to use effective antioxidants. However, lipid oxidation follows a complicated set of mechanisms, and no single antioxidant is effective in the prevention during all the stages of reaction. It may be advisable to use antioxidant combinations in which the antioxidants act in a synergistic way. $\alpha$-Tocopherol is a natural antioxidant most widely used and the improvement of its effectiveness when combined with other compounds such as the phospholipids PC and PE has been reported (Segawa et al., 1995). The mechanism involved is not clear but it seems to be related to the amino group of phospholipids.

The aim of this work was to obtain a deep knowledge about the synergistic mechanism responsible for this antioxidant protection, using aromatic and aliphatic amines as chemical models.

Materials and Methods

Raw material

Sardine oil was obtained from a fishmeal factory. $\alpha$-Tocopherol was added to fish oil (0.4 mg/g) together with different amines at two $\alpha$-tocopherol/amine molecular ratios: 1:20 and 1:40. In table 1 are shown the amine concentrations used. Oil samples without added $\alpha$-tocopherol were also used as a control.

Table 1 – Amine concentrations.

<table>
<thead>
<tr>
<th>Amine</th>
<th>Concentration (mg/g oil)</th>
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<tr>
<td>p-nitroaniline</td>
<td>2.6 (c) 5.1 (2c)</td>
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<tr>
<td>Aniline</td>
<td>1.7 3.5</td>
</tr>
<tr>
<td>p-toluidine</td>
<td>2.0 4.0</td>
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<tr>
<td>Cyclohexylamine</td>
<td>1.8 3.7</td>
</tr>
<tr>
<td>Propylamine</td>
<td>1.1 2.2</td>
</tr>
</tbody>
</table>

Sardine oil samples (5g) were poured into Petri dishes and allowed to oxidised for one month in the dark at 40 °C±2 °C. Samples were taken at regular intervals for different analyses.

Analytical methods

Peroxide value (PV) determination was performed by a titrimetric method with potassium iodide and sodium thiosulfate according to the Official Methods and Recommended Practices of the American Oil Chemists’ Society (1989). Anisidine value determination was carried out as described by Windsor and Barlow (1981). $\alpha$-Tocopherol analysis was done dissolving 9 mg of oil in 250 µl of hexane and 20 µl were injected in a system JASCO model PU 980 equipped with an auto-sampler JASCO AS-950-10 following the analytical conditions referred by Bandarra et al. (2003).

Synergistic effect (%) was calculated using the PV results obtained, following the equation indicated by Saito and Ishihara (1997).

$$\% \text{ Synergistic Effect} = \frac{S_{\text{oil}+\alpha-\text{tocopherol}} - S_{\text{oil}+\alpha-\text{tocopherol}+\text{amine}}}{S_{\text{oil}+\alpha-\text{tocopherol}}} \times 100$$

where: $S_{\text{oil}+\alpha-\text{tocopherol}}$ – slope of the plot of PV vs time; $S_{\text{oil}+\alpha-\text{tocopherol}+\text{amine}}$ – slope of the plot of PV vs time.
Results and Discussion

The fish oil used presented the usual high level of polyunsaturated fatty acids, which are easily oxidised. Their oxidation is clearly evidenced in Fig. 1A where it can be also observed the fast decrease of the endogenous \( \alpha \)-tocopherol level. Its initial value was 40 ppm, but completely disappeared after 4 days of storage. In order to avoid the fast oxidative process it is usual to add an antioxidant such as \( \alpha \)-tocopherol. This is illustrated in Fig. 1B where approximately 400 ppm was added. However, after 15 days it almost disappeared, which put into evidence the need for a combination of \( \alpha \)-tocopherol with other compounds enabling to keep it active as long as possible. Having this in mind it was studied the effect between \( \alpha \)-tocopherol and aromatic and aliphatic amines on the oxidation prevention of sardine oil.

The combined effect of \( \alpha \)-tocopherol and the different amines is shown in Figs. 2 and 3. The evolution of PV of oil samples with pNA show an induction period of 15 and 21 days for the concentration ratios 1:20 (c) and 1:40 (2c), respectively. These results indicate a possible synergism between this amine and \( \alpha \)-tocopherol and its evolution seem to be dependent on the amine concentration. Thus, when high concentration of pNA is used a depletion of \( \alpha \)-tocopherol in two stages seems to occur: (i) a fast initial step as a result of the possible pro-oxidant effect of pNA followed by (ii) a second one with a slower degradation where other formed antioxidant compounds (Maillard-like compounds) could be present. The secondary oxidation products were kept at low level in the sample oils with the higher amine concentration (Fig. 3).

The system with aniline/\( \alpha \)-tocopherol for the highest amine concentration showed longer induction period than the system pNA/\( \alpha \)-tocopherol. This may result from the non pro-oxidant activity of aniline compared to pNA. The evolution of AV is quite similar to that of PV (Fig. 3). Until the 15th day the AV was similar in both amine concentrations in oil samples but after that period the highest concentration was more effective.

In the case of p-toluidine a considerable increase of the induction period was recorded. The initial level of \( \alpha \)-tocopherol was maintained until the 5th day suggesting its regeneration, which was not observed in the other amine/\( \alpha \)-tocopherol systems. After this period the regeneration was not so effective but the level of \( \alpha \)-tocopherol was kept relatively high until the 21st day. Very low levels of secondary oxidation products (Fig. 3) were formed due to the limited formation of peroxides and also to their elimination by Maillard-type reactions with p-toluidine.

Cyclohexylamine/\( \alpha \)-tocopherol system also presented good antioxidant properties and it is possible that Maillard-type compounds play an important role to regenerate or “protect” \( \alpha \)-tocopherol. The evolution of \( \alpha \)-tocopherol in these trials was very different for both concentrations, showing a decrease very accentuated at the 5th day in the case of the highest concentration of this aliphatic amine whereas a very slow decrease was recorded in the system when lower concentration of cyclohexylamine was used. Furthermore, \( \alpha \)-tocopherol was present in the oil samples until the end of the experiments with both concentrations of cyclohexylamine. These results were unexpected and suggest different mechanisms involved. The level of secondary oxidation products was very low in both systems during all the experiment (Fig. 3).

The propylamine/\( \alpha \)-tocopherol system was also very effective although the PV were higher than in the previous system with cyclohexylamine. On the other hand the level of \( \alpha \)-tocopherol was kept relatively constant when the
The highest concentration of propylamine was used but in the system with less amine it disappeared after 21 days. It has also to be mentioned that aliphatic amines can act as oxygen scavengers, which is an important antioxidative mechanism to take into account. The AV was very low for the most concentrated amine samples whereas a gradual increase was measured in the oil samples with a lower propylamine level (Fig. 3). The complexity of these systems does not permit a clear picture of all mechanisms involved.

Figure 2 - Evolution of peroxide value and added α-tocopherol level in sardine oil with p-nitroaniline (pNA), aniline (A), p-toluidine (pT), cyclohexylamine (C), and propylamine (prA).
Figure 3 - Evolution of anisidine value in sardine oil with added tocopherol and p-nitroaniline pNA), aniline (A), p-toluidine (pT), cyclohexylamine (C),and propylamine (prA).

In table 2 are presented the induction periods of each amine/α-tocopherol system.
Table 2 – Induction periods of the different amine/α-tocopherol systems.

<table>
<thead>
<tr>
<th>System</th>
<th>Induction period (days)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Ratio 1:20 (c)</td>
</tr>
<tr>
<td>Oil + α-tocopherol</td>
<td>10</td>
</tr>
<tr>
<td>Oil + α-tocopherol + pNA</td>
<td>15</td>
</tr>
<tr>
<td>Oil + α-tocopherol + aniline</td>
<td>15</td>
</tr>
<tr>
<td>Oil + α-tocopherol + p-toluidine</td>
<td>25</td>
</tr>
<tr>
<td>Oil + α-tocopherol + cyclohexylamine</td>
<td>30</td>
</tr>
<tr>
<td>Oil + α-tocopherol + propylamine</td>
<td>25</td>
</tr>
</tbody>
</table>

Figure 4 – The synergistic effect of the different amine/α-tocopherol systems studied.

Conclusions

The results of the antioxidant synergy of α-tocopherol and the different amines is summarized in the Fig. 4 and the following conclusions could be draw:

- Both p-toluidine/α-tocopherol and cyclohexylamine/α-tocopherol systems presented the highest synergy for the ratio 1:40.
- High synergistic effect was also registered for p-toluidine and cyclohexylamine (ratio 1:20) and propylamine (ratio 1:40).
- The regeneration or “protection” of α-tocopherol in the systems p-toluidine/α-tocopherol, cyclohexylamine/α-tocopherol and propylamine/α-tocopherol seemed to have occurred.
- A number of simultaneous antioxidative mechanisms involving regeneration of α-tocopherol, Maillard-type compounds, oxygen scavenging and destruction of peroxides seem to be present in these systems.
Acknowledgements

Financial support (project ICA4-CT-2001-10032G “Improving the utilisation of low value fish by processing” and QCAIII project “Biotecnologia dos Organismos Marinhos”) is acknowledged.

References


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E-mails: narcisa@ipimar.pt ; irineu@ipimar.pt
1.3 ESSENTIAL AND TOXIC METALS IN SEAFOOD COMMERCIALISED IN PORTUGAL

Lourenço, H. M.; Assunção, J.; Raimundo, C.; Afonso, C.; Martins, M. F.; Nunes, M. L.;

Introduction

Seafood is considered an indispensable product in equilibrated diets, partly because almost elements, which have been mentioned to be essential for man, can be found in fish products at different levels. Nevertheless, such products can, in a certain extent, be contaminated with some chemicals, like mercury, lead and cadmium, coming of from several sources.

Species like small pelagic, tuna, hake, black and silver scabbard fish, swordfish, monkfish or wreckfish are important species in Portugal market not only in terms of economic value but also in what concerns the actual demand. Thus, it was aim of this work to characterise some seafood relatively to essential minerals composition and the possible accumulation of toxic metals. In this line, levels of calcium (Ca), copper (Cu), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), zinc (Zn) and also total mercury (Hg), lead (Pb) and cadmium (Cd) were quantified in 17 fish species and three products collected in retailer market.

Material and Methods

17 fish species and some products like hake and black scabbard fish eggs and monkfish liver, collected in several Lisbon retailers, were studied. Four samples of each fish or fish product were analysed, each one composed by five specimens. Depending on availability, samples were made up by portions or whole fish. Edible part was homogenised in a food blender and stored at –25ºC until further analysis. Phosphorus was analysed by UV-Visible spectrophotometry according to ISO/TC 34/SC6 N371 (1991). Ca, Cu, Mg, K, Na, Zn, Cd and Pb were performed by flame atomic absorption, following the method described in AOAC (1990). Total Hg was determined by cold vapour atomic absorption according to the procedure developed by Hatch and Ott (1968), described by Joiris et al. (1991).

Results and Discussion

In table I, the average concentration of analysed mineral elements for each studied species, in mg/kg of wet weight, are presented. In general, essential minerals in all studied fish species followed a series like K>P>Na>Mg>Ca>Zn>Cu, which suggests that these elements have the same importance whatever be the organism. Evident exceptions occurred in salted cod, in which sodium was detected in first place and in monkfish liver and black scabbard fish eggs in which phosphorus was the major element.

The highest average concentrations of potassium were found in tuna, sardine, swordfish and red fish, being the maximum value registered in tuna (3473 mg/kg wet weight). Concerning phosphorus, the major contents were detected in sardine and tuna, 2753 and 2553 mg/kg wet weight, respectively, but hake and black scabbard fish eggs also presented high concentrations. According to Lall (1995) fishery products are a good resource of this mineral. Sodium emerged as the most abundant element in salted cod and such value is certainly due to the preservation process. Nevertheless, in some species, like monkfish, red fish and smoothhound the level of this mineral was also appreciable. Salted cod, blackbelly rosefish and horse mackerel showed the highest levels of magnesium (481.0, 349.2 and 328.4 mg/kg wet weight, respectively). Among macro elements, calcium was the mineral that was presented in lower concentration in edible part of all studied species, which is in agreement with Lall (1995) results. Axillary seabream and wreckfish were the most rich fish species in calcium. Zinc was the microelement more abundant in all fish species, conger showed the highest concentration (12.3 mg/kg wet weight), followed by monkfish (7.4 mg/kg wet weight). On the other hand, hake and black scabbard fish eggs make a distinction by being a good source of this oligoelement. Sardine was the richest species in copper and monkfish liver presented the major level, about 10 mg/kg wet weight.

Relatively to toxic metals, some samples of axillary seabream, blackbelly rosefish, conger, wreefish, smoothhound and black scabbard fish exceeded the proposed EU limits (0.5 mg/kg wet wt. or 1.0 mg/kg wet wt., depending of species) for total mercury levels (EU, 2002). The highest total mercury concentration was found in
Table I – Average concentration of macro and micro elements (mg/kg of wet weight) in some fishery products.

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<th>Species</th>
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<th>Ca</th>
<th>Zn</th>
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<td>328.4</td>
<td>16.5</td>
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the liver of black scabbard fish (6.5 mg/kg wet wt.). Regarding cadmium, it has to be stressed the considerable levels registered in monkfish and black scabbard fish liver and in swordfish muscle. The levels of lead in all studied samples were considerably lower than the proposed EU limit, 0.2 mg/kg wet wt., (EU, 2002).

Conclusions

From this study it can be stressed that fish and fish products commercialised in Portugal can be a good source of various minerals and in general the levels of toxic metals do not exceed the limit values proposed by UE. Nevertheless, the consumption of black scabbard fish liver should be excluded of human diet and some fish like smoothhound and black scabbard fish should be consumed with moderation.

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ISO/TC 34/SC6 N371 (1991), 10p

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1.4 THE EFFECT OF HERRING MUSCLE PRESS JUICE ON THE GENERATION OF REACTIVE OXYGEN SPECIES FROM HUMAN MONOCYTES STIMULATED WITH PHORBOL MYRISTATE ACETATE

Guðjón Gunnarsson, Ingrid Undeland, Ann Lindgård, Ann-Sofie Sandberg and Bassam Soussi

Introduction

A protective effect of the aqueous extract from cod muscle (press juice) on oxidation has been shown (Undeland et al., 2003). In a model system consisting of washed minced cod muscle, the press juice from cod inhibited the onset of hemoglobin mediated oxidation during ice storage. This indicates the presence of a strong aqueous antioxidant in the cod muscle. Herring press juice has also shown protective effects against Fe-ascorbate stimulated oxidation of isolated fish muscle microsomes (Slabyj and Hultin., 1983). The antioxidant effect of herring press juice has been contributed to the high molecular weight fractions (>3500 Da), while the low molecular fraction (<1000 Da) asserted most of these effects in cod.

In this study, we wish to take these observations a step further and use a single cell system, isolated human monocytes. Human monocytes are equipped with an enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that creates a respiratory burst as a part of the phagocytosis process. The products of a respiratory burst are, among others, reactive oxygen species (ROS), superoxide anion, hydrogen peroxide and other radicals.

The objective is to study the effects of herring press juice on the generation of ROS in human monocytes stimulated with phorbol myristate acetate (PMA) using isoluminol-enhanced chemiluminescence (Lundqvist and Dahlgren., 1996).

Materials and methods

Isolation of human monocytes

The Ficoll-Hypaque procedure was used to collect mononuclear cells from buffy coats (Böyum., 1976). Monocytes were isolated from human blood with adhesions-step technique (Mattson-Hultén et al., 1999). They were then incubated in culture flasks, pretreated with human serum, for 1 h at 37°C in 5% CO2. The nonadherant cells were washed off with phosphate-buffered saline (PBS). The monocytes that had attached to the surface were detached with 5 ml PBS containing 5 mM ethylenediaminetetraacetate (EDTA) and 2% heat-inactivated fetal calf serum (FCS) for 20 min at 4°C. The monocytes were then collected, washed, and resuspended in Krebs Ringer phosphate buffer with glucose (KRG). They were kept in a bath of melting ice immediately after preparation.

Chemiluminescence measurements

The chemiluminescence (CL) was measured in duplicates of each sample at 37°C in a luminometer, using disposable polystyrene tubes with a reaction volume of 1 ml. The reaction mixture to measure extracellular CL contained 5,6 × 10^-5 M isoluminol (6-amino-2,3-dihydro-1,4-phthalazine-dione), 4 units horseradish peroxidase (HRP). The concentration of human monocytes in the mixture was 5 × 10^5 viable cells. To activate the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, phorbol myristate acetate (PMA) was added just before analyzing, final concentration 1 × 10^-6 M. Reaction volume was adjusted to 1 ml with KRG. The CL was recorded as a rate of production over time. The results were expressed in millivolts, produced from 5 × 10^5 viable cells.

Human monocytes were incubated with herring press juice, in different dilutions or buffer (control) and then stimulated with PMA. The resulting ROS production was measured as isoluminol enhanced chemiluminescence.
**Results**

The herring press juice lowered the max peak value and the total generated CL, shown as the rate of ROS production as a function of time. There was also an increase in the lag phase of the samples treated with herring press juice compared to controls. The lag phase increased from zero for controls and up to 10 minutes for samples treated with herring press juice. The results further show that the max peak value of produced ROS decreased up to 60% for human monocytes treated with herring press juice (dilution factor 100 in the assay). Looking at the total generated ROS, the decrease was also up to 60%. Different dilutions of herring press juice showed dose-response behavior in the model system.

**Conclusion**

We have shown that herring press juice suppresses the production of reactive oxygen species in human monocytes. Work is ongoing to elucidate the mechanism of these interesting observations in a number of model systems.

**References**


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**1.5 NOVEL NATURAL COMPOUNDS FROM GRAPE BYPRODUCTS AS INHIBITORS OF RANCIDITY IN FISH LIPIDS AND IN FROZEN FATTY FISH**

*M. Pazos, M. J. González, J. L. Torres, J. M. Gallardo and I. Medina.*

**Introduction**

Marine lipids are an important nutritional seafood particularly due to their high concentration of polyunsaturated fatty acids (PUFA) (*Ackman, 1989*). But, this high content of unsaturated lipids makes fish products very susceptible to loss of quality by development of lipid oxidation. Rancidity is specially faster in species like mackerel (*Scomber scombrus*) or horse mackerel (*Trauchurus trachurus*), in which muscle coexist large amounts of hemoglobin, a well-known activator of lipid oxidation, and of lipids (*Richards and Hultin, 2002*). The development of rancidity in fish oils, fish oils in water emulsions and frozen fatty minced muscle have been successfully retarded by natural antioxidants obtained from grape byproducts (*Vitis vinifera*) pomace. A total grape extract and purified fractions containing a wide variety of compounds as flavanol monomers, oligomers (procyanidins) and glycosylated flavonols were tested and their effectiveness was compared with propyl gallate, a synthetic antioxidant. The protection of endogenous antioxidant systems (α−tocopherol, ubiquinone-10, total glutathione) and n-3 polyunsatured fatty acids (PUFA) has been also investigated in frozen minced muscle.

**Materials and Methods**

Total phenolic extract, OW, was isolated by *Torres & Bobet (2001)* procedure and its fractionation was performed as described *Torres et al. (2002)*. OW and grape fractions were characterized by mean degree of polymerisation, mean molecular weight, antiradical power, portioning coefficients between oil /water, and percentage of galloylation. Fish oil was purchased by Fluka (New-Ulm, Switzerland). Oil-in-water emulsions containing 1% lecithin and 10% fish oil were prepared in water as previously was described by Huang et al. (1996b). Minced light muscle from fresh mackerel (*Scomber scombrus*) was used. Antioxidants were employed at concentration of 100 ppm. Bulk oil, oil in water emulsion and minced fish muscle were stored during experiments at 40, 30 and −10 °C, respectively. The rate of oxidation was monitored by at least two of followings methods: peroxides value (*Chapman and McKay, 1949*), conjugated diene and triene hydroperoxides (*Huang et al., 1996a*), TBA index (*Vyncke, 1970*) and measure of fluorescent compounds resulting from protein-oxidized lipid interactions (*Nielsen et al., 1985*). Analysis of PUFA were performance according to *Christie (1982)* method. Tocopherol and ubiquinone-10 was extracted as described *Burton et al. (1985)* and determined according to *Cabrini et al. (1992)*. Total glutathione was extracted by *Petillo et al. (1998)* and measured as described *Griffith (1980)*. Induction periods were calculated as the time (in days) required for a sudden change in the rate of oxidation by the method of tangents to the two parts of the kinetic curve (*Frankel, 1998*).

**Results**

Table 1 shows mean degree of polymerisation, partitioning coefficients between oil and water and percentage of galloylation of grape fractions.
Table 1. Mean degree of polymerization, percentage galloylation and partitioning coefficients between oil and water of grape phenolic fractions

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>Degree of polymerization</th>
<th>Galloylation (%)</th>
<th>Partitioning Coefficients (oil / water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total extract (OW)</td>
<td>1.7</td>
<td>15</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Monomers and oligomers flavanols (I)</td>
<td>1.4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Oligomers polymers flavanols (IV)</td>
<td>2.7</td>
<td>25</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Monomers flavanols (V)</td>
<td>1.0</td>
<td>&lt;1</td>
<td>0.19 ± 0.08</td>
</tr>
<tr>
<td>Oligomers flavanols (VI)</td>
<td>2.4</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Monomers flavonols (VII)</td>
<td>1.0</td>
<td>0</td>
<td>0.36 ± 0.03</td>
</tr>
<tr>
<td>Polymers flavanols (VIII)</td>
<td>3.4</td>
<td>34</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td>Propyl Gallate</td>
<td>1.0</td>
<td></td>
<td>0.83 ± 0.01</td>
</tr>
</tbody>
</table>

All grape phenolic extracts retarded lipid oxidation in fish oil as shown by the induction periods of conjugated dienes and trienes hydroperoxides (Table 2). Grape monomers were more effective than grape oligomers delaying oxidation in bulk fish oil. Monomeric flavanols (V) were more effective in fish oils than monomeric flavonols (VII). The synthetic antioxidant resulted more effective for inhibiting the formation of hydroperoxides, both conjugated dienes and trienes, than grape flavonoids. Considering induction periods (Table 2) total extract (OW) and fraction IV, containing oligomeric flavanols with intermediate degree of polymerization and percentage galloylation, were the most efficient antioxidants in fish oil in water emulsion. Propyl gallate had a inhibitory activity of rancidity similar to OW and fraction IV in emulsified fish oil. Monomers were less effective in this system than in bulk fish oil, compared with oligomers and polymers. Monomeric flavonols (VII) were the least efficient.

Table 2. Induction periods (in days) of formation of conjugated dienes and trienes hydroperoxides and fluorescent compounds in bulk fish oil and fish oil in water emulsion.

<table>
<thead>
<tr>
<th>Phenolic Antioxidants</th>
<th>Bulk oil</th>
<th>Oil in water emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dienes</td>
<td>Trienes</td>
</tr>
<tr>
<td>Control</td>
<td>2.8</td>
<td>3.9</td>
</tr>
<tr>
<td>OW</td>
<td>3.2</td>
<td>4.1</td>
</tr>
<tr>
<td>I</td>
<td>4.2</td>
<td>5.0</td>
</tr>
<tr>
<td>IV</td>
<td>3.7</td>
<td>5.1</td>
</tr>
<tr>
<td>V</td>
<td>4.9</td>
<td>5.3</td>
</tr>
<tr>
<td>VI</td>
<td>4.2</td>
<td>5.0</td>
</tr>
<tr>
<td>VII</td>
<td>4.4</td>
<td>5.3</td>
</tr>
<tr>
<td>VIII</td>
<td>3.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Propyl gallate</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

Induction periods of peroxides and aldehydes (TBA-i) obtained in minced mackerel muscle without antioxidant addition or treated with a 0.01% of antioxidant were respectively: control = 22 and 29 days; OW = 26 and 34 days; IV = 30 and 35 days; V = 32 and 37 days; VIII = 26 and 34 days and propyl gallate = 34 and 37 days. Table 3 shows values of peroxides and aldehydes after 39 and 66 days in minced muscle stored in frozen. These data demonstrated that all flavonoid fractions and propyl gallate were effective for retarding oxidation during frozen storage. The inhibition of formation of peroxides was higher than the inhibition of formation of volatiles. Grape phenolic fractions showed different effectiveness for decreasing the rate of oxidation and the amount of oxidation products formed. Fractions IV and V were the most efficient, and VIII gave the poorest results.
Table 3. Index of peroxides and aldehydes in minced muscle stored in frozen.

<table>
<thead>
<tr>
<th>Phenolic antioxidant</th>
<th>Peroxides (meq. O₂ / Kg lipid). Day 39</th>
<th>TBA-i (mg MDA / Kg muscle). Day 66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.8</td>
<td>4.1</td>
</tr>
<tr>
<td>OW</td>
<td>6.3</td>
<td>2.0</td>
</tr>
<tr>
<td>IV</td>
<td>4.6</td>
<td>1.7</td>
</tr>
<tr>
<td>V</td>
<td>3.5</td>
<td>2.0</td>
</tr>
<tr>
<td>VIII</td>
<td>6.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Propyl gallate</td>
<td>3.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Significant degradation of n-3 PUFA was observed in controls after 83 days of frozen storage. So, control muscle contained 22.6 ± 0.2 mg of 22:6 n-3 per g lipid and muscle treated with grape polyphenols and propyl gallate maintained the initial levels (about 25 mg of 22:6 n-3 per g lipid). There were not significant differences among antioxidants.

All polyphenolic fractions and propyl gallate achieved a huge preservation of level of α-tocopherol in minced muscle during frozen storage (Figure 1). Minced muscle with fraction IV and V maintained better α-tocopherol than total extract and fraction VIII. Propyl gallate was the best inhibitor of loss of tocopherol.

**Figure 1.** Levels of α-tocopherol in mackerel minced muscle with and without addition of exogenous compounds during storage at −10 °C.

In addition, all those exogenous compounds delayed the depletion of total glutathione and ubiquinone (data not shown), endogenous antioxidants, though less efficiently than inhibition of loss of tocopherol. All grape polyphenols and propyl gallate had comparable efficacy delaying loss of ubiquinone.

**Discussion**

In bulk fish oils, monomeric flavanols and propyl gallate showed the highest efficiency. A previous study in bulk corn oil has also indicated a higher effectiveness of monomers flavanols than polymers (Torres et al., 2002). Since fractions IV, VIII, and OW showed the same efficiency the degree of polymerization and percentage galloylation of procyanidins did not show any relation to the activity in bulk oils. Glycosylated flavonol monomers (fraction VII) were less efficient than the non-galloylated monomeric flavanols (V, mostly catechin). In oil-in-water emulsions, grape oligomers were more efficient than monomers. Propyl gallate was also highly effective in emulsions and it is in agreement with the observation that hydrophobic compounds are efficient in emulsions because they are largely accumulated in oily drops and oily/aqueous interface (Frankel, 1998) due to capacity of procyanidins to establish hydrophobic and/or hydrophilic interactions, depending on the
environment, as already suggested Torres et al., (2002). The components of these fractions have hydrophobic cores with hydrophilic hydroxyl groups and may accumulate in oily/aqueous interface. Fraction IV and V were the most effective natural compounds for inhibiting oxidation and rancidity in mackerel muscle during frozen storage. The total extract, OW, and fraction VIII showed significant efficiency as well. Propyl gallate was the major inhibitor of peroxides and aldehydes. It is remarkable the antioxidant activity of OW and fraction IV, in minced muscle and fish oil emulsion, since were present in lower molar concentrations than synthetic antioxidant and monomeric flavanols, fraction V. Molar concentration corresponding of concentration of 0.01 % of OW, fraction IV, fraction V and propyl gallate is, respectively: 0.18, 0.11, 0.34 and 0.47 µM.

Both, grape polyphenols and propyl gallate preserved α−tocopherol, ubiquinone-10 and total glutathione, endogenous substances of fish muscle with antioxidant properties. The inhibition of depletion of these endogenous compounds could be due basically to actuation of exogenous compounds as first barrier against oxidation, preserving therefore endogenous antioxidants, or to their possible regeneration by exogenous antioxidants.

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References


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1.6 PREVENTING SEAFOOD LIPID OXIDATION AND TEXTURE SOFTENING TO MAINTAIN HEALTHY COMPONENTS AND QUALITY OF SEAFOOD (LIPIDTEXT A SEAFOODPLUS PROJECT)


Seafood products are healthy foods due to their high content of n-3 lipids, antioxidants, valuable proteins and other healthy components. However, the nutritional value and sensory quality of seafood products may deteriorate during processing and storage due to oxidation processes and other post mortem changes in the fish muscle. The objective of the LIPIDTEXT project is to secure and maintain nutritional value (high level of antioxidants, n-3 lipids, and low levels of potentially toxic oxidation products) and high sensory quality (colour, flavour, texture parameters) of seafood products including fresh and frozen fish fillets, fish based products and fish oil enriched systems. The LIPIDTEXT project is part of the SEAFOODplus programme under the EU FP6.

The project aims at understanding the mechanisms and kinetics of the processes leading to rancidity and texture changes, and to develop new technologies to maintain quality. To date, post mortem changes in the lipid and protein fractions of muscle have largely been looked upon as separate problems. The LIPIDTEXT project is focusing on sensory and nutritional degradation of fish muscle arising from chemical interactions between oxidation products of lipids/proteins with intact lipids/proteins. Such reactions not only destroy valuable lipids, antioxidants and proteins, but can also result e.g. in production of volatiles (rancidity), protease activation (muscle softening), protein cross-linking (muscle toughening) and pigment formation (discoloration).

The LIPIDTEXT project is composed of three parts: 1) one aiming at establishing model systems to be used for studies of oxidation and for mathematical modelling of oxidation; 2) one aiming at elucidating interaction reactions between proteins and lipids, the relationship between protease activity and texture, and the effect of antioxidants on lipid/protein oxidation, and 3) one aiming at finding new predictors for the critical reactions in relation to texture changes.

The research work is organised in 6 work blocks as shown in Fig. 1. The figure also illustrates the interactions between the different work blocks.

![Fig. 1. Organisation of research work in LIPIDTEXT](image-url)
More details about the research to be carried out in each research block are given below.

**Block 1. Experimental system establishment and**

This block will focus on development of experimental systems for detailed studies of lipid/protein oxidation and proteolysis in different types of fish products. We will focus on establishing experimental systems that will allow for studies on interactions between oxidised lipids/proteins with intact lipids/proteins in fish oil emulsions, liposomes, washed mince and whole muscle. We will move forth and back between simple and more complex model systems.

**Block 2. Modelling of oxidation in defined/real food emulsions and fish muscle**

Data on oxidation of marine lipids from block 1 and 4 will be analysed and mathematical models describing the oxidation kinetics in different systems containing marine lipids will be developed. This work will elucidate how lipid oxidation is dependent upon pro-oxidants. The mathematical models will be developed from relatively simple model systems to complex systems and might in future be used to design production methods for seafood with minimum lipid oxidation.

**Block 3. Relationship between protease activity and texture**

Rapid assays of the main proteases present in fish muscle will be developed using fluorescent-labelled substrates in an ELISA plate type of assay. The proteases active post-mortem in fish and their correlation with texture will be determined. Differences in the rate/extent of post mortem softening between species will be utilised in the study. In model systems based on mixtures of fractions from different fish the regulation of the proteases will be studied. Moreover, the relationship between lipid/protein oxidation and protease activity will be studied.

**Block 4. Interactions between reactants in experimental systems**

To obtain an understanding of protein/lipid oxidation mechanisms in fish products the interactions between the reactants responsible for the oxidative deterioration will be studied at the molecular level in simple model systems related to real foods. Interactions between proteins and lipids will be studied including oxidised hemoproteins, antioxidants, low molecular weight pro-oxidants. We will also study how oxidised lipids will interact with structural proteins to explain how this affects protein functional properties. In the model emulsions, we will study how the choice of emulsifier will affect oxidative stability and we will use this knowledge to improve oxidative stability in real fish oil enriched foods. On the basis of results in block 5, additional antioxidant experiments will be performed in model systems to elucidate the antioxidant mechanisms.

**Block 5. Effect of natural antioxidants in fish products**

The antioxidant capacity of natural compounds (also evaluated in model systems in block 4) will be investigated in frozen fish products and the effect of added antioxidants on the loss of endogenous antioxidants will be determined. Methodologies for antioxidants application will be tested in order to select the most efficient antioxidant. Selected antioxidants and antioxidant concentrations will be tested. Experiments will be carried out in both small and large scale.

**Block 6. Effect of protein expression pattern on texture**

The protein expression pattern will be used as a tool to find markers that can predict the quality (texture) of the final product from the raw material. Protein expression will be evaluated by proteome analysis using computer-aided image analysis in order to identify isoforms of muscle-expressed proteins. We will relate protein expression to protein and lipid oxidation.
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1.7 OCCURRENCE OF PEPTIDES IN TROUT MUSCLE DURING POST MORTEM STORAGE AND COOKING

Caroline Bauchart, Didier Rémond, Christophe Chambon, Martine Morzel.

Introduction

Dietary proteins are known to carry a wide range of nutritional, functional and biological properties. Many of those are attributed to bioactive peptides (3 to 20 amino acids). Such peptides are inactive within the sequence of parent protein and can be released during food processing or gastrointestinal digestion to exert different activities (Korhonen and Phlanto, 2003). Milk is the most studied source of bioactive peptides, though fish proteins are also known to contain potential bioactive sequences (Kitts and Weiler, 2003). Peptides can be present in fish muscle intra vitam or could be produced by proteolysis occurring during post mortem storage. Therefore, our objective was to investigate the occurrence of peptides in trout muscle during ice storage and cooking.

Materials and Methods

Fish and experimental procedure

Rainbow trout (Onchorynchus mykiss) (mean weight 300 g) were obtained from a local fish farm. Fish were sacrificed by a blow on the head, gutted and stored in ice. Samples of white dorsal muscle were taken from 3 fish approximately 1 h 30 after slaughter (T0) and after 6 days of ice storage (T6). Dorsal fillets were taken from another 3 fish stored for 6 days in ice. Fillets were vacuum-packed and cooked in a water bath: after reaching a core temperature of 70 °C, cooking was prolonged for 5 min. Raw and cooked samples were stored at –80 °C until experimental use.

Peptide extraction

Four extraction protocols of low molecular compounds were tested. 2.5 g of muscle were homogenised in 12.5 ml of (1) 3% perchloric acid (PCA), (2) 2% trichloroacetic acid (TCA), (3) 4% sulfosalicylic acid (SSA) and (4) 0.9% NaCl, using an ultra-turrax. In protocol 4, 1.35 ml of 40% SSA was then added to the homogenate. All the homogenates were centrifuged at 10,000g for 20 min at 4 °C. The supernatant was ultra-filtered (Vivaspin, cut-off 5 kDa, Vivascience) at 3,000g for 2 to 3 h at 4 °C. Extracts were stored at –20 °C until experimental use.

Nitrogen content and amino acids analysis

Total nitrogen content was determined by the Kjeldahl method on muscle and extracts. For free amino acids (FAA) analysis, extracts were applied to AG-50 resin in the H+ form followed by deionised water wash. The amino acids were then eluted with NH4OH. Eluate was evaporated and resuspended in 0.1 M lithium buffer (pH 2.2). For total amino acids (TAA) analysis, extracts were hydrolysed in 6 N HCl at 110 °C for 24 h and the dry residue was resuspended in 0.1 M lithium buffer (pH 2.2). Samples were stored at –20 °C until analysis. Amino acids were analysed by ion-exchange liquid chromatography on a HPLC System BioTek (Kontron). Postcolumn derivatisation with ninhydrin yielded amino acid derivatives which were detected at 570 nm et 440 nm. The peptidic amino acids (PAA) fraction was calculated by difference between TAA and FAA.

Mass spectrometry (MS) analysis

Nano-Electrospray-Ionisation-MS/MS (Nano-ESI-MS/MS) was carried out on a mass spectrometer LCQ Ion Trap equipped with a nanoelectrospray source (Thermofinnigan). The nanoelectrospray capillaries (Protana) were loaded with 3 µl of extract in 50% acetonitrile (ACN) in water with 2% TCA. Ionisation was performed with a liquid junction and a noncoated capillary probe (New Objective). Data acquisition was performed in a
manual mode and the collision-induced dissociation (CID) of selected precursor ions was performed using 35% relative collision energy.

Reverse phase – high performance liquid chromatography (RP-HPLC) analysis

Chromatographic separation was carried out using a 522 Pump System (Kontron Instruments), on a Nucleosil C18 100 Å 5 µm (4.6 x 250 mm) column (CIL Cluzeau), at 40 °C and at a flow rate of 1 ml/min. The gradient was performed using two solvents (A: 0.1% trifluoroacetic acid (TFA) in water, B: 0.1% TFA in 100% ACN), and formed as follows: 0% B in 0-5 min, 0-60% B in 5-35 min, 60% B in 35-60 min, 60-100% B in 60-63 min, 100% B in 63-68 min. Detection was performed at 220 nm.

Results

Nitrogen content

The four tested extraction methods were not significantly different (ANOVA), with 3.11 to 3.51 mg of extracted nitrogen/g of muscle (Figure 1). Moreover, no significant difference was obtained between T0, T6 and cooked fish.

![Fig. 1: Nitrogen content (mg/g of muscle) in extracts of raw (T0, T6) and cooked ( ) muscle for each protocol. Data are means of 3 trout ± SD.](image)

Amino acid analysis

Whatever the extraction method, TAA were not significantly different and accounted for about 40% (w/w) of extracted nitrogen (Table 1). On a molar basis, the PAA concentration decreased from T0 (57.3% of TAA) to T6 (42.8% of TAA) concomitantly with a rise in FAA. Only a few amino acids were represented in PAA: l-methylhistidine, beta-alanine, and to a lesser extent glycine and glutamic acid (Figure 2). Between T0 and T6, glycine concentration in PAA remained unchanged whereas a decrease of about 40% for glutamic acid and 20% for both l-methylhistidine and beta-alanine was observed.

Table 1: Amino acid quantification in T0 and T6 extracts. Data are means of the amino acid concentration for one trout for all protocols.

<table>
<thead>
<tr>
<th></th>
<th>N total</th>
<th>TAA (mg N/g)</th>
<th>FAA</th>
<th>PAA</th>
<th>TAA (µmol/g)</th>
<th>FAA</th>
<th>PAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>3.18</td>
<td>1.21</td>
<td>0.35</td>
<td>0.86</td>
<td>54.23</td>
<td>23.25</td>
<td>31.06</td>
</tr>
<tr>
<td>T6</td>
<td>3.20</td>
<td>1.23</td>
<td>0.51</td>
<td>0.71</td>
<td>60.14</td>
<td>34.33</td>
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</tbody>
</table>
Fig. 2: Peptidic amino acid quantification in T0 and T6 extracts. Data are means (± SD) of the amino acid concentration for one trout for all protocols.

MS and RP-HPLC analysis

The ESI mass spectrum showed two major peaks at m/z 241.1 and m/z 262.9 (Figure 3). The second peak (m/z 262.9) corresponded to the adduct of a sodium group from the precursor ion (m/z 241.1). The MS/MS spectrum for the mono charged m/z 241.1 ion showed a major peak at m/z 170.1 (data not shown).

Fig. 3: ESI-MS profile of 2% TCA extract at T6.

Apart from hydrophilic compounds not retained by the RP column, HPLC chromatograms exhibited a 13.9 min peak at T6 and in cooked fish that did not exist at T0 (data not shown). This putative peptide remains unidentified.

As regards technical constraints, SSA interfered greatly with HPLC detection before the beginning of the gradient.
Discussion

Based on extracted nitrogen quantity, the different tested extraction methods were equivalent, and there was no significant ice storage and cooking effect. However, in our chromatography conditions, PCA and TCA appeared to be more suitable than SSA. Therefore, in further work, peptide extraction will be performed by either PCA or TCA protocols.

In our study, TAA accounted for about 40% of extracted nitrogen. Non-protein nitrogen compounds, such as guanidine compounds, trimethylamine oxide (TMAO), urea, nucleotides and compounds related to nucleotides (Ikeda, 1980), could constitute the remaining nitrogen fraction.

At T0, very few low molecular weight (LMW) peptides were present in the muscle extracts of rainbow trout. The most abundant PAA of the extracts were 1-methylhistidine and beta-alanine, which correspond to the amino acids of the dipeptide anserine. The measured mass values (MS and MS/MS) corresponded to the anserine mass (240 Da) and to 1-methylhistidine mass (169 Da), thus confirming measurements of amino acid quantification. Therefore, it is likely that anserine (β-Ala-1-methyl-His) is present in the extracts, in accordance with many studies on fish muscle. Anserine is an endogenous peptide in the white muscle of rainbow trout at the concentration of about 9 µmol/g (Abe, 1991). After 6 days of ice storage, a loss of about 20% of anserine amino acids concentration was observed. This indicates anserine hydrolysis during post mortem storage, in accordance with results showed by Ruiz-Capillas and Moral (2001). Moreover, the presence of glycine and glutamic acid in PAA led to the presence of glutathione (γ-Glu-Cys-Gly) in trout muscle extracts but other analyses would be necessary to confirm this hypothesis. Glutathione has been found to decrease during storage (Brannan and Erickson, 1996; Petillo et al., 1998), which is consistent with the decrease of glutamic acid concentration in PAA after 6 days of ice storage. Both anserine and glutathione could be considered as bioactive peptides since they display antioxidant properties (Wu et al., 2003; Decker and Xu, 1998).

Very few other LMW peptides were present in the muscle extracts after ice storage and cooking. Since relatively little post mortem proteolysis was observed in fish muscle proteins (Kjærgård and Jessen, 2003), a few peptides would be generated, which could explain our results. Fish muscle generally shows little post mortem change in myofibrils (Ladrat et al., 2003). For instance, no degradation of myosin heavy chain, troponin T, desmin, α-actinin and actin occurred during post mortem storage of different fish species (Verrez-Bagnis et al., 2001; Verrez-Bagnis et al., 1999; Lund and Nielsen, 2001). In our storage and cooking conditions, protease activity could be impaired by low or high temperatures, which would explain the low generated peptide concentration during ice storage and cooking. Alternatively, post mortem proteolysis would generate only larger protein fragments.

Most fish bioactive peptides have been detected in in vitro hydrolysates. Specific conditions of hydrolysis associated to selected enzymes appear to be important to produce specific types of bioactive peptides (Gilmartin and Jervis, 2002). Thus, our storage and cooking conditions did not promote peptide generation in rainbow trout muscle.

Conclusions

In the muscle of rainbow trout, we have shown that anserine, a major endogenous dipeptide of white fish muscle, is hydrolysed, resulting in a loss of about 20%, after 6 days of ice storage. In addition, very few LMW peptides, if any, were generated after 6 days of ice storage and after cooking. Our storage and cooking conditions appear unfavourable for peptide generation in rainbow trout muscle. Further work will be performed to study digestion of fish muscle in animal model, in order to evidence bioactive peptides in fish protein digesta.

References

Decker E, Xu Z (1998) Food Technol 52 (10), 54-59
Desirable nutritive components in seafood


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1.8 INFLUENCE OF HERRING (CLUPEA HARENGUS) ON BIOMARKERS FOR CARDIOVASCULAR DISEASE

Helen Allenström, Anna Maria Langkilde, Ingrid Undeland, Ann-Sofie Sandberg

Objective:

To investigate if consumption of baked herring (Clupea Harengus) have positive effects on biomarkers for cardiovascular diseases in high-risk individuals.

Methodology:

A crossover intervention study in 14 obese humans, with mildly elevated levels of blood cholesterol was performed. During a four-week period they received one meal a day, five days a week containing 150g baked herring (approximately 2.5 g n-3 fatty acids/day) or baked chicken/lean pork as a reference meal. All accompanying food items to the meat/fish were identical in the meals. Otherwise they consumed their normal diet. Fasting blood samples were collected every second week and analyzed for fatty acids, triglycerides, total cholesterol, LDL, HDL, Apo A, Apo B, Lp A, fibrinogen and CRP. Study design is shown in the figure below:

![Study Design Diagram]

Fasting blood samples were collected every second week and were frozen in -80°C and analyzed at the same time after the study end. They were analyzed for fatty acids, triglycerides, total cholesterol, LDL, HDL, Apo A, Apo B, Lp A, fibrinogen and CRP.

Results:

All fourteen subjects completed the study and increased levels of the n-3 fatty acids EPA (20:5) and DHA (22:6) in their serum confirmed compliance.

Among men and postmenopausal women (three fertile women excluded), a statistical significant increase in HDL during the herring period was shown. This increase was due to a rise in HDL₃. No significant differences were found between the two groups in any of the other variables.

Discussion/Conclusion:

HDL₃ is shown to be raised after fish consumption in a few previous studies. In Sanders study from 1984, both marine and plant PUFA raised HDL₃ in twelve men among which most had hyperlipoproteinaemia and were overweight (Sanders et al. 1984). When twenty male patients with hypertriglyceridemia were treated with 6 g PUFA /day for four weeks HDL₃ was increased (Sanders et al. 1985). Also Deck saw a small rise in HDL₃, when eight overweight men and women with hypertriglyceridemia that were given 4.6g n-3/day (Deck et al. 1989). A rise in total HDL could be due to a higher fat intake or because saturated fat raise HDL (West et al. 1990, Sanchez-Muniz 2002).

Marine n-3 fatty acids have been shown to lower TG in numerous studies, especially in patients with hypertriglyceridaemia. The results have however not been significant in all studies due to a low number of patients (Harris 1997). It was somewhat unexpected that the herring consumption did not lower TG in this study.
However, when n-3 is provided via fish through diet, different protein sources are substituted with the fish and there is a multiplicity of variables changed. Harris (1997) proposed that this is the reason for mixed results in some studies. There is also a possibility that the preparation of the fish influence the health beneficial effects. Intervention studies in humans seldom precisely explain how the fish is prepared.

There are a few other studies where TG is not decrease by n-3-intake in the literature. These are in most cases studies providing low amounts of n-3 fatty acids (less than 1g n-3 a day), studies having few subjects and/or without controls. In Tidwell’s study 17 healthy men with BMI from 23-36 was included (Tidwell et al. 1993). They ate a control diet rich in dietary fibre the first 21 days and then they had a fish diet for 19 days. The TG decreased during the first 21 days, but did not continue to decrease on the fish diet. The lack of effect on TG by fish in that case was, according to the authors, perhaps because it was a short-term study.

The length of the study, the diversity of the group and the fact that the herring contained a high content of total fat compared to the chicken/pork may have contributed to the lack of differences between the groups on biomarkers, other than HDL₃, that previously have been proven affected by fish.

References

West et al. (1990) Am J Epidemiology 131:271-282

Authors

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2.1 PRE-RIGOR FILLETING AND QUALITY OF FED ATLANTIC COD (GADUS MORHUA L.)

Silje Kristoffersen, Torbjørn Tobiassen, Lars A. Godvik, Margrethe Esaiassen, Ragnar L. Olsen

Atlantic cod (Gadus morhua L.) has for centuries been one of the most economically important species in the North Atlantic fisheries. Today, both natural variations and over-exploitation have caused decline in the stocks of cod. In an international fresh fish market it is important to be able to supply the buyers with right amount and quality regardless of season. Also the general positive consumer trends for seafood and in particular fresh seafood, have led to increased focus on the possibility of large-scale cod farming, including net-pen feeding of live caught cod.

In intensive aquaculture the fish are normally fed to satiation, giving them a high growth rate, but also depositing large amounts of lipids and increasing the glycogen content in the liver and muscle. The amount of glycogen in the muscle is important for meat quality since it determines the glycolytic potential and thereby the ultimate post-mortem pH of the muscle. A low ultimate muscle pH in cod is associated with increased fillet gaping, high liquid loss and poor texture quality. After slaughter the muscle pH decreases and reaches the ultimate level in less than 24 hours depending on ante-mortem activity. Fillets of gadoid species like haddock, hake and cod are particular prone to gaping and softening and may because of this often be unsuitable for mechanical processing like filleting and skinning post-rigor.

The aim of this work has been to study the effects of pre-rigor processing on the fillet quality of fed cod. Two experiments were carried out using commercial size cod (3-4 kgs) in very good biological condition typical for cod fed to satiation. Fish were filleted manually or by industrial filleting equipment either pre-rigor (2 hrs post-mortem) or post-rigor (4-5 days post-mortem). In one of the experiments wild caught cod of similar size but of typical lower biological condition, was used as control.

Post-rigor filleted fed cod showed very much gaping while pre-rigor filleting gave a similar low gaping score as found in wild cod filleted pre- or post-rigor. The difference in gaping was also observed after 10 days storage in ice. Pre-rigor filleting resulted in a permanent shortening of the fillets and lower water content. This did not affect the whiteness of the fish flesh. However, a correlation was seen between whiteness and individual ultimate muscle pH.

Authors

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2.2 A STUDY ABOUT FLESH QUALITY OF WILD AND CULTURED COMMON DENTEX (DENTEX DENTEX, LINNAEUS, 1758)

Şükran Cakli, Tolga Dince., Asllii Cadun, Kürşat Firat, Şahin Saka

Introduction

Fish culture in the Mediterranean is essentially based on two species (sea bream and sea bass) with productions which have increased in a spectacular way in recent years, from 37, 179 mt in 1994 to 76, 000 mt in 1998. This increase in production has led to market saturation and a fall in price. One of the forms in which market supply may be increased and a contribution be made to development and/or expansion of aquaculture is to diversify the species being cultured. In this regard, dentex is one of the candidate species, which offers good possibilities. This species is still located in Mediterranean sea and have culture activities in Greece, Italy and Spain. And this species has the same market price as the preferred fish.

Fishery production of Turkey is approximately 627, 847 tons in a year. Aquaculture production is 61, 165 tons. The most preferred species are trout in fresh water culture (33, 707 tons), sea bass in aquaculture (14, 339 tons) and sea bream in aquaculture (11, 681 tons). Aquaculture of sea bass and sea bream has been done successfully in Turkey since 1990. The culture activity of dentex began in 2000 with a few foundations and nowadays still in activity with a successful production. Capture production of dentex is 79 tons per year (Anonym, 2002).

In this study, comparison of fish quality in cultured dentex (Dentex dentex) and wild dentex (Dentex dentex) has done from larva to portion size in a pilot aquaculture foundation. According to this aim proximate composition (protein, fat, moisture and ash), fatty acid composition, color measurements and biometrical measurements have determined for 9 months in monthly periods.

Material and methods

Brood stock and egg incubation

Common dentex brood stock, 8 females (2.4 kg mean weight) and 8 males (1.3 kg mean weight), were selected from wild breeders and stocked in 8 m³ tank with a seawater supply of 35 l min⁻¹. Frozen cuttlefish (Sepia officinalis) and Leander squilla (Palaemon elegans) were provided daily as the primary food source. The fish were subjected to natural photoperiod of natural rearing seasons (16 h light: 8 h dark), and the water temperatures varied throughout the experimental period between 15.5-21.0 °C. Eggs spawned by fish group were immediately collected in recuperator. Following the fertilization, the viable buoyant eggs were separated from the dead sinking eggs.

Eggs were incubated in 50 litre incubators at an initial density of 2500 eggs.l⁻¹ with a gentle flow of seawater of 15.5±0.5 °C. Oxygen saturation was over 85%, salinity was 37 ppt and pH was around 7.65. Ammonia and nitrite components were always <0.012 mg 1⁻¹.

Larval rearing

Larvae were stocked at density of 80 ind.l⁻¹ in a cylindironical tank (6 m³). The color of the tanks was dark-grey. Larval rearing was carried out in a closed sea water system. Water temperature, dissolved oxygen, salinity, pH, ammonia and nitrite levels were monitored daily. Water temperature was maintained between 15.5 and 21.0 °C (temperature increased day by day from 15.5 to 19 °C between 0 and 7th days, 19 to 20 °C between 8 and 26th days, from 20 to 21 °C between 27 and 32nd days). During larval culture period, oxygen, salinity and pH were maintained at > 85 %, 37% and 7.6, respectively. Ammonia and nitrite were kept constant always below 0.01 mg 1⁻¹.

Newly hatched larvae fed from day 4 (when the mouth opened) to day 12-14 with rotifers (Brachionus plicatilis but mainly with Brachionus rotundiformis) cultured with algae and enriched (DHA Protein Selco, Artemia Systems SA, Ghent, Belgium) at a density of 10–15 individuals ml⁻¹ plus green-water composed of Nannochloropsis sp., Chorella sp., and Isochrysis sp. at a density of 150,000–200,000 cells ml⁻¹. From day 9 to day 17, Artemia nauplii grade (AF480 INVE Aquaculture) at 4–7 individuals.ml⁻¹ and from day 15 until day 38, Artemia metanauplii at 2-4 individuals.ml⁻¹(EG, Artemia Systems SA, Gent, Belgium), both enriched with Protein Selco (Artemia Systems SA, Ghent, Belgium).
Larvae were stocked at volume of 12 m$^3$ in a cylindironical tank in the 40th day. Fish was being stocked according to 2unit/litre per a tank and 18 h light was applied. Cages were in a circle shape and has a 12 m radius and 8 m water depth. In these cages according to largeness of fish 8-18 mm nets without a bow was used. Fish which were in 4.02±0.3 gr weight were stoked in 40 individual/m$^3$. Half-wet bait was used in feeding and feeding was done in three times a day. Pellets were distributed slowly, allowing all fish to eat. Temperature ranged from 12.1 to 25.8 °C with a calculated mean of 18.9±0.4 °C for the entire period. Monthly water temperature values and fish body weights can be seen at table 1. Salinity (37–38‰) and dissolved oxygen (range 5.8–6.4 mg l-l) were monitored weekly. Fish were sampled in the cage conditions in the middle of every month.

**Analytical Methods**

**Proximate composition**

Moisture (Ludorff and Meyer, 1973), crude fat (Bligh and Dyer, 1959), crude protein (AOAC, 1984) and fatty acid were performed as proximate composition analysis of wild and cultured common dentex were determined during 9 months.

**Biometrical measurements and Condition factor**

Final weight, initial weight, proportion of initial weight and final weight, total length, fork length, standard length, head length, head-anal fin height, height and width measurements have done in captured from nature and cultured fish according to the monthly period. And also condition factor of dentex have calculated in a monthly period according to (CF) W/ L$^3$ x 100 formula.

**Analysis of fatty acid compositions**

Total lipid (TL) was extracted and purified according to Bligh and Dyer (1959), and TL content was determined gravimetrically. The lipids were saponified and esterified for fatty acid analysis by the method of IUPAC II D19. Separation of fatty acid methyl esters was achieved on a SP-2330 Fused Silica Capillary Column (30 m x 0.25 mm i.d.,0.20µm). The oven temperature was 120 °C for 5 min, programmed to 180 °C at 10° C/min, then programmed to 220 °C at 20° C/ min and then held there for 20 min. The injector and detector temperatures were maintained at 240 and 250° C, respectively. The carrier gas was a high purity helium with a linear flow rate 0.5 ml/min and split ratio of 1/ 150. Fatty acid methyl esters were identified using marine lipid methyl esters as standards( Sigma : 189-19 lipid standard).

**Colour measurement**

The colour measurement on fish samples trials were carried out with the spectral colour meter Spectro- pen ® (Dr. Lange, Dusseldorf, Germany). The colour was measured on homogenates prepared from each fish. The homogenate was placed in plastic Petri dishes and the colour measurement was repeated ten times. In the CIELab system L’ denotes lightness on a 0 to 100 scale from black to white; a’, (+) red or (-) green; and b’, (+) yellow or (-) blue (Schubring, 2002).

**Statistical analysis**

Results are presented as means ± SD ( n: 3 or 4 ). Differences between means were analysed by one- way analysis of variance( ANOVA) followed by Tukey and Duncan tests.

**Results and Discussion**

Biometrical measurements of wild and cultured dentex according to monthly period are given in Table 1 and Table 2.
### Table 1. Biometrical measurements of wild dentex

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<td>280±42,42</td>
<td>274±73,5</td>
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<td>2,6±0,09</td>
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</table>

The initial weight of wild dentex is 6,98±0,96 and 10,36±1,07 % of body weight. But in cultured dentex proportion of initial weight to body weight is between 8,32±0,29 and 10,83±3,01 %. Condition factor determined between 0,75±0,85 and 1,61±0,27 in wild dentex. CI values are determined between 1,42±0,03 and 1,60±0,03 in cultured dentex.

### Table 2: Biometrical measurements of cultured common dentex

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<td>4,7±0,14</td>
<td>3,9±0,14</td>
<td>4±0,21</td>
<td>4,6±0,13</td>
<td>4,5±0,10</td>
</tr>
</tbody>
</table>

### Table 3. CI and initial weight/ body weight proportion values in wild and cultured dentex

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>CI Wild Dentex</td>
<td>1,61±0,27</td>
<td>0,75±0,85</td>
<td>1,28±0,02</td>
<td>1,46±0,13</td>
<td>1,29±0,03</td>
<td>1,36±0,37</td>
<td>1,21±0,12</td>
<td>1,40±0,12</td>
<td>1,3±0,12</td>
</tr>
<tr>
<td>%</td>
<td>10,36±1,07</td>
<td>8,83±1,18</td>
<td>6,98±0,96</td>
<td>7,83±0,20</td>
<td>8,57±1,28</td>
<td>8,48±3,15</td>
<td>6,98±0,96</td>
<td>8,30±1,4</td>
<td>8,68±2,17</td>
</tr>
<tr>
<td>CI Cultured Den</td>
<td>1,53±0,02</td>
<td>1,60±0,03</td>
<td>1,42±0,03</td>
<td>1,44±0,02</td>
<td>1,45±0,22</td>
<td>1,55±0,01</td>
<td>1,43±0,20</td>
<td>1,50±0,11</td>
<td>1,42±0,2</td>
</tr>
<tr>
<td>%</td>
<td>10,57±3,17</td>
<td>9,05±3,06</td>
<td>9,44±0,24</td>
<td>8,93±0,33</td>
<td>10,8±3,01</td>
<td>9,32±0,29</td>
<td>9,85±1,10</td>
<td>10,5±2,15</td>
<td>10,3±0,19</td>
</tr>
</tbody>
</table>
Wild dentex which was captured from nature chemical composition values were determined as follows; moisture between 73,30±0,23 % and 75,51±0,57 %, crude fat between 0,76±0,12 % and 4,33±0,38 % and protein between 18,98±0,54 % and 23,43±1,10 % during 9 months study. Cultured chemical composition values were determined as follows; moisture between 72,23±1,02 % and 73,91±0,90 %, crude fat between 2,40±0,26 % and 5,29±0,43 % and protein between 21,70±0,94 % and 23,67±0,43 % during 9 months study. Statistical analyze has done according to each months and between wild and cultured dentex. (Table 4)

Table4. Proximate composition of wild and cultured dentex

<table>
<thead>
<tr>
<th></th>
<th>Moisture %</th>
<th>Fat %</th>
<th>Protein %</th>
</tr>
</thead>
<tbody>
<tr>
<td>September</td>
<td>74,29±0,55 a</td>
<td>2,34±0,15 adf</td>
<td>22,93±0,73 ad</td>
</tr>
<tr>
<td>October</td>
<td>74,79±0,97 a</td>
<td>4,33±0,38 cj</td>
<td>18,98±0,54 ad</td>
</tr>
<tr>
<td>November</td>
<td>73,83±0,01 a</td>
<td>2,46±0,87 adf</td>
<td>19,42±0,31 bc</td>
</tr>
<tr>
<td>December</td>
<td>73,43±0,23 a</td>
<td>0,76±0,12 bcj</td>
<td>23,14±1,04 ad</td>
</tr>
<tr>
<td>January</td>
<td>75,51±0,57 a</td>
<td>2,24±0,27 af</td>
<td>23,43±1,10 abd</td>
</tr>
<tr>
<td>February</td>
<td>74,84±0,18 a</td>
<td>1,77±0,88 acj</td>
<td>23,32±0,71 d</td>
</tr>
<tr>
<td>March</td>
<td>74,39±1,65 a</td>
<td>2,03±0,36 ag</td>
<td>20,18±0,39 abc</td>
</tr>
<tr>
<td>April</td>
<td>74,50±0,09 a</td>
<td>1,64±0,72 ab</td>
<td>21,95±0,31 abd</td>
</tr>
<tr>
<td>May</td>
<td>73,30±1,18 a</td>
<td>1,79±0,21 abhin</td>
<td>22,10±0,20 abd</td>
</tr>
<tr>
<td>September</td>
<td>72,23±1,02 a</td>
<td>4,30±0,52 cij</td>
<td>22,93±0,73 d</td>
</tr>
<tr>
<td>October</td>
<td>73,83±2,24 a</td>
<td>3,36±0,43 ddf</td>
<td>22,39±0,98 ad</td>
</tr>
<tr>
<td>November</td>
<td>72,44±2,92 a</td>
<td>4,04±1,38 ddk</td>
<td>21,90±3,01 abd</td>
</tr>
<tr>
<td>December</td>
<td>73,02±2,70 a</td>
<td>2,40±0,26 adf</td>
<td>23,67±0,43 d</td>
</tr>
<tr>
<td>January</td>
<td>73,84±0,52 a</td>
<td>4,75±0,24 j</td>
<td>21,70±0,94 abd</td>
</tr>
<tr>
<td>February</td>
<td>73,40±0,83 a</td>
<td>5,29±0,43 ckl</td>
<td>22,56±0,52 abd</td>
</tr>
<tr>
<td>March</td>
<td>72,65±0,80 a</td>
<td>2,75±0,18 dfghabc</td>
<td>21,70±0,83 c</td>
</tr>
<tr>
<td>April</td>
<td>73,91±0,22 a</td>
<td>2,78±0,02 dfghabc</td>
<td>21,65±0,88 abd</td>
</tr>
<tr>
<td>May</td>
<td>73,47±1,68 a</td>
<td>3,61±0,22 cdfj</td>
<td>21,90±0,35 abd</td>
</tr>
</tbody>
</table>

*Arithmetic means and standard deviation, different superscripts between columns characterize significant differences (p<0,05).

Wild and cultured dentex colour measurement values of 9 months study, can be seen in Table 5.
### Table 5: Wild and Cultured Common Dentex colour measurements

<table>
<thead>
<tr>
<th></th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wild dentex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.month (Sept)</td>
<td>49.9</td>
<td>-2.0</td>
<td>8.6</td>
</tr>
<tr>
<td>2.month (Oct)</td>
<td>51.4</td>
<td>-0.9</td>
<td>10.4</td>
</tr>
<tr>
<td>3.month (Nov)</td>
<td>50.1</td>
<td>-1.9</td>
<td>8.9</td>
</tr>
<tr>
<td>4.month (Dec)</td>
<td>52.0</td>
<td>-1.7</td>
<td>8.9</td>
</tr>
<tr>
<td>5.month (Jan)</td>
<td>49.6</td>
<td>-2.0</td>
<td>8.6</td>
</tr>
<tr>
<td>6.month (Feb)</td>
<td>48.7</td>
<td>-1.7</td>
<td>7.6</td>
</tr>
<tr>
<td>7.month (Mar)</td>
<td>50.2</td>
<td>-1.9</td>
<td>9.8</td>
</tr>
<tr>
<td>8.month (Apr)</td>
<td>51.1</td>
<td>-1.1</td>
<td>8.9</td>
</tr>
<tr>
<td>9.month (May)</td>
<td>49.8</td>
<td>-2.0</td>
<td>8.8</td>
</tr>
<tr>
<td><strong>Cultured common dentex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.month (Sept)</td>
<td>54.9</td>
<td>-1.8</td>
<td>9.1</td>
</tr>
<tr>
<td>2.month (Oct)</td>
<td>54.6</td>
<td>-1.1</td>
<td>8.6</td>
</tr>
<tr>
<td>3.month (Nov)</td>
<td>53.4</td>
<td>-0.8</td>
<td>9.1</td>
</tr>
<tr>
<td>4.month (Dec)</td>
<td>53.9</td>
<td>-0.7</td>
<td>12</td>
</tr>
<tr>
<td>5.month (Jan)</td>
<td>54.2</td>
<td>-2.0</td>
<td>9.7</td>
</tr>
<tr>
<td>6.month (Feb)</td>
<td>55.2</td>
<td>-1.7</td>
<td>8.2</td>
</tr>
<tr>
<td>7.month (Mar)</td>
<td>53.6</td>
<td>-0.9</td>
<td>9.0</td>
</tr>
<tr>
<td>8.month (Apr)</td>
<td>54.3</td>
<td>-1.1</td>
<td>8.7</td>
</tr>
<tr>
<td>9.month (May)</td>
<td>53.8</td>
<td>-0.8</td>
<td>9.8</td>
</tr>
</tbody>
</table>

L* (lightness), a* (redness), b* (yellowness)

### Conclusion

Dentex culture commenced very recently, in terms of the culture of traditionally Mediterranean species such as the gilthead sea bream and sea bass, which have increased in a spectacular way in recent years. Market supply may be increased and a contribution be made to development of aquaculture is to diversify the species being cultured. Relatively few references are available on this fish.

The high mortality rate during larval and post-larval culture is one of the most notable problems facing the development of dentex culture. The low survival rates appear to be caused by pathological problems, absence of swim bladder, malformations, cannibalism, bladder hyperinflation, etc. In order to solve these problems, it is essential to develop specific culturing techniques, at all stages, which allow for improvement of the survival and quality of larvae and juveniles.

Research on dentex culture is underway in several Mediterranean countries, and although the results obtained to date, using techniques very similar to those used in the culture of other sparidae, are promising (ease of reproduction in captivity, favorable adaptation to inert food, high growth rates both at the larval stage and in pre-fattening and on growing, and very favorable food conversion rates), culture of this species presents serious difficulties which must be overcome in order to commence mass rearing, and for it to become a real alternative to other species produced on an industrial scale.

### Acknowledgments

We thank the staff of the Teknomar Sea Fish Broodstock Centre where the experiments were conducted (Akvatur Marine Product, İzmir, Turkey) for their technical support.

### References


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firatmk@yahoo.co.uk
2.3 FROM POND TO TABLE – TRANSPARENCY IN AQUACULTURE PRODUCTION WITH REGARD TO FISH HEALTH, ANIMAL WELFARE AND FARM MANAGEMENT

Kleingeld, DW, R Kruse and F Feldhusen

Introduction

A continuous increase of fish production in aquaculture systems has been recorded for several years. According to data of the Food and Agriculture Organisation of the United Nations (FAO) the total aquaculture production of marine and fresh water organisms (excluding aquatic plants) reached 37 million tonnes in 2001 (Vannuccini, 2003). Aquaculture belongs to the fastest growing agricultural branches. In Germany this trend has not been observed until now, but for the future an increase in aquaculture production must be expected. The growth prospect of aquaculture in Germany is mainly restrained by nature protective directives and building licensing regulations, but is also affected by animal welfare considerations. The production of fish in aquaculture systems however provides a very effective source of human protein supply, due to the fact that fish are able to produce protein in a very sufficient way.

Following the annual report on German Fisheries (2003) a total of 40 500 tonnes fish are produced in aquaculture systems in Germany. About 24 000 tonnes salmonids and 16 000 tonnes cyprinids are raised in traditional pond farms. 500 tonnes fish, primarily eel (Anguilla anguilla) and sheatfish (Silurus glanis), are produced in heated re-circulation systems. In the Federal State of Lower Saxony approximately 200 professional and semi-professional fish farmers produce nearly 2 200 tonnes salmonids (mainly rainbow trout - Oncorhynchus mykiss) and 400 tonnes cyprinids (mainly common carp – Cyprinus carpio) in pond farms as well as 400 tonnes eel and sheatfish in re-circulation systems.

In 2003 the Lower Saxony Federal State Ministry of Agriculture incited to carry out a research project in order to gain knowledge of existing aquaculture problems related to fish as a human food source as a basis for future official surveillance in this area. The Veterinary Institute Cuxhaven was charged with the organisation of this aquaculture project. An interdisciplinary co-operation between several institutes and services of the Lower Saxony Federal State Office for Consumer Protection and Food Safety (LAVES) as well as the Federal Research Centre of Fisheries in Hamburg and the School of Veterinary Medicine in Hanover was established. In order to gain an initiating view on this topic, the transparency of aquaculture production should be presented by monitoring 15 trout farms in Lower Saxony. The Fish Epizootics Control Service of the Veterinary Task-Force covered a considerable part in this project and was responsible for the monitoring of fish health, animal welfare and farm management in the fish farms involved. In the following the results of these examinations and inquiries are presented.

Material and Methods

During the winter period 2003/2004 a total of 15 fish farms situated in the Federal State of Lower Saxony were visited. The examination on the farm site included anamnesis, clinical examination of 10 fresh slaughtered rainbow trout in a marketable size of about 350 g, dissection of these fish, anatomic-pathological and microscopic examination as well as organ sampling for virological and bacteriological examination (including inhibition test). Water samples were taken at the inflow and outflow of the ponds in which the fish samples were collected. Furthermore a questionnaire was carried out in order to gain a subjective view on farm management (eg holding / production / purchasing of fish, water control, hygienic aspects, feeding intensity) and animal welfare conditions (eg stocking density, holding conditions, handling, hygienic aspects, killing and slaughtering methodology).

Prior to clinical dissection the fish were manually stunned using a club, delivering a single hard blow midline behind the eyes of the fish followed by cutting the spinal cord. The total and average weight as well as length of the fish were determined. The fish and in particular the gills were macroscopically investigated. Samples for microscopic examination were taken from the skin and fins, from the gills and from the intestine. Smear preparations were done from the blood, liver and kidney. Only native preparations have been examined. A sterile sampling of the gills and an organ pool consisting of liver, kidney and spleen, was established for later bacteriological examination. A piece of muscle tissue (~1 cm³) was sampled in order to perform a three-plate-test. Sterile sampling of brain tissue, spleen, head kidney, heart and pyloric caeca was carried out for virological examination. The samples for bacteriological examination were
transported for further examination at 4°C. Samples for virological examination were placed in sterile plastic tubes containing transport medium and transported for further examination at 4°C. The bacteriological as well as the virological examinations took place at the Veterinary Institute Hannover of the Lower Saxony Federal State Office for Consumer Protection and Food Safety.

Water samples were taken at the inflow and outflow of the pond. Analysis of ammonium, nitrite, nitrate content was done at the spot using rapid test kits and a transportable Spectroquant® Photometer NOVA 60. Furthermore the carbonate hardness, temperature, oxygen content, oxygen saturation, total gas saturation, pH and conductivity were measured at the farm site. For the purpose of COD-measurement water samples were transported to Hannover at 4°C. The carbon dioxide content of the water was calculated from the pH and carbonate hardness measurement.

On each farm a questionnaire viewed on farm management and animal welfare aspects was carried out. With regard to farm management the fish farmer had to answer questions related to the production size, reproduction and purchasing of live fish, production form and aim of production, water management and water supply. An index ticking in a questionnaire form was done by the farmer and by the Fish Epizootics Control Service. With regard to animal welfare the fish farmer had to answer questions related to stocking density, holding conditions, handling, hygienic aspects, killing and slaughtering methodology. Also here an index ticking was done. At the end of the farm visit a first review of the farm was established by the Fish Epizootics Control Service with regard to the first impressions on fish health, farm management and animal welfare aspects. This review was expressed by an index ticking in the questionnaire form.

Sampling of fresh slaughtered rainbow trout and smoked rainbow trout, fish feed, pond soil sediments and water for further examinations enhanced to the project aiming on fish as a human food source, was carried out by an officer of the Veterinary Institute Cuxhaven.

Results

A total of 150 rainbow trout were sampled by the Fish Epizootics Control Service in order to perform clinical, bacteriological and microbiological analysis. 30 water samples were sampled for water analysis. The average weight and size of the fish ranged between 269.0 and 464.1 g respectively 28.0 and 32.8 cm which resulted in average weight of 352.1 g and an average length of 30.8 cm. The water temperature ranged between 1.7 and 9.7 °C.

Results of the fish health examinations

The findings of the clinical and microbiological fish health examinations are shown in table 1. With the exception of one farm, no severe parasitic, bacterial, mycotic or viral infestations could be observed. Exoparasites (Epistylis sp., Gyrodactylus sp. and Trichodina sp.) were found on the skin and gills of the rainbow trout at low infestation rates of minor importance only in five farm sites. In one farm a significant infestation with endoparasites belonging to the genus Acanatocephalus could be registered. Swelling of the gill tissue and damage of the fins, was observed in 11 respectively 7 farms. These findings were of minor importance. Changes in the liver (degenerative processes) and spleen tissue (swelling) were registered in 6 respectively 2 farms. In one farm the latter findings were of severe importance. Here the notifiable fish epizootic disease Viral Haemorrhagic Septicaemia (VHS) could be determined. In this case also characteristic petechiae were observed in the visceral fat tissue, on the pyloric caeca and swim bladder. Bacteria, belonging to the genera Aeromonas hydrophila and Pseudomonas sp. were found in 6 farms. Nine out of 15 samples of muscle tissue tested for inhibition (three-plate-test) turned out to be positive. The size of the inhibition areas ranged between 2 and 5.5 mm at pH6.
Table 1: results of the fish health examinations

<table>
<thead>
<tr>
<th></th>
<th>exoparasites (skin)</th>
<th>exoparasites (gills)</th>
<th>endoparasites</th>
<th>gill damage</th>
<th>fin damage</th>
<th>spleen changes</th>
<th>liver changes</th>
<th>organ petechiae</th>
<th>virological infestation</th>
<th>bacteriological infestation</th>
<th>mycological infestation</th>
<th>inhibition test</th>
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<tr>
<td>No. of findings</td>
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<td>3</td>
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<td>11</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>2</td>
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<td>0</td>
<td>0</td>
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<td>2</td>
<td>6</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</tr>
</tbody>
</table>

Results of the water analysis

With the exception of one farm, the results of the water analysis showed no findings of significant importance. These findings are presented in table 2. In one farm findings of severe importance were determined with regard to ammonium/ammonia content as well as nitrite content. The oxygen content of the pond water also proved to be below the minimum recommended level in this case.

Table 2: results of the water analysis

<table>
<thead>
<tr>
<th></th>
<th>ammonium content</th>
<th>nitrate content</th>
<th>COD</th>
<th>carbonate hardness</th>
<th>pH</th>
<th>oxygen content</th>
<th>total gas saturation</th>
<th>carbon dioxide content</th>
<th>conductivity</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
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<td>0</td>
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<td>7</td>
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<td>significant importance</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Results of the questionnaire

The indexed results of the questionnaire, implicating fish health and water analysis findings are summarised in table 3. The farm management index was determined by the Fish Epizootics Control Service depending on the source of fish purchase, on reproduction, water and hygienic management as well as feeding intensity. The
animal welfare index was evaluated by subjective impressions with regard to the handling of the fish through netting and killing as well as slaughtering. With regard to the killing methodology in 14 out of 15 farms the fish are routinely killed by manual stunning using a club. After Bretzinger (2001) this methodology still performs the most satisfactory way of killing trout with regard to animal welfare. In four farms a device for electric killing of trout was existing. This device however was routinely used in only one farm. Other factors, as to mention the holding factors (e.g., concrete ponds, stocking density), hygienic management and water amount and water quality, were also implicated in the animal welfare index. The fish health, three-plate-test and water analysis index were ranged by the examination results. The first review was indexed during the farm visit by the Fish Epizootics Control Service. The final review was calculated by summatting all indexes mentioned above. Higher values in the indexes represent a negative sense. In this context the highest summation (farm 12) was indexed at 100. The final review index varies between 21.3 and 100.0. The ranking after the final review is presented in table 3.

Table 3: results of the questionnaire and ranking (indexed)

<table>
<thead>
<tr>
<th></th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<th>7</th>
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<th>9</th>
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<th>12</th>
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<td>12.5</td>
<td>37.5</td>
<td>12.5</td>
<td>12.5</td>
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<td>37.5</td>
<td>12.5</td>
<td>37.5</td>
<td>12.5</td>
</tr>
<tr>
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<td>53.0</td>
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</table>

Results of further examinations

The results of the other studies within this project can not be discussed within this presentation. Food hygiene examinations of fresh and smoked trout turned out negative, no remarkable findings could be registered. Residual examinations showed minor and expectable positive findings with regard to antibiotic substances in pond sediments. Estrogenic examinations also proved to be partially positive. Isotopic examinations showed a clear correlation between water and fish samples.

Discussion and Conclusions

The part of the project related to the transparency in aquaculture carried out by the Veterinary Task-Force, Fish Epizootics Control Service of the Lower Saxon Federal State Office for Consumer Protection and Food Safety (LAVES) includes a wide range of subjective impressions and objective measurements with regard to fish health, animal welfare and farm management. It is very hard to reveal these numerous factors within one final review. Within this study this has been done in order to present a ranking of the fish farms. The only conformity of the farms participating in this study represents the fact that in all farms rainbow trout are produced and have been sampled with regard to this project. There are great variations with respect to farm management, water quality as well as handling of the fish. The final review however is mainly affected by the fish health (index varies between 8.0 and 48.8) as well as water quality (between 0 and 75.0). It is well known that the fish health depends in a high degree on the environmental quality. The environmental quality is mainly influenced by the water quality. In this context the water quality represents the most important issue with regard to the outbreak of fish diseases. Within the aim of the project it is important to prove if farm management, animal welfare, fish health and environmental quality affect the quality of the processed fish (fresh slaughtered or smoked). With regard to that question, no conclusions can be drawn at this stage of the study. First results show that the food hygiene quality at this period of sampling was not affected by the fish health index or water quality index. The
water temperature at sampling varied between 1.7 and 9.7 °C. It can be expected, that during the summer where higher water temperatures occur, food hygiene examinations might be of greater significance. For that reason the project will be continued in the summer season 2005 after evaluation of all examination results with regard to the sampling period winter 2003/2004.

The high number of positive findings with regard to the inhibition tests (nine out of 15 samples) is rather surprising taking into account that only in four farms antibiotic treatments took place within 12 months prior to examination. Merely in one of these four farms a treatment took place within three months prior to examination. These results might indicate an unspecific reaction. Residual analysis of antibiotic substances in fresh slaughtered fish resulted in negative findings. Fish feed samples will be tested on inhibitive activity in the course of this project.

The final indexes range between 21.3 and 100. Only one farm could be ranked below 25 (21.3). Eight farms showed a final index between 25 and 50 (28.6 - 45.2). Five farms vary between 50 and 75 (50.1 – 59.0). One farm was indexed at 100. The index differences between the farms ranked between 2 and 14 were rather low. In general, with exception of two farms, the fish health and environmental quality was satisfactory. In order to gain more information with regard to the aim of this project, it is necessary to establish further sampling and examination during the summer season.

References


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2.4 THE USE OF ANTI-MOUSE TYROSINASE ANTIBODY TO INVESTIGATE MECHANISMS OF MELANISATION IN FARMED COD  

Marie Cooper and (K. Midling)

Patterns of superficial and non-integumentary melanin distribution in farmed cod have been observed to differ from those found in wild cod. The appearance of melanin containing cells in the muscles and blood vessels of farmed cod could affect the quality of the product. Whilst melanin has no cited human toxicity the appearance of black lines in fish fillets detracts significantly from the sensory acceptability of the filet. The aims of this study are to compare melanin deposition patterns in farmed and wild cod, to identify the mechanisms underlying deposition of melanin in blood vessel walls and tissues other than the skin and scales and to investigate the factors that may influence melanogenesis. In preliminary studies we have looked at the distribution of melanin synthesising capacity in the tissues of farmed cod. Furthermore we have examined the influence of soya based diets on melanin synthetic capacity in farmed cod populations.

The age, gender, origin and diet of the fish used in the study were recorded. The techniques selected for the study included Western Blot analysis of protein extracts derived from a variety of tissues. The principle enzyme involved in the production of melanins is tyrosinase which catalyses the first two steps in the biynthetic pathway leading to melanin pigments. Although no antibodies specific to the teleost isotype of tyrosinase are available this protein is highly conserved. Sequence analysis using genomic and proteomic databases (BLAST) revealed significant amino acid sequence homology, greater than 60% in the catalytic domain, between mammalian (human) and teleost (zebra fish) isotypes of this protein. As a result Western Blots on extracts from fish tissues were performed using a mouse anti-human tyrosinase antibody previously shown to have sensitivity to a range of mammalian tyrosinase isotypes and cross reactivity with TRP2 (tyrosine related protein 2).

In addition to Western Blotting to identify the location and relative quantities of tyrosinase present in selected tissues we analysed tyrosinase activity present in the same extracts using a spectrophotometric assay. The assay determines the activity of tyrosinase by measuring the change in absorbance at 505nm caused by the conversion of L-Dopa to dopachrome in the presence of the electron transfer facilitator molecule MBTH. Activity is expressed as units of evolved product /min/ mg protein measured during the linear phase of the reaction. Preliminary results from these studies indicate that melanophores are widely distributed throughout the tissues of both farmed and wild cod. The presence of tyrosinase and tyrosinase related protein in these tissues is indicative that the melanogenic capacity is contained within widely distributed, specialised, pigment producing cells. However the finding that tyrosinase protein and tyrosinase activity were present in the melano-macrophage centres isolated from muscle tissues indicates that the previously held assumption that this enzyme was specific to skin and eye melanocytes in this species may be incorrect. The presence of melanogenic activity was unrelated to gender in farmed cod, furthermore the appearance of melanin in the tissues did not show any consistent relationship to age despite reports of age related melano-macrophage accumulation. The role of diet in the appearance of non-integumentary melanisation requires further study.

Authors

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2.5 MINIMAL PROCESSING OF NEW FARMED FISH SPECIES

Rosnes, J.T., Kleiberg, G.H., Lunestad, B.T., and Lorentzen, G.

The seafood species cod (Gadus morhua), halibut (Hippoglossus hippoglossus), spotted wolf-fish (Anarhichas minor), sea urchins (Strongylocentrotus droebachiensis), and blue mussels (Mytilus edulis) are new species that are under investigation for commercial farming in temperate waters. The biological and technical bottlenecks are in many cases solved, but optimized production and processing has been requested. In the period 2002-2004 the Norwegian Research Council financed the project “Minimal processing of new farmed species” run by the Norwegian Institute of Fisheries and Aquaculture Research (Tromsø) in cooperation with The National Institute of Nutrition and Seafood Research (NIFES) (Bergen) and Norconserv (Stavanger). The aim of the project was to examine processing and packaging methods that can increase the shelf life and maintain a high microbiological and sensory quality as well as the safety of the new farmed seafood species. The project was divided into three parts; i) examination of main spoilage and pathogenic bacteria on raw materials from farmed seafood products, ii) examination of the growth potential of both spoilage flora and pathogens when added at elevated levels in raw materials and iii) examination of minimal processing of raw materials from the species. Results from experiments with spotted wolf-fish are presented as an example of the new farmed species.

Materials and methods

The wolf-fish fillets were cut into small portions (130 g ± 2 g each) at the production area at Norconserv and individually packaged in modified atmosphere or in overwrap packages with exposure to air. The MA packages were high-density polyethylene (HDPE) semi rigid trays (Dynopack, Polymoon, Kristiansand, Norway). The air was evacuated and the gas mixture (60 % CO₂ and 40 % N₂) introduced into the package before heat sealing (lidding film: 15 my PE/74 my PA, Dynoseal ST 1575, Polimoon) on a semiautomatic packaging machine (Dyno VGA 462, Polimoon).

Samples of 25 g fish tissue were taken at random and homogenized in 250 ml of 0.9 % NaCl (w/v) and 0.1% peptone (w/v) for 120 sec in a Stomacher 400 Laboratory Blender (Seward Medical, London, U.K.). Total viable counts measured as aerobic plate counts (APC) and H₂S-producing bacteria were measured after a suitable dilution had been added to melted and tempered (44 °C) iron agar (Agar Lyngby, IA, Oxoid CM 867, Basingstoke, Hampshire, U.K.) supplemented with L-cysteine, and stored at 20±1 °C for 3 days. The content of psychrotrophic bacteria was determined by a spread plate count method with plate count agar (PCA, Merck, Darmstadt, Germany) added 1 % NaCl, and incubated at 8 °C for 5-7 days. Average results of duplicate measurements are presented as log colony-forming units (cfu) per gram wolf-fish.

Vibrio cholerae (CCUG 33379), V. parahaemolyticus (CCUG 14474 T), Bacillus cereus (Norconserv 11/98 1203) and Listeria monocytogenes (CCUG 15527) were inoculated in five parallels into vials containing sterile juice made from muscle of farmed Spotted wolf-fish. The fish juice was prepared in accordance with a method described by (Dalgaard 1995). Vials were incubated aerobically at 4 and 8ºC, and examined with respect to bacterial numbers at fixed intervals. The most recent version of relevant methods described by NMKL (Nordic Committee on Food Analysis) was applied.

Chemical analysis

Trimethylamine-oxid (TMAO), trimethylamine (TMA) and total volatile basic nitrogen (TVN) were determined in duplicate using a modified Conway microdiffusion method (Conway and Byrne 1933). Homogenised fish fillets (25 g) were expressed as mg N/100 g product. The pH of the fish tissue was determined in triplicate using a pH meter (Beckman 72) on 25 g of homogenate of wolf-fish muscle with 25 ml of 0.1 M KCl in distilled water.

Sensory analysis

Samples (15-20 mm wide cut of the stored samples) for cooked wolf-fish evaluation were packaged in cook-plastic pouches (PA/PE 20/50) under slight vacuum (95%) and cooked in water vapour (80°C in 10 min) without any salt or spice addition. Using a descriptive test adopted from (Shewan et al. 1953) with a scale from 10 (fresh seaweedy odour, fresh sweet flavor and firm texture) to 1 (putrid odor and flavor, sloppy texture) the odor, flavor and texture (firmness and juiciness) of the wolf-fish samples were evaluated. All cooked sensory evaluations
were carried out in duplicate. For both raw and cooked wolf-fish a score of 5 was chosen as the minimum acceptance level. Texture analysis had a scale from 5 (firm, blue-white muscle, no discolor) to 1 (very soft, clear browning of muscle).

Gas analysis

The headspace gas composition in the MA-packages was determined in triplicate by injecting an aliquot (30 ml) of the headspace gas of the trays using an oxygen and carbon dioxide analyser (M.A.P. Test 4000, Hitech Instruments, Luton; UK). The gas was collected with a syringe after intrusion of the top foil. The analyser was calibrated against a certified gas mixture (O₂:CO₂:N₂ 1.1:44.1:54.8) and air before each sampling.

Driploss/waterloss

Driploss/waterloss was measured gravimetrically in triplicate. The mass of the drip (g) was divided by the initial mass of product (g) and reported as a percentage (%).

Results and discussion

Microbiological, chemical and sensory data on the new farmed species are poorly reported. In this paper wolf-fish is used as an example of one the new farmed fish species. Wolf fish fillets were packaged in air and modified atmosphere (MA) and stored at -1 and 4 °C. Selected results are shown in Figure 1. Comparison of fish packaged in air at -1 and 4 °C showed no significant differences in APC after 1 and 4 days, but the numbers were lower at -1 °C (p<0.001) after 6, and 8 days (Figure 1A). The same trend in temperature influence were found on MA packaged products, with no significant difference between -1 and 4 °C during the first 4 days, but the numbers were lower (p<0.001) at day 8 and 11 for products at -1. In general the APC were lower, up to two log units in MA packages, compared to air stored products.
Figure 1 A-E: Microbiological and chemical analysis on packaged wolf-fish. MAP – 1 °C (- ■ -), MAP + 4 °C (-●-), Air – 1 °C (-○-), Air + 4 °C (-○○-).
Differences in packaging methods, MAP vs. air, gave a more evident difference in APC, and at -1 °C storage the numbers were lower (p<0.001) for MA packaged fish at 6,8,11, 13, and 15 days. They were also lower in MAP at 4 °C (p<0.001) measured at 6, 8, 11, and 13 days. This shows that MA had a greater inhibiting effect than temperature.

The numbers of psychrotrophic bacteria closely mimicked the APC numbers (data not shown). In a separate storage experiment, wolf-fish fillets were analysed specific for *Photobacterium phosphoreum* using a Malthus method as described by (Dalgaard *et al.* 1996). Fillets with skin on, stored at 4 °C showed ratios of log APC/log *P.phosphoreum* at day 4 (5.3/ 5.3), day 8 (6.6/ 7.1) and day 16 (9.3/ 7.1). Characterisation of specific strains at superchilled temperatures and in MA should be included in further studies.

*S.putrefaciens* would form black colonies on iron agar, but only a few, 13 out of 54 of the samples, contained detectable levels (> 10 CFU/g). Samples with H2S-producing bacteria varied between log 1 and log 5 CFU/g wolf-fish, and growth appeared irrespectively of storage temperature and atmosphere. The findings indicate that this H2S producing bacteria can grow on wolf-fish, but they were only sporadically found on the fillets.

Sparse degradation of TMAO was seen after 8 days of storage and after 11 days, degradation of TMAO and production of TMA started (Figure 1 B-C).

In packages with MAP combined with superchilled temperatures the lowest levels of TMAO-reduction took place. In general there is no significant difference in MAP and air stored wolf-fish in either reduction of TMAO, or production of TMA or TVN at -1 °C. Differences between MA and air are, however, found at 4 °C for these chemical parameters.

There were no significant differences in pH compared to parameters storage time or packaging method. pH varied between 6.2 and 6.8 for wolf-fish in air during the storage period (data not shown). There was a general trend of increased pH from day 10 for all variants except for superchilled MAP. Superchilled MA packaged wolf-fish was between pH 6.4 and 6.5 during the whole storage period.

Driploss from the samples increased by temperature and temperature-MAP interaction. The driploss was highest in the MAP samples at 4 °C (3.2 to 8.6%), and lowest in the superchilled, overwrapped samples (~2%). It has been suggested by several authors that dissolved CO2 in products would decrease the water holding capacity (Sivertsvik *et al.* 2002). However, for salmon this effect is not pronounced (Randell *et al.* 1999), typical drip loss around 2% under 60% CO2 atmospheres as compared to approximately 0.5% in air storage. Our results on wolf-fish suggest that the driploss of MAP products stored at -1 °C was about 2% higher than in those stored in air and that the CO2 had a negative effect at 4 °C in increasing the driploss to 6 to 8 %.

Sensory evaluation results (Figure 1 D-E) confirmed the effect observed for the microbiological counts. The temperature effect was significant (p<0.01) for all sensory scores, and the use of MAP significantly increased sensory scores (p<0.005 for raw odor, p<0.01 for flavor). Sensory scores decreased as a function of storage time, however, the effect of using short storage time was less important than using superchilling and MAP on the sensory quality of wolf-fish. The wolf-fish exposed to air had a self life of less than 8 days at 4 °C and 11 days at -1 °C, for both odor and flavor. MA packaged wolf-fish at 4 °C had a shelf life of 13 days, an extension of 5 days compared to air. At -1 °C the shelf life was not reached at day 15.

In a separate experiment pathogenic bacteria were inoculated in fish juice from wolf-fish. *Vibrio cholerae* and *V. parahaemolyticus* did not multiply in the wolf-fish juice, and died of during the experiment. The numbers of *Bacillus cereus* decreased during incubation on 4 °C, whereas this bacterium showed able to multiply when kept at 8°C. A substantial increase in bacterial numbers was observed for *Listeria monocytogenes* at an incubation of both 4 and 8°C.

**Concluding remarks**

This work has revealed some unexpected results which influence the packaging method for spotted wolf-fish. Used in a proper manner this knowledge can further develop MAP as a suitable packaging method for wolf-fish, and subsequently pull the marked for farmed wolf-fish a step further. The MAP in combination with superchilling increased the shelf life with at least 5 days but this combination was not as effective as seen in storage of salmon at such conditions. MA was effective in reducing the growth rate of bacteria, and the use of MA had more effect than the use of superchilled temperature. *Listeria monocytogenes* grew well on wolf-fish at 4 °C and psychrotrophic *Bacillus cereus* increased in numbers at 8 °C.
References

Conway EJ, Byrne A (1933) LXI. *Biochemical Journal* 27: 419.

Authors

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2.6 EFFECTS OF VEGETABLE DIETARY LIPID SOURCES ON FAT CONTENT AND FATTY ACID PROFILE IN TURBOT (*PSETTA MAXIMA*)

S. Lois, E. Silva, S. Cabaleiro, M. V. Ruiz Osenda, A. Teijido and I. Medina.

Introduction

Fish farmed diets are characterised by high dietary fat levels which gives rise an excessive lipid content in farmed flesh fish. Fish oils and meals are becoming more and more scarce and there is a strong interest in replacing fish oil by vegetable oils. Such substitution must guarantee good nutritional and organoleptic qualities and a healthy growth.

Sensorial characteristics of fish are very related to their fatty composition (Flick and Martin, 1992). This composition is highly rely on dietary. Most marine fish species, for example turbot, have an essential fatty acid required, especially eicosapentaenoic acid (EPA: 20:5ω3) and docosahexaenoic acid (DHA: 22:6ω3), because they can not synthesize these fatty acids from precursors in significant amounts (Owen et al., 1975). This must be taken into consideration when vegetable oils are used in the diets.

The aim of the study was to evaluate the effects of partial replacement of fish oil by vegetable oil, and the effect of a washout with a return to fish oil (the last month), on fat content and fatty acid profile in farmed turbot, making a comparison with wild turbot.

Materials and Methods

All samples, commercial feed, farmed and wild turbots were supplied by Cluster de la Acuicultura de Galicia. Two isoproteic (52 %) and isolipidic (20 %) diets were studied: a control diet with 100 % fish oil and a replacement diet with 80 % fish oil and 20 % soybean oil. Diets were stored at ambient temperature and were grinded the day before analysis. Three individuals per each group (farmed turbot fed with 100 % fish oil: FO, farmed turbot fed with 80 % fish oil and 20 % soybean oil: SO, farmed turbot fed with replacement diet and washout: SO+FO, wild turbot: WT) were stored at -20 ºC for analysis. The mean weight of turbots were 1.500 g. Liver and dorsal muscle were studied. All samples were homogenised before analysis.

Lipid content

Total lipid content of fish and diet samples were extracted by Bligh & Dyer method (1959) and were quantified by gravimetric analysis (Herbes and Allen, 1984). Additionally, the fat content of diets was measured following the standard method (extraction by Soxhlet, OAOC 963.15).

Fatty acid composition

Fatty acid composition was determined by gas chromatography (Christie, 1992). Previously, fatty acids were methylated with a solution of sulphuric acid in methanol.

Results

Lipid composition

It was observed that lipid content of dorsal muscle of farmed turbots fed with different diets (FO, SO, SO+FO) was not significant different (Table 1). Lipid content of liver was high, and SO and SO+FO turbots showed higher content than FO turbot. Lipid content of wild turbots was remarkably smaller than farmed turbots.
Table 1: Lipid content of farmed turbot with different diets and wild turbot.

<table>
<thead>
<tr>
<th></th>
<th>FO</th>
<th>SO</th>
<th>SO+FO</th>
<th>WT</th>
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<tr>
<td>Liver</td>
<td>27.46 ± 0.29</td>
<td>34.60 ± 1.75</td>
<td>39.19 ± 0.68</td>
<td>15.39 ± 2.79</td>
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<tr>
<td>Dorsal muscle</td>
<td>0.87 ± 0.10</td>
<td>0.90 ± 0.13</td>
<td>0.90 ± 0.10</td>
<td>0.55 ± 0.07</td>
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Fatty acid composition

FO diet showed a very high EPA and DHA content, whereas replacement diet, SO diet, showed a high concentration of linoleic acid (18:2\(\omega_6\)) and lower contents of EPA and DHA (Table 2).

Table 2: Lipid content and fatty acid composition of experimental diets.

<table>
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<th>Fatty acids</th>
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<th>SO</th>
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<tr>
<td>16:0</td>
<td>9.51 ± 0.02</td>
<td>6.95 ± 0.09</td>
</tr>
<tr>
<td>16:1(\omega_7)</td>
<td>20.47 ± 0.01</td>
<td>18.80 ± 0.19</td>
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<tr>
<td>18:0</td>
<td>4.38 ± 0.04</td>
<td>4.34 ± 0.07</td>
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<tr>
<td>18:1(\omega_9)</td>
<td>7.46 ± 0.09</td>
<td>10.51 ± 0.03</td>
</tr>
<tr>
<td>18:1(\omega_7)</td>
<td>2.61 ± 0.08</td>
<td>2.21 ± 0.04</td>
</tr>
<tr>
<td>18:2(\omega_6)</td>
<td>3.10 ± 0.03</td>
<td>13.31 ± 0.03</td>
</tr>
<tr>
<td>18:3(\omega_3)</td>
<td>1.15 ± 0.14</td>
<td>2.49 ± 0.03</td>
</tr>
<tr>
<td>18:4(\omega_3)</td>
<td>2.71 ± 0.04</td>
<td>2.15 ± 0.02</td>
</tr>
<tr>
<td>20:1(\omega_9)</td>
<td>1.48 ± 0.05</td>
<td>1.19 ± 0.03</td>
</tr>
<tr>
<td>20:4(\omega_6)</td>
<td>0.93 ± 0.03</td>
<td>0.77 ± 0.02</td>
</tr>
<tr>
<td>20:4(\omega_3)</td>
<td>0.78 ± 0.02</td>
<td>0.65 ± 0.00</td>
</tr>
<tr>
<td>20:5(\omega_3)</td>
<td>14.84 ± 0.17</td>
<td>11.45 ± 0.12</td>
</tr>
<tr>
<td>22:1(\omega_11)</td>
<td>0.55 ± 0.10</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td>22:1(\omega_9)</td>
<td>0.20 ± 0.00</td>
<td>0.14 ± 0.00</td>
</tr>
<tr>
<td>22:5(\omega_3)</td>
<td>2.19 ± 0.01</td>
<td>1.49 ± 0.00</td>
</tr>
<tr>
<td>22:6(\omega_3)</td>
<td>21.00 ± 0.12</td>
<td>18.32 ± 0.18</td>
</tr>
</tbody>
</table>

The main fatty acids of farmed and wild turbots were palmitic acid (16:0), oleic acid (18:1\(\omega_9\)), linoleic acid (18:2\(\omega_6\)), eicosapentaenoic acid (20:5\(\omega_3\)) and docosahexaenoic acid (22:6\(\omega_3\)). These fatty acids represented 67-70% in liver turbot (Table 3) and 77-81% in dorsal muscle turbot (Table 4).

Table 3: Liver fatty acid composition of turbot.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>FO</th>
<th>SO</th>
<th>SO+FO</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>12.90 ± 0.40</td>
<td>13.15 ± 0.32</td>
<td>14.24 ± 0.05</td>
<td>19.02 ± 3.87</td>
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<tr>
<td>18:1(\omega_9)</td>
<td>13.68 ± 0.31</td>
<td>21.74 ± 0.23</td>
<td>16.37 ± 0.17</td>
<td>17.01 ± 2.74</td>
</tr>
<tr>
<td>18:2(\omega_6)</td>
<td>4.01 ± 0.10</td>
<td>10.25 ± 0.08</td>
<td>12.15 ± 0.15</td>
<td>0.78 ± 0.27</td>
</tr>
<tr>
<td>20:5(\omega_3)</td>
<td>11.05 ± 0.27</td>
<td>5.87 ± 0.18</td>
<td>6.47 ± 0.04</td>
<td>6.24 ± 1.79</td>
</tr>
<tr>
<td>22:6(\omega_3)</td>
<td>26.18 ± 0.94</td>
<td>18.64 ± 0.23</td>
<td>20.02 ± 0.09</td>
<td>23.93 ± 1.74</td>
</tr>
</tbody>
</table>
Table 4: Dorsal muscle fatty acid composition of turbot.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>FO</th>
<th>SO</th>
<th>SO+FO</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>19.64 ±1.13</td>
<td>19.40 ± 0.79</td>
<td>20.47 ± 0.54</td>
<td>22.40 ± 0.87</td>
</tr>
<tr>
<td>18:1ω9</td>
<td>8.81 ± 0.81</td>
<td>10.59 ± 0.59</td>
<td>9.10 ± 0.67</td>
<td>7.67 ± 0.83</td>
</tr>
<tr>
<td>18:2ω6</td>
<td>2.89 ± 0.42</td>
<td>9.47 ± 0.20</td>
<td>8.49 ± 0.47</td>
<td>0.51 ± 0.05</td>
</tr>
<tr>
<td>20:5ω3</td>
<td>11.16 ± 0.48</td>
<td>8.31 ± 0.07</td>
<td>9.02 ± 0.14</td>
<td>8.01 ± 1.39</td>
</tr>
<tr>
<td>22:6ω3</td>
<td>35.15 ± 3.94</td>
<td>32.30 ± 1.98</td>
<td>33.92 ± 1.45</td>
<td>39.56 ± 2.96</td>
</tr>
</tbody>
</table>

In all cases, there was a significant influence of dietary lipid on fatty acids composition of tissues, liver and muscle. Principally, this influence was observed in linoleic acid. The percent of linoleic acid in FO turbot was 4.01 % in liver and 2.89 % in muscle, in SO turbot was 10.25 % in liver and 9.47 % in muscle. However this replacement was not enough to observe significant differences in the amounts of EPA and DHA in muscle (Tables 3 and 4).

Between SO and SO+FO turbots, slight differences have been observed (Table 4 and 5). Apparently, although one month with FO diet was influencing the lipid composition of both liver and muscle, one month was not a sufficient period to achieve the level of FO turbot (see EPA content in Tables 3 and 4).

Fatty acid profile of muscle of wild turbots was very close to the profile of muscle of FO turbots. In this case, the significant differences were in the content of linoleic acid, EPA and DHA. Fatty acid profile of liver in both turbots followed the same tendency, although in the liver there were more differences in other fatty acids, like: palmitic acid and oleic acid.

Discussion

A replacement of 20 % of fish oil by soybean oil, did not effect on the lipid content of muscle in farmed turbot. Turbot has the ability to store large amounts of lipid in the liver (Bell et al., 1999). This was also observed in this work in farmed and wild turbots. The lipid content of farmed turbots was approximately twice higher than in wild turbots in liver and muscle.

The fatty acid composition of liver and muscle reflected the fatty acid composition of the diet. The efect of dietary was very marked with high amounts of linoleic acid in SO and SO+FO turbots, against to FO turbot (Regost et al., 2003). Wild turbot are carnivore, eat fish and crustaceans, so they had high concentration of EPA and DHA and low linoleic acid (Serot et al., 1998). All diets employed in this work provide acceptable levels of EPA and DHA in farmed turbots, as can be seen by comparing with wild turbot.

Conclusion

The fatty acid composition of liver and muscle of farmed turbot was clearly influenced by the fatty acid composition of the diets.

Farmed and wild turbots had a similar proportion of total polyunsaturated fatty acids, but lipid content were much higher in farmed turbot than in wild turbot.

A replacement of 20 % was not sufficient to note a very significant change in the content of EPA and DHA. Differences were observed in linoleic acid. When the last month SO turbots were fed with a fish oil diet, these increased EPA and DHA amounts. However this period was not enough to reach the concentrations of polyunsaturated fatty acids achieved by FO turbots.

Acknowledgements

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References


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2.7 RELATIONSHIP BETWEEN SENSORY AND INSTRUMENTAL
TEXTURE ANALYSIS OF FARMED COD

Turid Mørkøre, Hanne Morkemo og Trine Galloway

Texture is one of the most important parameters that determine the overall quality perception of seafood products. Soft flesh leads to reduced acceptability by the consumers, and quality downgrading in the processing industry. Farmed cod are generally firmer than wild-caught cod, and cutlets of farmed cod generally maintain their original shape better during heat treatment. However, farmed cod occasionally are tough and dry after frozen storage. The ability to control and improve texture of cod products requires reliable and reproducible analytical methods. Therefore it is important to identify objective measurements that show high correlations with sensory attributes that are interesting to the processing industry and to consumers.

The aim of this study was to investigate the possibility to predict firmness of heat-treated farmed cod cutlets by mechanical analyses of raw or heat-treated cutlets. A trained panel (12 assessors) performed the sensory analyses. The instrumental analyses were performed using either a flat-ended cylinder (12.5 mm dia) or a Warner-Bratzler knife. Mechanical analyses of raw cutlets showed significant correlation with sensory firmness when using the cylindrical probe. Hence, sensory firmness of heat-treated cod could be predicted by performing instrumental analyses of raw cutlets. Mechanical analyses of heat-treated cutlets showed significant correlation with sensory firmness when using the cylindrical probe and the WB-knife.

Authors

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\textsuperscript{b}BioMar, N-7484 Trondheim, Norway
2.8 VARIATION IN COPPER LEVEL, TEXTURE AND GAPING OF FARMED SALMON. SAMPLING TIME SHOWED HIGHER IMPACT THAN FEED COMPOSITION

Turid Mørkøre

Flesh softening and fillet gaping in fish are believed to be associated with reduced strength of the connective tissues. Copper (Cu) is essential for cross-linking of collagen, the major component of connective tissues. In the present study Cu content in muscle, serum and liver was studied during spring, a time period when salmon farmers at the Norwegian West-coast frequently experience problems with soft texture and fillet gaping. Two feeds with notably different formulation were used: 1) commercial extruded dry feed (DF; 27.7 MJ kg\(^{-1}\) dry matter), and 2) alginate based moist feed (MF; 19.8 MJ kg\(^{-1}\) dry matter).

The fieldwork was conducted in seawater during the period January- May in duplicated net-pens (800m\(^{3}\)) per diet with adult Atlantic salmon (BW 2.3 kg). Salmon were sampled for analyses of fillet gaping, texture and Cu-level in March and April. In March, both fish groups had less gaping, firmer fillets and higher Cu level in serum and muscle. In April, the MF group had more gaping than the DF group. In March the MF group had highest serum Cu level. Otherwise no significant variation was found due to dietary treatment. Variation in serum Cu-content explained 32% (r = 0.57) of the variation in fillet hardness, and increasing serum and muscle Cu level related negatively with gaping. The dry matter content (DM) in the muscle of the MF group was stable at about 32% throughout the experimental period, while the DM of the DF group increased linearly from 32% in January to 35% in May. At the end of the experiment, the condition factor, and fillet- and visceral fat content were higher in the DF group. Sensory hardness and tastiness were similar for both dietary fish groups.

In conclusion, sampling time had a greater impact on Cu-level, texture and gaping than dietary treatment during springtime in adult Atlantic salmon.

Authors

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2.9 CONTRACTION OF PRE-RIGOR SALMON FILLETS. EFFECT OF FEEDING AND STRESS.

Turid Mørkøre, Pablo Mazo, Reidun Lilleholt, Vildana Tahirovic og Olai Einen

The interest for filleting salmon immediately after slaughtering (pre-rigor) has increased significantly during recent years. Pre-rigor fillets can reach the market in a fresher state, and they generally have more intense red colour, firmer texture, and less gaping than post-rigor fillets. The shrinkage of pre-rigor fillets is the most possible reason to the improved colour and texture. Pre-rigor filleting requires that rigor-mortis occurs after a certain period of time. Controlling factors that accelerate rigor contraction is therefore essential. Further knowledge is needed on rigor development of salmon with different nutritional status, and on the interaction between stress and nutritional status.

The experimental period of the present study was 70 days (September-December 2003) and two groups of salmon were used (initial BW 2.8 kg). Group A) was fed to satiation during the whole period. Group B) was starved for 35 days, thereafter fed for 35 days. Salmon were sampled for analyses at day 0, 7, 35 and 70. At the sampling d 35, two different slaughtering methods were used, stress or non-stress. Fillet contraction was recorded regularly during a period of 72h following filleting. Furthermore, analyses of ATP, glycogen/lactate, pH and texture were performed continuously after slaughtering. The results showed that the fillet contraction was faster in stressed than in non-stressed salmon, and faster in fed than in starved salmon.

Authors

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2.10 COMPARISON OF PERCUSSIVE STUNNING AND ASPHYXIATION OF FARMED SOLE (SOLEA SOLEA) WITH RESPECT TO DEVELOPMENT OF RIGOR MORTIS AND PRODUCT QUALITY

Hans van de Vis, Karin Kloosterboer, Martine Veldman and Bert Lambooij

Since the last decade supermarkets, governments, farmers and processors of fish and welfare associations have become increasingly concerned about welfare of fish. This concern is also focused on welfare aspects of slaughter procedures and how the applied techniques may influence product quality.

The objective of the study was therefore to compare percussive stunning in combination with gutting to asphyxiation (by flushing the water with nitrogen gas) followed by gutting. Farmed sole (Solea solea) was used as species in the study.

Effects of the slow (asphyxiation) and fast stunning method (percussion) were investigated by analysis of onset and resolution of rigor mortis (rigor index values). Changes in quality profile during storage of the fillets at 0 °C were assessed by analysis of colour (L, a, and b values), pH and sensory properties of the fillets, using QDA.

Stress at slaughter promoted the onset of rigor mortis, as it was observed for percussion and asphyxiation that maximal rigor index values were recorded after 53.3 and 17.4 h of storage, respectively. For both methods a complete resolution of rigor mortis did not occur, as after 7 days of storage at 0 °C the rigor index curve levelled off at 61% and 66% for percussion and asphyxiation, respectively. These data suggest that the flesh was tough. This process is called cold shortening and has been reported for warm-blooded slaughter animals. In order to increase tenderness of the flesh electrostimulation in combination with so-called high temperature conditioning (storage 4 h at 9 °C and 44 h at 4 °C, prior to storage at 0 °C) was applied after percussion and asphyxiation. It was observed that 4 days after gutting the average rigor index values were 32 and 30 % for percussion and asphyxiation, respectively. These data suggest that resolution of rigor mortis was complete. Analysis of the fillets revealed that asphyxiation resulted in significantly higher pH values (p <0.05) in the caudal region of the fillets for day 1 up to day 12 after gutting, than for percussion. No significant differences were observed for measured L, a and b values. For the QDA attribute watery taste only, the batch that was stunned by asphyxiation scored significantly lower at day 9 than the percussion batch (4.1 vs. 6.2). After 5 days of storage QDA revealed no significant differences for the batches.

To summarize, stress at the time of slaughter had a substantial effect on onset and resolution of rigor mortis. The effect on quality of the fillets, however, was little.

Authors

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2.11 TRADITIONAL AND INNOVATIVE STUNNING/SLAUGHTERING METHODS FOR EUROPEAN SEA BASS COMPARED BY THE COMPLEX OF THE ASSESSED BEHAVIOURAL, PLASMATIC AND TISSUE STRESS AND QUALITY INDEXES AT DEATH AND DURING SHELF LIFE


Introduction

The fish welfare, even at time of death, is calling interest and concern both at consumer and producer level, analogous to what already happened for farmed mammals and birds. From an animal welfare point of view, slaughter methods must cause immediate loss of consciousness, rendering the fish quickly insensitive to pain and suffering until death, or causing the death of a fish preventively anaesthetised or effectively stunned (Lambooij et al., 2002a). Some investigations on catching and slaughtering methods were performed, especially on salmonids (trout and salmon) (Azam et al., 1989; Kestin et al., 1995; Sørensen and Carlehoeg, 1999; Robb et al., 2000; Otter et al., 2001; Robb et al., 2002) and eels (Van der Vis et al., 2001; Lambooij et al., 2002a, b; Morzel and Van der Vis, 2003), but very little information is available on “Mediterranean” species like sea bass (*Dicentrarchus labrax*) (Parisi et al., 2002; Poli et al., 2003). Moreover, among killing methods, little consideration had the method widely used in Italy for this species, the immersion in water and ice, even pointed out as a method to be forbidden because considered not “human”, after the results of a few experiments (Farmed Animal Welfare, 1996).

The aim of this research was to find a practical low stressing slaughter method which at the same time allows better fish quality, by testing in parallel various traditional (the most used slaughter methods in European fish farms such as asphyxia, cold stunning in ice-water mixture, spiking, knocking, CO2 narcosis, and electrical stunning) and innovative methods (gas addition - N2-CO2 - to ice-water) at the same time and evaluating them through the study of the relative behavioural and physiological responses of the fish at death, evolution of post mortem biochemical processes, shelf life and quality/freshness of the product.

Materials and methods

Six subsequent experiments were carried out on 393 sea bass (*Dicentrarchus labrax*), 300-600 g l.w. commercial size range, reared in farms of the co-operative Coo.P.A.M., Ansedonia (Grosseto). For the first four trials, concerning the evolution of the stress and quality parameters in the first 24 hours after death, 10 days before each trial fish were transferred to the research recirculating system, subdivided into 400 l tanks (n.6) containing sea water (23°C temperature, 7 ppm DO, 24% salinity, 14 kg/m3 density) and let to recover. For the last two trials, concerning mainly the evolution of quality parameters during shelf life, fish were stunned and slaughtered directly at the rearing plant. Stunning/slaughter methods compared were: water ice mixture 2:1 (WI - normally utilised for sea bass by Italian aquaculture farms), asphyxia (AS), narcosis in carbon dioxide saturated water (CD), electrical stunning on whole body in fresh water for 2 minutes at 24 V, 50 Hz a.c. (EL) using Fishkill EG10002 (Scubla Aquaculture, Italy), spiking (SP), knocking (KN), ice-water mixture saturated with 100% N2 (WI-N2), or 100% CO2 (WI-100CD), or 40% N2 and 60% CO2 (WI-60CD), or 60% N2 and 40% CO2 (WI-40CD). These methods were subdivided as follows, to compare fish from the same batch and always using water and ice 1:2 as control method:
- trial 1: WI, AS, KN, CD, EL, using 29 sea bass (534.7±112.3 g b.w.);
- trial 2: WI, AS, KN, CD, EL, SP, using 35 sea bass (383.0±81.4 g b.w.);
- trial 3: WI, AS, KN, CD, EL, using 85 sea bass (321.5±82.8 g b.w.);
- trial 4: WI, SP, WI-N2, WI-60CD, using 44 sea bass (493.3±97.3 g b.w.);
- trial 5: WI, AS, EL, SP, using 44 sea bass (500 g b.w.);
- trial 6: WI, WI-100CD, WI-60CD, WI-40CD, using 80 sea bass (483.7±100.9 g b.w.).

All stunning methods were followed by 30 minutes in ice covering. The relative fish behavioural responses, death times and hormonal changes linked to the various conditions of stress and consequent biochemical processes post mortem were investigated by the evaluation at death of the following parameters: 1) fish behaviour and death times, 2) hematic parameters - glucose, lactate, cortisol and hematocrit of blood collected...
from caudal vein; 3) eye liquor and muscle pH (pHe, pHm); 4) muscle lactic acid. Moreover, 5) muscle isometric contraction was evaluated in continuous during the first 24 hours after death and at death and during the shelf life of fish were determined 6) muscle ATP and related catabolites (ADP, AMP, IMP, inosine- HxR, hypoxanthine- Hx) (HPLC); 7) rigor index (also evaluated at 3, 6, 9 and 24 hours after death); 8) dielectric properties; 9) compactness by Instron machine; 10) quality involution at 1°C with ice covering following the EU scheme (Rule 2406/96 EEC - freshness class: Extra-3 (very fresh), A-2 (fresh), B-1 (bad quality), evaluated daily by a trained panel of 5 judges, until the fish were considered spoiled. Data were analysed by ANOVA (slaughter methods). To compare the complex of parameters for each method in the different trials a sort of “demerit score” was plotted.

Results and Discussion

The evaluation of the behaviour during slaughter and death times (Table 1) showed that KN and SP gave immediately death when correctly performed, even if difficult to be done properly. AS, at room temperature (15-20°C) resulted the more stressful and less ethical method, due to the long time needed for the stunning (loss of movements only after more than one hour) and for the violent reactions in the first 3 minutes. WI resulted in some way a not resolved question from the point of view of the animal welfare because, if the not short time of stunning (20.0±5.5 min) is considered, the absence of fast movements and the gradual passage to the state of unconsciousness, practically a cold anaesthesia, has also to be underlined, suggesting on the whole that the method is only a little violent and not so stressful. The gases insufflated in the water subtract oxygen, allowing a faster death due to hypoxia. The use of CO2 has negative effects on fish behaviour and stunning (7.0±1.4 min but fish showed quick violent spasms). The presence of ice in the water had an additive effect on the CO2, accelerating the second phase of stunning, when the fish turns upside down and loses the ability to react to the external stimuli (3.5±0.5 min). 100% of nitrogen in water and ice gave a rather limited effect (17.0±1.0 min). The mixtures of nitrogen and carbon dioxide in ice-water reduced the time of stunning to 3.5±0.5 min, possibly mainly due to a CO2 effect. Stunning with different voltage has been tried for EL fish, finding that 24V for 2 min caused immediate loss of consciousness in nearly all fish without causing carcass and flesh damage.

Table 1 - Effect of different stunning /killing methods on the loss of reaction to external stimuli.

<table>
<thead>
<tr>
<th>Method</th>
<th>Fish Behaviour</th>
<th>Loss of /no reaction to external stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI</td>
<td>Slow and little violent stunning</td>
<td>20.0±5.5 (Trial 1)</td>
</tr>
<tr>
<td>CD</td>
<td>Violent agony in the 1st minute</td>
<td>7.0±1.4 (Trial 1)</td>
</tr>
<tr>
<td>EL</td>
<td>Not evaluated</td>
<td>2</td>
</tr>
<tr>
<td>AS</td>
<td>Prolonged agony and violent activity</td>
<td>70.0±27.6 (Trial 1)</td>
</tr>
<tr>
<td>KN</td>
<td>Violent struggle before death</td>
<td>0</td>
</tr>
<tr>
<td>SP</td>
<td>Violent struggle before death</td>
<td>0</td>
</tr>
<tr>
<td>WI-N2</td>
<td>Slow and not violent stunning</td>
<td>17.0±1.0 (Trial 4)</td>
</tr>
<tr>
<td>WI-60CD</td>
<td>Violent agony in the first 30 seconds</td>
<td>3.5±0.5 (Trial 6)</td>
</tr>
<tr>
<td>WI-40CD</td>
<td>Violent agony in the first 30 seconds</td>
<td>3.5±0.5 (Trial 6)</td>
</tr>
<tr>
<td>WI-100CD</td>
<td>Violent agony in the first 30 seconds</td>
<td>3.5±0.5 (Trial 6)</td>
</tr>
</tbody>
</table>

Among the plasmatic stress indicators (Table 2), WI gave generally lower and more constant values of cortisol than the other methods. In Trial 3, AS and EL provoked an evident increase of haematocrit levels (51 and 49% vs 36, 34 and 26% for WI, CD and SP, respectively), EL provoked the highest concentration in plasma cortisol (112 ng/ml) and, together with AS the highest plasma lactate (100 and 104 mg/dl), confirming the results obtained for haematocrit, all due to the violent and/or prolonged movements. On the contrary, the SP fish showed the lowest plasma lactate (33 mg/dl), while the CD and WI fish had intermediate levels (48 and 63 mg/dl). Also as regards glycemia, AS and EL (251 and 158 mg/dl) seem to be more stressful than SP, CD, and WI (74, 94, and 117mg/dl). Also in Trial 2, AS provoked the highest values of glycemia (350 mg/dl) and plasma lactate (124 mg/dl).

In Trial 4 no difference emerged for glucose, while SP fish showed higher cortisol (48 ng/ml) and lactate (70 mg/dl) than the WI fish and lower haematocrit than WI-N2 fish, revealing that possibly in this trial the spiking procedure was not done so properly. In Trial 6 WI-100CD gave intermediate values of cortisol, while WI-60CD gave better results, not different from water and ice.
The main results for biochemical parameters are summarised in Table 3. In Trial 2 WI and EL presented the highest muscular pH at death. In Trial 3 the highest ocular pH at 0 h after death was measured in SP and WI and EL, the lowest in AS while in CD it was intermediate. The highest pH at death of SP, WI and EL fish possibly indicated a low anaerobic glycolytic activity before death. On the contrary, asphyxia, releasing high amounts of lactic acid before death, resulted in lower pH both at muscular and eye liquor level. This was confirmed, in Trial 3, by the fact that AS fish had the highest muscle L-lactic acid (42 µmol/g), while the WI fish had the lowest one (28 µmol/g). The CO₂ addition to water and ice (Trial 4 and 6) slightly lowered pH only at eye liquor level.

The ATP and Adenylate Energy Charge (AEC) measures at death are the most indicative of a stress condition before death. In Trial 2 highest ATP levels were those of KN and SP fish, while the lowest ones were those of AS and CD fish. AS also showed the highest values of IMP and inosine.

The AEC value (Trial 3) confirmed that the asphyxia was the most stressful method, together with EL, showing the lowest values for the great consumption of energetic resources and the highest levels in SP. In contrasts with the findings of Trial 2 also CD showed high AEC. In Trial 6 the best AEC levels were showed by WI and WI-N₂ to water/ice mixtures significantly decreased the death time (3.5 vs 20 min) but did not change AEC at death.

Contemporaneously to the gradual depletion of the ATP reserves there is the gradual set up of the *rigor mortis* therefore the *rigor* index can give good information on the stress status of the fish before death. As results in Table 4, in Trial 2 AS fish reached 100% *rigor* index at 3h after death (together with CD fish), compared with...
the 6h necessary in the case of EL and KN fish or the 9h necessary for the SP and WI fish. In fact, at 3h after death WI and SP fish showed rigor index of 45% and 60% versus the 100%, 97% and 85% found in AS, CD and KN. In Trial 3, together AS fish also CD and EL fish reached 100% rigor index at 3 h after death. The delayed rigor onset in fish stunned with ice-water confirmed what already seen for haematic and biochemical parameters, namely that this method, being not much violent, allows the fish to preserve a great part of its own cellular energetic reserves at death. The addition of 100% CO2 in ice water mixture (Trial 6) provoked 100% rigor index already at 3 h after death, while fish only stunned in WI or in WI added with nitrogen and CO2 mixtures showed lower values. A more compact texture has been found in WI sea bass (Trial 3), at 6 hours from death. In Trial 3 the muscular isometric contraction of muscle indicated an earlier strips contraction in AS and EL fish. Fish Tester scores and the K1 value were not able to discriminate the different methods. The sensory analysis on raw fish indicated in Trial 5 1 day shorter shelf life in asphyxiated fish and 1 day longer shelf life in WI fish of Trial 6; the EL stunning method, also stressful according to some plasmatic and tissue parameters, did not forfeited concerning to the shelf-life. The gas addition generally did not improve the shelf life with respect to water and ice, only the mixture WI-60CD giving better or analogous results, the other mixtures even slightly lowering the score in some cases (Trial 4 and 6).

### Table 4 – Main quality parameters evaluated

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Trial</th>
<th>WI</th>
<th>AS</th>
<th>KN</th>
<th>SP</th>
<th>CD</th>
<th>EL</th>
<th>WI-100CD</th>
<th>WI-60CD</th>
<th>WI-40CD</th>
<th>WI-N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rigor Index at 3h hour after death (%)</td>
<td>2</td>
<td>45.3c</td>
<td>100.0a</td>
<td>85.1ab</td>
<td>60.4bc</td>
<td>97.0a</td>
<td>85.1ab</td>
<td>25.1</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time of maximum IC (min)</td>
<td>3</td>
<td>385.0a</td>
<td>137.5b</td>
<td>436.0a</td>
<td>391.0a</td>
<td>163.0b</td>
<td>96.8</td>
<td>***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish Tester at 10th day after death</td>
<td>5</td>
<td>55.3</td>
<td>55.3</td>
<td>57.3</td>
<td>60.0</td>
<td>58.7</td>
<td>59.3</td>
<td>65.3</td>
<td>3.7</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Muscular compactness a 6th hour after death</td>
<td>3</td>
<td>2239.5a</td>
<td>1506.3bc</td>
<td>1388.2bc</td>
<td>1956.0ab</td>
<td>1169.6e</td>
<td>324.4</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU scheme synthetic score at 7h day after death</td>
<td>4</td>
<td>1.04e</td>
<td>1.71b</td>
<td>1.97a</td>
<td>1.63b</td>
<td>0.33</td>
<td>0.45</td>
<td>n.s.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU scheme synthetic score at 10th day after death</td>
<td>6</td>
<td>1.23a</td>
<td>1.47</td>
<td>1.47</td>
<td>0.34</td>
<td>0.31</td>
<td>0.37</td>
<td>0.42</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EU scheme synthetic score at 7h day after death</td>
<td>6</td>
<td>1.33a</td>
<td>0.39b</td>
<td>0.83b</td>
<td>0.80b</td>
<td>0.83b</td>
<td>0.31</td>
<td>***</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* for 0.01<p<0.05; ** for 0.001<p<0.01; *** for p<0.001; a,b,c for p<0.05

To have a sort of summary of all analytical results, the mean values of the different parameters for each stunning/ killing method have been plotted in a sort of “demerit score”, ranging from 0 to 100 (0= better value of the parameter, 100= worst value), which is shown in Table 5. The worst score was that of asphyxiation, due to the most prolonged agony, a remarkable physical activity, a relevant mobilisation of energy reserves with earlier rigor onset and shorter shelf life, especially in comparison to spiking, knocking and live chilling; to follow the scores of EL, WI-100CD and CD, SP, WI-40CD, and KN showed the intermediate scores. Live chilling did not result particularly stressful, giving constants results in all trials. The use of 60% CO2 with 40% N2 during fish live chilling got the best scores, being able to shorten stunning time (from 20’ to 3-4’), without big differences in stress and quality indicators of live chilling alone.
Table 5 - Summary of analytical results for the 6 trials by a "demerit score". The last column reports, the correlation value (and its significance) between each parameter and the “final” or “mean” demerit score.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WI-60CD</th>
<th>WI-N2</th>
<th>WI</th>
<th>KN</th>
<th>WI-40CD</th>
<th>SP</th>
<th>CD</th>
<th>WI-100CD</th>
<th>EL</th>
<th>AS</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death times</td>
<td>5.7</td>
<td>24.3</td>
<td>31.4</td>
<td>0.0</td>
<td>5.7</td>
<td>0.0</td>
<td>10.0</td>
<td>5.7</td>
<td>2.9</td>
<td>100.0</td>
<td>0.733*</td>
</tr>
<tr>
<td>Blood haematocrit</td>
<td>48.0</td>
<td>50.8</td>
<td>56.3</td>
<td>62.3</td>
<td>0.0</td>
<td>0.0</td>
<td>30.3</td>
<td>74.6</td>
<td>92.6</td>
<td>100.0</td>
<td>0.659ns</td>
</tr>
<tr>
<td>Blood cortisol</td>
<td>0.0</td>
<td>4.6</td>
<td>9.6</td>
<td>22.5</td>
<td>39.2</td>
<td>51.0</td>
<td>25.3</td>
<td>34.5</td>
<td>100.0</td>
<td>25.7</td>
<td>0.412ns</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>32.7</td>
<td>5.7</td>
<td>26.2</td>
<td>0.0</td>
<td>55.0</td>
<td>3.5</td>
<td>11.8</td>
<td>51.2</td>
<td>31.7</td>
<td>100.0</td>
<td>0.741*</td>
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<tr>
<td>Blood lactate</td>
<td>4.9</td>
<td>0.0</td>
<td>25.0</td>
<td>9.9</td>
<td>100.0</td>
<td>22.6</td>
<td>30.6</td>
<td>30.5</td>
<td>61.8</td>
<td>85.3</td>
<td>0.636*</td>
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<tr>
<td>Muscle lactate at death</td>
<td>0.0</td>
<td>30.7</td>
<td>45.4</td>
<td>57.1</td>
<td>100.0</td>
<td>0.962**</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Eye pH at death</td>
<td>43.9</td>
<td>24.0</td>
<td>0.0</td>
<td>14.6</td>
<td>36.6</td>
<td>6.5</td>
<td>53.7</td>
<td>70.7</td>
<td>13.4</td>
<td>100.0</td>
<td>0.623ns</td>
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<tr>
<td>Muscle pH at death</td>
<td>0.0</td>
<td>32.4</td>
<td>34.3</td>
<td>60.0</td>
<td>55.3</td>
<td>48.6</td>
<td>28.6</td>
<td>37.7</td>
<td>100.0</td>
<td>0.664*</td>
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<tr>
<td>ATP at death</td>
<td>0.0</td>
<td>47.8</td>
<td>27.6</td>
<td>19.9</td>
<td>29.7</td>
<td>61.9</td>
<td>50.8</td>
<td>64.5</td>
<td>100.0</td>
<td>0.905***</td>
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</tr>
<tr>
<td>IMP at death</td>
<td>0.0</td>
<td>39.8</td>
<td>60.6</td>
<td>9.0</td>
<td>30.2</td>
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<td>15.5</td>
<td>61.8</td>
<td>100.0</td>
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<td>HSr at death</td>
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<td>13.6</td>
<td>0.0</td>
<td>19.8</td>
<td>11.5</td>
<td>0.0</td>
<td>12.4</td>
<td>100.0</td>
<td>0.876***</td>
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<tr>
<td>AEC at death</td>
<td>0.0</td>
<td>49.6</td>
<td>43.6</td>
<td>15.4</td>
<td>37.2</td>
<td>33.1</td>
<td>33.3</td>
<td>78.2</td>
<td>100.0</td>
<td>0.847**</td>
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<tr>
<td>Rigor index at 3rd h. a.d.</td>
<td>9.5</td>
<td>17.7</td>
<td>7.5</td>
<td>64.7</td>
<td>46.3</td>
<td>0.0</td>
<td>96.5</td>
<td>78.8</td>
<td>100.0</td>
<td>0.683*</td>
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<tr>
<td>Time of maximum IC</td>
<td>41.1</td>
<td>25.4</td>
<td>15.0</td>
<td>0.0</td>
<td>21.9</td>
<td>0.0</td>
<td>89.9</td>
<td>100.0</td>
<td>0.776*</td>
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<td></td>
</tr>
<tr>
<td>Fish Tester at 10th d. a.d.</td>
<td>60.0</td>
<td>83.0</td>
<td>0.0</td>
<td>80.0</td>
<td>66.0</td>
<td>53.0</td>
<td>100.0</td>
<td>0.417ns</td>
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<tr>
<td>Muscular compactness a 6th hour after death</td>
<td>0.0</td>
<td>100.0</td>
<td>33.3</td>
<td>87.0</td>
<td>86.1</td>
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<tr>
<td>K1 value at 9th d. a.d.</td>
<td>100.0</td>
<td>0.0</td>
<td>63.6</td>
<td>75.1</td>
<td>0.0</td>
<td>100.0</td>
<td>3.4</td>
<td>81.8</td>
<td>100.0</td>
<td>0.735**</td>
<td></td>
</tr>
<tr>
<td>EU scheme synthetic score at 3rd d. a.d.</td>
<td>0.0</td>
<td>50.7</td>
<td>47.1</td>
<td>8.8</td>
<td>54.7</td>
<td>58.8</td>
<td>3.4</td>
<td>81.8</td>
<td>100.0</td>
<td>0.735**</td>
<td></td>
</tr>
<tr>
<td>EU scheme synthetic score at 10th d. A. D.</td>
<td>5.8</td>
<td>35.3</td>
<td>0.0</td>
<td>66.0</td>
<td>57.7</td>
<td>61.5</td>
<td>100.0</td>
<td>0.834*</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Final (mean) demerit score</td>
<td>20.7</td>
<td>22.6</td>
<td>24.8</td>
<td>30.1</td>
<td>32.6</td>
<td>33.9</td>
<td>40.8</td>
<td>43.6</td>
<td>59.1</td>
<td>94.3</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

The 6 different trials showed that for sea bass the most constant results along the several experiments were found with asphyxia (always the worst methods both for the stress condition and the qualitative point of view) and with water ice mixture 2:1 (simple to apply and not particularly stressful), while the most variable results were found with CO2 narcosis and electrical stunning. This last, even if showing biochemical parameters values indicating a stressful effect, gave positive responses as regard qualitative parameters and shelf life. The adaptation of electrical stunning for sea bass needs more studies, to reduce the application time and to increase the amount of current provided. Spiking and knocking were the fastest and generally less aversive slaughtering methods, but not practical for this species. It is worth to underline that the stunning in water and ice mixture, followed by half an hour permanence in ice, did not result particularly stressful, causing a pre-slaughtering cooling, reduced the movements at death, making the fish easy to handle and not able to recover when put in ice; the time needed for the sure stunning is not short but the rapid body cooling has many advantages, mainly from a qualitative point of view (late rigor mortis, better flesh texture and prolonged shelf life) and both the haematic and muscular stress indicators gave good responses, comparable in some cases to the spiking responses. The addition of gas mixture to ice water was able to significantly shorten the death time without big differences in stress and quality indicators. In particular, the use of WI-60CD had the general best score. The cost of carbon dioxide and nitrogen makes the use of these mixtures less economically convenient in comparison to the only ice water mixture; the problem will be to give a right value to the shortening in stunning times, both from an ethic and a business management point of view.

References


Authors

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2.12 TAILORING THE FATTY ACID COMPOSITION OF TROUT FILLETS FOR HEALTH PURPOSES
PRELIMINARY RESULTS

Pier Paolo Gatta, Silvia Testi, Marina Silvi, Giampiero Pagliuca, Alessio Bonaldo,
Arjen Roem, Anna Badiani

Introduction

Adequate intakes of alpha-linolenic acid (LNA) and eicosapentaenoic plus docosahexaenoic acids (EPA + DHA) for adults on a 2,000 kcal diet are currently estimated to be 2.22 and 0.65 g/day, respectively (Simopoulos et al., 1999). According to the American Heart Association, a food-based approach to meet those requirements is preferable, at least for patients without documented coronary heart disease. A recommendation stemmed from that to eat a variety of (preferably oily) fish at least twice weekly, and to include oils and foods rich in LNA (Krauss et al., 2000). Yet in the opinion of Sinclair (2000) and Kris-Etherton et al. (2000) this is quite an underestimation and 3–4 meals/week of fatty fish would be necessary at least to meet EPA + DHA daily requirements. Most probably, many individuals would prefer to consume fewer servings of fish, leading to the need for the development of fortified products. Aquaculture could give a significant contribution to achieve this aim.

Rainbow trout (Oncorhynchus mykiss Walbaum, 1792) are now widely used around the world for fish farming and restocking of angling fisheries. Rainbow trout are adaptable and farmed in a wide variety of situation. They have high flesh yields and usually commands medium-to-low prices, often becoming cheaper than other prime fish. As a consequence of these assets, rainbow trout could prove an obvious candidate for selective n-3 polyunsaturated fatty acid (PUFA) enrichment.

An attempt was therefore made to fortify rainbow trout flesh through feeding EPA + DHA enriched diets, in order to: a) attain an EPA/DHA ratio close to 2:1, significant intakes of EPA being regarded as protective against inflammatory bowel disease (Belluzzi et al., 2000), colorectal cancer (Nkondjock et al., 2003), and possibly bipolar disorder (Stoll et al., 1999); b) attain an EPA/DHA ratio close to 1:2, to tailor fillets to the higher DHA needs of expectant or nursing mothers (Hornstra, 2001; Hibbeln, 2002), besides tentatively ameliorating stress-related disease (Hamazaki et al., 1999). Besides those mentioned, a third treatment was examined, based on LNA enrichment of trout flesh through feeding. This treatment served as a control, LNA being possibly considered an economic means of trout flesh fortification with n-3 PUFA in Blueprint specifications for quality fish, a type of product which is increasing in popularity. At the same time, the LNA treatment was considered “functionally” warranted, given that: 1) LNA seems to have a more important role than formerly suspected on cardiovascular disease, up to the point that it was recently reclassified as the only “conditionally indispensable” PUFA in adulthood (Cunnane, 2000); 2) both the consumption of LNA and the dietary ratio between linoleic acid (LA) and LNA are far from being ideal in most European countries and the United States (Kris-Etherton et al., 2000; Lanzmann-Petithory, 2001).

Materials and Methods

Rainbow trout from the same parental stock, initial weight 150–160 g, were randomly and evenly assigned to 9 fibreglass tanks (600 L capacity) supplied with aerated well water at 13–14°C (52 fish per tank, three tanks per dietary treatment). After two weeks of adaptation to Diet 1, fish were fed (to apparent satiation twice daily, Monday to Saturday, and fasted on Sunday) the same basal diet at 42% crude protein and 26% lipid, 15% of which was as follows: Diet 1 (D1), linseed oil; Diet 2 (D2), EPAX 4510 TG (a glyceride containing min. 44% EPA and max. 15% DHA; Pronova Biocare, Lysaker, Norway); Diet 3 (D3), EPAX 1050 TG (a glyceride containing max. 17% EPA and min. 50% DHA; ditto). Each diet was supplemented with an equal amount of vitamin E (300 mg/kg). Fish from each diet group were sampled and analysed at the start of the trial and every 15 days for lipid content and fatty acid composition of the fillets (Folch et al., 1957; Christie, 1989). In the present paper special importance was attached to selected PUFA concentrations (expressed as % fatty acid methyl esters, FAME) and ratios found in the fillets within the first 60 days of the trial.
Results and Discussion

The diets were selectively fortified as expected (D1:D2:D3 = 3.15:1.04:1 for LNA; 1:2.56:1.48 for EPA; 1:1.23:1.85 for DHA) (Table 1). As a consequence, D1, D2 and D3 differed in a number of fatty acid ratios, most notably LA/LNA, LNA/n-3 and EPA/DHA (1.66, 4.39, 4.62; 0.41, 0.12, 0.11; 0.63, 1.30, 0.50, respectively).

Table 1. Fatty acid composition (% FAME) of the diets (mean ± s.d.)

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 14:0</td>
<td>3.92 ± 0.049</td>
<td>4.06 ± 0.051</td>
<td>4.55 ± 0.088</td>
</tr>
<tr>
<td>C 16:0</td>
<td>15.47 ± 0.008</td>
<td>14.93 ± 0.047</td>
<td>16.27 ± 0.252</td>
</tr>
<tr>
<td>C 18:0</td>
<td>3.68 ± 0.002</td>
<td>4.03 ± 0.024</td>
<td>3.37 ± 0.0003</td>
</tr>
<tr>
<td>C 16:1 n-7</td>
<td>3.85 ± 0.002</td>
<td>3.99 ± 0.011</td>
<td>4.38 ± 0.103</td>
</tr>
<tr>
<td>C 18:1 n-9</td>
<td>18.34 ± 0.143</td>
<td>16.86 ± 0.141</td>
<td>16.01 ± 0.053</td>
</tr>
<tr>
<td>C 18:1 n-7</td>
<td>1.99 ± 0.015</td>
<td>2.64 ± 0.051</td>
<td>2.08 ± 0.009</td>
</tr>
<tr>
<td>C 20:1 n-9</td>
<td>3.61 ± 0.574</td>
<td>2.17 ± 0.091</td>
<td>1.75 ± 0.024</td>
</tr>
<tr>
<td>C 18:2 n-6</td>
<td>18.76 ± 0.095</td>
<td>16.36 ± 0.084</td>
<td>16.40 ± 0.097</td>
</tr>
<tr>
<td>C 18:3 n-3</td>
<td>11.32 ± 0.870</td>
<td>3.73 ± 0.002</td>
<td>3.59 ± 0.032</td>
</tr>
<tr>
<td>C 18:4 n-3</td>
<td>0.18 ± 0.003</td>
<td>0.23 ± 0.003</td>
<td>0.24 ± 0.005</td>
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<td>C 20:4 n-6</td>
<td>2.61 ± 0.0004</td>
<td>2.93 ± 0.058</td>
<td>3.00 ± 0.075</td>
</tr>
<tr>
<td>C 20:5 n-3</td>
<td>3.95 ± 0.071</td>
<td>15.21 ± 0.043</td>
<td>8.82 ± 0.207</td>
</tr>
<tr>
<td>C 22:5 n-3</td>
<td>0.84 ± 0.008</td>
<td>1.19 ± 0.028</td>
<td>1.77 ± 0.023</td>
</tr>
<tr>
<td>C 22:6 n-3</td>
<td>9.48 ± 0.0027</td>
<td>11.66 ± 0.274</td>
<td>17.58 ± 0.276</td>
</tr>
</tbody>
</table>

Fortnightly sampling of fish revealed temporal changes in lipid content of the flesh, which increased from an overall mean of 5.68 % to 8.11% over the 60 day feeding period, the diet effect having no bearing on the data (Table 2). Alteration of the source of supplemental dietary lipid resulted in some differences in fatty acid profile of trout. Significant changes in fatty acid composition, notably in LNA, EPA and DHA, occurred between fish fed different diets. From the start of the trial to the 60th day, a 58% increase was observed for LNA percentage in the D1 group, as against a more modest 44% increase for EPA in the D2 group and a decidedly lower 9% increase for DHA in the D3 group. These changes were paralleled by a significant decrease in LNA content of both the D2 and D3 groups (–29% and –31%, respectively) and in EPA and DHA levels of the D1 group (~18% and ~22%, respectively).
## Table 2. Lipid content (%) and selected fatty acid composition (% FAME) of trout fillets

<table>
<thead>
<tr>
<th>Trait</th>
<th>Time(^{(1,2)}) (T)</th>
<th>Diet (D)(^{(3)})</th>
<th>Overall mean (om)</th>
<th>MSE</th>
<th>Stat. Sign.(^{(4)})</th>
<th>D</th>
<th>T</th>
<th>D*T</th>
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<tr>
<td>Lipid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>1</td>
<td>b 4.78</td>
<td>b 5.90</td>
<td>b 6.37</td>
<td>b 5.68</td>
<td>1.9785</td>
<td>ns</td>
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<tr>
<td></td>
<td>2</td>
<td>a 7.19</td>
<td>ab 6.69</td>
<td>ab 7.53</td>
<td>a 7.14</td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>a 8.28</td>
<td>ab 7.51</td>
<td>a 8.30</td>
<td>a 8.03</td>
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<tr>
<td></td>
<td>4</td>
<td>a 8.11</td>
<td>ab 7.47</td>
<td>a 8.79</td>
<td>a 7.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>a 8.18</td>
<td>a 8.41</td>
<td>ab 7.74</td>
<td>a 8.11</td>
<td></td>
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</tr>
<tr>
<td></td>
<td><strong>om</strong></td>
<td>7.31</td>
<td>7.20</td>
<td>7.57</td>
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<tr>
<td>C18:2 n-6</td>
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<td></td>
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<tr>
<td>(LA)</td>
<td>1</td>
<td>c 13.72</td>
<td>14.03</td>
<td>b 13.72</td>
<td>c 13.83</td>
<td>0.1918</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>b 14.40</td>
<td>14.18</td>
<td>ab 14.12</td>
<td>b 14.23</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>a 15.18</td>
<td>14.39 (y)</td>
<td>a 14.42</td>
<td>a 14.66</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>ab 14.90</td>
<td>14.07</td>
<td>a 14.57 (xy)</td>
<td>ab 14.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>ab 14.79</td>
<td>14.15</td>
<td>a 14.40 (xy)</td>
<td>ab 14.45</td>
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<tr>
<td><strong>om</strong></td>
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<td>14.60</td>
<td>14.17 (y)</td>
<td>14.25 (y)</td>
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<td>a 4.70</td>
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<td>a 5.18</td>
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<td>a 6.99</td>
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<td>bc 3.70</td>
<td>ab 4.77</td>
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<tr>
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<td>c 3.32 (y)</td>
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<td>b 4.65</td>
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<td>a 3.00</td>
<td>ab 2.80</td>
<td>a 2.87</td>
<td>0.1465</td>
<td>ns</td>
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<td>ab 2.58</td>
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<td>a 2.65</td>
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<td>a 2.50</td>
<td>ab 2.64</td>
<td>a 2.93</td>
<td>a 2.69</td>
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<td>4</td>
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<td>a 2.77</td>
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<td>b 2.23</td>
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<td>c 4.08</td>
<td>3.90</td>
<td>c 4.02</td>
<td>0.0862</td>
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<td>***</td>
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<td>d 4.54 (x)</td>
<td>3.87 (y)</td>
<td>c 4.01</td>
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<td>c 4.96 (x)</td>
<td>4.00 (y)</td>
<td>bc 4.12</td>
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<td>b 3.40 (z)</td>
<td>b 5.32 (x)</td>
<td>4.05 (y)</td>
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<td>4.16 (y)</td>
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<td>3.57 (z)</td>
<td>4.96 (x)</td>
<td>4.00 (y)</td>
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<td></td>
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<tr>
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<td>1</td>
<td>a 17.90</td>
<td>a 16.90</td>
<td>ab 16.28</td>
<td>a 17.03</td>
<td>1.9388</td>
<td>***</td>
<td>***</td>
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<td></td>
<td>2</td>
<td>b 14.99</td>
<td>ab 15.50</td>
<td>b 15.90</td>
<td>b 15.46</td>
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<tr>
<td></td>
<td>3</td>
<td>b 13.51 (y)</td>
<td>b 14.13 (xy)</td>
<td>b 15.82</td>
<td>c 14.49</td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>b 14.48 (y)</td>
<td>ab 15.61 (xy)</td>
<td>ab 17.13</td>
<td>b 15.74</td>
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<td></td>
<td>5</td>
<td>b 13.93 (y)</td>
<td>ab 15.35 (y)</td>
<td>a 17.81</td>
<td>b 15.70</td>
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<td></td>
</tr>
<tr>
<td><strong>om</strong></td>
<td></td>
<td>14.96 (y)</td>
<td>15.50 (y)</td>
<td>16.59 (x)</td>
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</tbody>
</table>

\(^{(1)}\) 1 = start of the trial; 2 = 15 days; 3 = 30 days; 4 = 45 days; 5 = 60 days;\(^{(2)}\) Within group: a, b, c, d, e (P ≤ 0.05);\(^{(3)}\) Between groups: x, y, z (P ≤ 0.05);\(^{(4)}\) ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05; ns = not significant.

In each diet group there was an elevation of flesh DHA and rather a dramatic reduction in EPA compared to the respective dietary levels. As to LNA, the difference between flesh and dietary levels was notable for the D1 group only, the former level being lower. On the whole, the three groups did not differ as to the sum of n-3 PUFA (for D1, D2 and D3, 26.64%, 27.24% and 27.58%, respectively, at the 60\(^{th}\) day of the trial). Percentages of flesh n-3 PUFA were lower than the respective dietary levels; this was more evident for the D2 and D3 groups than for the D1 group (32.02%, 32.00% and 27.77%, in that order).
The same held true for total n-6 PUFA (17.02%, 16.36% and 16.74%, respectively, in the flesh of D1, D2 and D3 trout at the 60th day, as against the respective dietary content which was 21.37%, 19.29% and 19.60%). From the start of the trial to the 60th day, LA level marked a slight incremental change (+8%, +1% and +5% in the flesh of D1, D2 and D3 trout), whereas arachidonic acid (AA) content underwent a sudden drop between the 45th and the 60th day of the trial (so that the overall decrease from the start of the trial amounted to –21%, –26% and –16% for D1, D2 and D3 trout, respectively).

The only health-related index that did not alter from the start of the trial to the 60th day was the n-6/n-3 ratio. Moreover, the values found for the D2 and D3 trout were identical to those obtained in the respective diets (see Table 3, where only the values at the 60th day are to be found, to economise on space).

Table 3. Health-related indices of lipid from trout fillets at the 60th day of the trial

<table>
<thead>
<tr>
<th>Trait(1)</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>MSE</th>
<th>Stat. Sign. (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-6/n-3</td>
<td>0.64</td>
<td>0.60</td>
<td>0.61</td>
<td>0.0013</td>
<td>ns</td>
</tr>
<tr>
<td>LA/LNA</td>
<td>2.03 y</td>
<td>4.30 x</td>
<td>4.32 x</td>
<td>0.0612</td>
<td>***</td>
</tr>
<tr>
<td>EPA/AA</td>
<td>1.52 z</td>
<td>2.67 x</td>
<td>1.79 y</td>
<td>0.0376</td>
<td>***</td>
</tr>
<tr>
<td>LNA/n-3</td>
<td>0.27 x</td>
<td>0.12 y</td>
<td>0.12 y</td>
<td>0.0002</td>
<td>***</td>
</tr>
<tr>
<td>LNA/EPA+DHA</td>
<td>0.42 x</td>
<td>0.16 y</td>
<td>0.15 y</td>
<td>0.0005</td>
<td>***</td>
</tr>
<tr>
<td>EPA/DHA</td>
<td>0.24 y</td>
<td>0.38 x</td>
<td>0.23 y</td>
<td>0.0004</td>
<td>***</td>
</tr>
</tbody>
</table>

(1) Between groups: x, y, z (P ≤ 0.05); (2) ***P ≤ 0.001; ns = not significant.

All other indices did change during the same time span, the most considerable variations being those involving LNA in the D1 group (LA/LNA = –32%; LNA/n-3 = +69%; LNA/EPA+DHA = +100%) and EPA in the D2 group (EPA/AA = +96%; EPA/DHA = +58%), whereas those involving DHA in the D3 group were less noticeable (LNA/n-3 = –33%; LNA/EPA+DHA = –38%) or nearly absent (EPA/DHA). At the 60th day, the three groups of trout seemed to be best discriminated by the EPA/AA ratio, an estimate of the competitive inhibition exerted by eicosanoids formed from EPA on those derived from AA, which was significantly higher for the D2 group.

On the whole, after two months of experimental feeding, the composition of trout flesh lipids did not straightforwardly reflect that of the dietary fats, an observation already made by others (Greene and Selivonchick, 1990; Sowizral et al., 1990). The prospects of selectively fortifying trout flesh with n-3 PUFA seemed more promising for LNA and EPA than for DHA.

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Financial support provided by the Ministry of Education, University and Research of Italy (ex-60% funds). The valuable cooperation of Dr. Alessio Pecchini (Skretting Italia, Mozzecane, Verona) is gratefully acknowledged.

References


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2.13 SELECTED FATTY ACID CONTENTS OF COOKED N-3 PUFA-ENRICHED TROUT FILLETS

Anna Badiani, Silvia Testi, Marina Silvi, Elisa Zironi, Alessio Bonaldo, Alessio Pecchini, Pier Paolo Gatta

Introduction

Fatty fish have been recently included in the list of functional foods by the American Dietetic Association on account of their content of n-3 polyunsaturated fatty acids (PUFA) as bioactive components (Hasler, 2002). A statement by the American Heart Association (Kris-Etherton et al., 2002) substantiated that inclusion and advocated increasing n-3 PUFA intake through a dietary approach, at least for patients without documented coronary heart disease, which would imply up to 4 meals/week of oily fish.

This may prove quite a remarkable feat to accomplish on a weekly basis for those consumers who do not enjoy eating fish, especially if associated with heavy, gamey aromatics as fatty fish frequently are. In many respects, enriching mild-tasting medium-fat fish with n-3 PUFA could provide a welcome option. For that kind of value-added product, more than for “plain” fish, it would be sensible to ascertain what could be the most suitable cooking method to better retain those beneficial nutrients. This paper will present the results of an experiment which was designed to investigate the effects of two cooking procedures on selected fatty acids of rainbow trout enriched with n-3 PUFA.

Materials and Methods

The research was centred on the rainbow trout (Oncorhynchus mykiss Walbaum, 1792) selectively fortified with n-3 PUFA and examined during the first two months of dietary treatment as described by Gatta et al. (2004). An unforeseen lack of trained personnel, combined with the fact that this part of the project was expected to be rather labour intensive, meant that experimental cooking had to be postponed for a further two weeks. In spite of this, it was deemed that this delay would not have undermined the soundness of the whole procedure, given that two cooking methods were to be compared, hence the necessity of a “twin fillet” approach without any raw reference.

At the 75th day of the trial, 6 batches of 3 fish each were randomly sampled for each diet (2 batches/tank). Fish were immersed in ice-cold water for slaughter, gutted, filleted and boned. Within batch, 3 skin-on fillets were destined for baking-in-foil in a preheated forced air convection oven, to exemplify “convection + moist-heating” (oven baking, OB), while their counterparts were pan-fried in a Teflon-coated pan without any added cooking fat, to exemplify “conduction + dry-heating” (pan frying, PF). Cooking was discontinued when flesh temperature reached 65–70°C as checked either with an iron-constantan thermocouple connected to a digital potentiometer (OB) or with a digital thermometer (PF). Cooking time was recorded and heating rate calculated.

After cooking, fillets were allowed to drain and cool, and total weight losses were determined. All fillets from each subsample of fish (i.e. cooking method within batch) were skinned, ground together and thoroughly mixed to provide a homogeneous composite paste, which was analysed for lipid and fatty acid contents (g/100 g edible portion).

Results and Discussion

The dietary treatments the trout had been subject to had no bearing on the processing parameters and cooking losses. The cooking methods adopted differed significantly in terms of processing parameters [cooking time: OB = 18 min, PF = 15 min (P = 0.0338); heating rate: OB = 3.88 °C/min, PF = 4.66 °C/min (P = 0.0406)], though average cooking losses expressed as a percentage of the initial raw mass were almost identical [OB = 15.52%, PF = 15.66% (P = 0.7796)] and roughly centred between the ranges of values collected by Matthews and Garrison (1975) for rainbow trout, either baked (8–10%) or broiled (20–27%).
The lipid content of the cooked fillets did not differ significantly among diets \([OB = 7.39, \text{ PF} = 7.19 (P = 0.6694)]\), thereby qualifying these fish as medium-fat (Stansby, 1973). The values obtained were higher than the average value given by Holland et al. (1993) for grilled rainbow trout (5.40), although similar to the figure recently published by USDA (2004) for dry-heat cooked rainbow trout (7.20), thus allowing for direct comparison about fatty acid contents.

Table 1. Selected fatty acid content (g/100 g edible portion) and health-related ratios of oven-baked (OB) or pan-fried (PF) trout fillets

<table>
<thead>
<tr>
<th>Trait</th>
<th>Cooking (C)</th>
<th>Diet (D)(1)</th>
<th>Overall mean (om)</th>
<th>MSE</th>
<th>Stat. Sign(2)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>D1</td>
<td>D2</td>
<td>D3</td>
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<tr>
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<td>OB</td>
<td>1.03</td>
<td>1.19</td>
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</tr>
<tr>
<td></td>
<td>PF</td>
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<td>1.22</td>
<td>1.07</td>
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<td>om</td>
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<td>1.21</td>
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<tr>
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<tr>
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<td>PF</td>
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<td></td>
<td>PF</td>
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<td>1.96</td>
<td>1.70</td>
<td>1.73</td>
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<td>1.54 y, 1.95 x, 1.76 xy</td>
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<tr>
<td>C18:3 n-3 (LNA)</td>
<td>OB</td>
<td>0.45 x</td>
<td>0.23 y</td>
<td>0.21 y</td>
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</tr>
<tr>
<td></td>
<td>PF</td>
<td>0.44 x</td>
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<td>0.20 y</td>
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<td>0.23 y</td>
<td>0.20 y</td>
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<td>0.43 x</td>
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<td>0.30</td>
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<tr>
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<td>PF</td>
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<td>0.26 y</td>
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<tr>
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<td>0.19 z</td>
<td>0.43 x</td>
<td>0.27 y</td>
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<td>C22:6 n-3 (DHA)</td>
<td>OB</td>
<td>0.80 y</td>
<td>1.08 xy</td>
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<td>1.13 x</td>
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<td>1.51 x</td>
<td>1.46 x</td>
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</tr>
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<td>1.55 x</td>
<td>1.35 xy</td>
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<tr>
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<td>om</td>
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<td>1.53 x</td>
<td>1.40 x</td>
<td></td>
</tr>
<tr>
<td>EPA/DHA</td>
<td>OB</td>
<td>0.25 y</td>
<td>0.40 x</td>
<td>0.23 y</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>PF</td>
<td>0.24 y</td>
<td>0.38 x</td>
<td>0.24 y</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>om</td>
<td>0.24 y</td>
<td>0.39 x</td>
<td>0.24 y</td>
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<tr>
<td>n-6/n-3</td>
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<td>0.62</td>
<td>0.63</td>
</tr>
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</table>

(1) Between groups: x, y, z \((P \leq 0.05)\); (2) ***\(P \leq 0.001\); **\(P \leq 0.01\); +\(P \leq 0.10\); ns = not significant.
Oven baking and pan frying proved to be indistinguishable as to the effect on the PUFA content of these rainbow trout fillets (Table 1). This observation was in line with the conclusions drawn by both Ågren and Hänninen (1993) for three freshwater fish species (rainbow trout included), provided that cooking methods without additional oils were used, and Johansson (2001) in her work on eating quality of farmed rainbow trout.

The dietary treatment did not affect the content of the n-6 PUFA in the cooked fillets, more specifically that of linolenic acid (LA) and arachidonic acid (AA). A significant diet effect was observed for the content of each of the tabulated n-3 fatty acid, as well as for the whole n-3 family of PUFA, although in this case the difference between diets was only marginally significant. As expected, D1 cooked fillets contained a significantly higher amount of alpha-linolenic acid (LNA) than D2 and D3 fillets (D1:D2:D3 = 2.20:1.15:1). The same held true for the content of eicosapentaenoic acid (EPA) of D2 cooked fillets compared to those of D1 and D3 fillets (D1:D2:D3 = 1:2.26:1.42), whereas the content of docosahexaenoic acid (DHA) of D3 cooked fillets differed significantly from that of the D1 fillets only (D1:D2:D3 = 1:1.39:1.43). No difference emerged between diets as to the n-6/n-3 ratio, whereas D2 cooked fillets had a significantly higher EPA/DHA ratio (D1:D2:D3 = 1:1.63:1).

The D1, D2 and D3 cooked fillets, each considered for the fatty acid they had been selectively fortified with, were superior to the dry-heat cooked rainbow trout examined by USDA (2004), with an average content of LNA, EPA and DHA equal to 0.082, 0.334 and 0.820 g/100 g, respectively. With reference to the adequate intake of LNA and EPA + DHA (2.22 and 0.65 grams/day, respectively) recently reasserted by Simopoulos (2003) for adults on a 2,000 kcal diet, a 100-g serving of cooked fillets was able to give the following average contribution: D1 = 20 and 151%; D2 = 10 and 235%; D3 = 9 and 216%, respectively. If attention is focussed solely on EPA + DHA, the D2 cooked fillets appeared to be the most suitable option, since three 100-g servings per week would be able to satisfy the combined weekly requirements of these n-3 PUFA.

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References


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2.14 NUTRITIONAL TRAITS OF DORSAL AND VENTRAL FILLETS FROM FARMED EUROPEAN SEA BASS, GILTHEAD SEA BREAM AND RAINBOW TROUT

Silvia Testi, Alessio Bonaldo, Anna Badiani, Pier Paolo Gatta

Introduction

Quality differences due to fillet region have been quite extensively researched in farmed Atlantic salmon, mostly in connection with fat and texture variation (Aursand et al., 1994; Zhou et al., 1996; Refsgaard et al., 1998; Sigurgisladottir et al., 1999; Katikou et al., 2001). This was much less the case with rainbow trout (Fjellanger et al., 2001; Mørkøre et al., 2002), and even less with other important farmed species, namely European sea bass and gilthead sea bream.

The paucity of information on quality variations within fillet is most likely due to the relatively small fillet size of these three species, yet in general it deserves attention as a potential source of difficulties in sampling planning in view of nutritional analyses (Fjellanger et al., 2001), sensory evaluation with either trained panellists or plain consumers (Lawless and Heymann, 1999), and possibly storage trials. Moreover, an increasing interest in this issue can be easily envisaged, as the consumer demand for a greater variety of value-added fish products is growing steadily.

This preliminary study was therefore conducted to determine if and to what extent compositional differences exist between dorsal and ventral fillets from farmed European sea bass (Dicentrarchus labrax Linnaeus, 1758), gilthead sea bream (Sparus aurata Linnaeus, 1758) and rainbow trout (Oncorhynchus mykiss Walbaum, 1792).

Materials and Methods

Five ready-for-sale specimens for each species [European sea bass (ESB in the following), gilthead sea bream (GSB) and rainbow trout (RT)] were randomly selected from stocks of fish intensively reared in Italian commercial farms producing for the Italian market. Fish were weighed, eviscerated and filleted. Each fillet was weighed with skin, then cut along the insertion line of the ribs to obtain a dorsal and a ventral fillet. After skinning, the two dorsal fillets from each fish were joined, their sum being named “dorsal portion” (DP), and weighed. The same was made with the two ventral fillets, which yielded a “ventral portion” (VP). Both DP and VP obtained from each fish were analysed in duplicate for proximate composition and fatty acid content, both expressed as g/100 g flesh.

Results and Discussion

Despite wide differences in body weight (mean ± standard error: ESB = 226 ± 20 g; GSB = 273 ± 18 g; RT = 519 ± 35 g), the three species did not differ as to the yield of either DP (range: 22.11–22.80%) or VP (range: 16.08–18.20%), both expressed as a percentage of body weight.

ESB proximate composition proved to be the most widely affected by fillet location, followed by GSB, and then RT (Table 1). Lipid content was by far the most variable trait within species, the ratio between DP and VP averaging 1:2.92 for ESB, 1:1.68 for GSB, and 1:1.66 for RT. To our knowledge, information about the proximate composition of DP and VP from ESB and GSB is lacking. In RT a clear trend towards increasing fat levels was observed in the caudal-cranial direction, whereas the gradient from the dorsal to the ventral part of the fish, though similar, seemed to be less pronounced (Fjellanger et al., 2001).
Table 1. Proximate composition of flesh (g/100 g edible portion)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Site(1) (Si)</th>
<th>Species(2) (S)</th>
<th>p(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sea bass</td>
<td>Sea bream</td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>D</td>
<td>x 75.60 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>y 68.31 ab</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>D</td>
<td>x 19.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>y 17.71 b</td>
<td></td>
</tr>
<tr>
<td>Lipid</td>
<td>D</td>
<td>y 4.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>x 12.99 ab</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>D</td>
<td>x 1.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>y 1.06 b</td>
<td></td>
</tr>
</tbody>
</table>

(1) D = dorsal portion; V = ventral portion; (2) Between groups: a, b (P \leq 0.05); within group: x, y (P \leq 0.05); (3) ***P \leq 0.001; **P \leq 0.01; ns = not significant.

Much more statistically significant differences between DP and VP as to the fatty acid composition of flesh lipids (expressed as % total fatty acid methyl esters) emerged for ESB than for GSB and RT (data not shown). This was to be anticipated, given the much wider difference in lipid content found in the former species between the two portions and therefore the different percentages of triglycerides and phospholipids expected in them (Opstvedt, 1984).

Species effect within location was examined to compare ESB, GSB and RT flesh lipids especially for their content of health-related n-3 polyunsaturated fatty acids (PUFA), expressed as g/100 g flesh, and their susceptibility to oxidation, evaluated through the Peroxidisability Index (PI), as suggested by Erickson (1992) (Table 2).

DP from GSB was significantly richer in saturated fatty acids (SFA), as well as in monounsaturated (MUFA) and n-6 PUFA than DP from ESB and RT. That superiority was maintained for alpha-linolenic acid (ALA) and the sum of n-3 PUFA, whereas for eicosapentaenoic acid (EPA) GSB did not differ from ESB and for docosahexaenoic acid (DHA) the difference between the three species was only marginally significant. On the whole, the n-3 PUFA content of DP from ESB was similar to that of DP from RT.

As to VP, the contents of SFA, MUFA, n-6 and n-3 PUFA did not differ significantly between ESB and GSB, being always higher than their counterparts in VP from RT. Still, the difference between the three species as to the DHA content was not significant and that observed for the sum of n-3 PUFA was only marginally significant, in spite of quite a lower figure for RT.

The ratio between EPA and DHA was higher in ESB than GSB and RT, both in DP and in VP. Within each species n-6/n-3 was lower, i.e. more favourable, in DP than in VP, RT oil emerging as the most healthful in that respect. The higher n-3 PUFA percentage in RT oil (most notably that of DHA) was also responsible for its higher PI, both in DP and in VP.
Table 2. Selected fatty acid content (g/100 g edible portion) and indices of nutritional and technological quality of flesh lipid

<table>
<thead>
<tr>
<th>Trait(1)</th>
<th>Dorsal portion</th>
<th>Ventral portion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sea bass</td>
<td>Sea bream</td>
</tr>
<tr>
<td><strong>∑ saturated</strong></td>
<td>1.08 b</td>
<td>2.07 a</td>
</tr>
<tr>
<td><strong>∑ monounsaturated</strong></td>
<td>1.40 b</td>
<td>2.88 a</td>
</tr>
<tr>
<td>18 : 2 n6 (LA)</td>
<td>0.22 b</td>
<td>0.53 a</td>
</tr>
<tr>
<td>20 : 4 n6 (AA)</td>
<td>0.04 b</td>
<td>0.07 a</td>
</tr>
<tr>
<td><strong>∑ n6</strong></td>
<td>0.28 b</td>
<td>0.63 a</td>
</tr>
<tr>
<td>18 : 3 n3 (ALA)</td>
<td>0.05 b</td>
<td>0.10 a</td>
</tr>
<tr>
<td>20 : 5 n3 (EPA)</td>
<td>0.29 ab</td>
<td>0.43 a</td>
</tr>
<tr>
<td>22 : 6 n3 (DHA)</td>
<td>0.60</td>
<td>0.97</td>
</tr>
<tr>
<td><strong>∑ n3</strong></td>
<td>1.05 b</td>
<td>1.83 a</td>
</tr>
<tr>
<td>n6/n3</td>
<td>0.26 b</td>
<td>0.35 a</td>
</tr>
<tr>
<td>EPA/DHA</td>
<td>0.49 a</td>
<td>0.44 a</td>
</tr>
<tr>
<td>Perox. Index (PI)(2)</td>
<td>192 ab</td>
<td>170 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>∑ saturated</strong></td>
<td>3.15 a</td>
<td>3.49 a</td>
</tr>
<tr>
<td><strong>∑ monounsaturated</strong></td>
<td>4.27 a</td>
<td>4.79 a</td>
</tr>
<tr>
<td>18 : 2 n6 (LA)</td>
<td>0.71 a</td>
<td>0.90 a</td>
</tr>
<tr>
<td>20 : 4 n6 (AA)</td>
<td>0.09 a</td>
<td>0.10 a</td>
</tr>
<tr>
<td><strong>∑ n6</strong></td>
<td>0.86 a</td>
<td>1.06 a</td>
</tr>
<tr>
<td>18 : 3 n3 (ALA)</td>
<td>0.16 a</td>
<td>0.18 a</td>
</tr>
<tr>
<td>20 : 5 n3 (EPA)</td>
<td>0.81 a</td>
<td>0.70 ab</td>
</tr>
<tr>
<td>22 : 6 n3 (DHA)</td>
<td>1.36</td>
<td>1.41</td>
</tr>
<tr>
<td><strong>∑ n3</strong></td>
<td>2.68</td>
<td>2.82</td>
</tr>
<tr>
<td>n6/n3</td>
<td>0.32 b</td>
<td>0.38 a</td>
</tr>
<tr>
<td>EPA/DHA</td>
<td>0.59 a</td>
<td>0.49 b</td>
</tr>
<tr>
<td>Perox. Index (PI)(2)</td>
<td>163 b</td>
<td>155 b</td>
</tr>
</tbody>
</table>

(1) ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05; +P ≤ 0.10; ns = not significant; between groups: a, b, c (P ≤ 0.05).

(2) Peroxidisability index = (0.025 × % monoenes) + (1 × % dienes) + (2 × % trienes) + (4 × % tetraenes) + (6 × % pentaenes) + (8 × % hexaenes).

From a nutritional standpoint, two regular fish burgers (105–110 g each) per week of VP from either ESB or GSB would be able to meet the weekly EPA + DHA requirements of an adult on a 2,000 kcal diet [4.55 g/week, according to Simopoulos et al. (2003)], while at the same time providing 200÷220 kcal each. Though probably fatter than the usual muscle food entrée, a patty of this type would be much leaner than an ordinary burger (ND, 2004) and it could be easily assembled into a healthy meal with foods having a low nutrient density for lipids. On the other hand, a market for DP from ESB and GSB might be found as fresh, unprocessed products because of better appearance and a higher susceptibility to lipid oxidation compared with their ventral counterparts. On the grounds of the present results, a separate use of DP and VP from RT would not seem to be warranted, because of their relatively modest difference in lipid content.
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References


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3.1 COMPARISON OF HISTAMINE CONTENTS OF SARDINE (SARDINA PILCHARDUS) CAUGHT IN DIFFERENT SEASON DURING REFRIGERATED STORAGE

Nalan Gokoglu and Pınar Yerlikaya

Introduction

Fish are rich sources of high-quality protein, essential vitamins and healthful polyunsaturated fatty acids. The high content of proteins, on the other hand, represents a risk in the decomposition processes. The disintegration of proteins yields peptides and amino acids, which are susceptible to further decay, resulting in biogenic amines, that can be widely distributed in proteinaceous foods (Krijzek et al., in press). The presence of biogenic amines in food is important from a health or toxicological perspective, since the consumption of foods containing amines has been associated with some cases of food poisoning (Taylor, 1985; Bardócz, 1995). The most frequent foodborne intoxications caused by biogenic amines involve histamine. Histamine poisoning is also referred to as “scombroid fish poisoning” because of the association of this illness with the consumption of scombroid fish (Halasz et al., 1994). Histamine is a product of the microbial degradation of the amino acid histidine due to the action of histidine decarboxylase (Cinquina et al., 2004). Immediately after catching, fresh fish contains very low levels of histamine, but the content increases with the progress of fish decomposition. Therefore, histamine has also been proposed as a chemical index of freshness of fishes and poor hygienic quality of raw materials used and/or poor manufacturing conditions (Hwang et al., 2003). In this study, the changes in histamine contents of sardines caught in different seasons during refrigerated storage were compared.

Materials and methods

The fish were caught from the gulf of Antalya, Turkey in February and July. Fresh fish was purchased from fisherman in 5-6 h after harvesting and transferred to the laboratory within 2 h of purchase. After initial histamine concentrations were determined they were stored in a refrigerator (4°C). Samples were taken for analyses at 24 h intervals during the storage.

Histamine (HI) content was determined by the modified method of Mietz and Karmas (1977, 1978). A Varian Star Model 9050 with model 9010 solvent delivery system, Marathon auto sampler and UV-VIS detector was used with a Supelcosil LC-18 (25 cm x 4.6 mm, 5µm).

Histamine dihydrochloride (41.40 mg) was used for standard solution. Dansyl chloride reagent was prepared by dissolving 10 mg dansylchloride in 1mL acetone. Ten grams of sample was transferred to a 250 mL tube and homogenized with 90 mL 5% trichloracetic acid using an ultraturrax homogenizer. The homogenate was centrifuged at 6000 rpm and filtered through whatman filter paper. After derivatization with dansyl-chlorid, sample extract and working standard solution were injected into liquid chromatograph. A linear solvent program (gradient elution) was used from 60% solvent B in A to 100% B in 30 min, at a constant flow rate of 1.0 mL/min. The injection volume was 10 µL. Dansylated amines were detected by measuring the absorbance at 254 nm.

Sensory analyses were performed at each sampling point. Sensory properties of raw and cooked fish were assessed by a panel of five experienced panelists. In raw state, the appearance and odor were evaluated. Then the fish were cooked in a closed jar over boiling water without adding of any water. The panelists evaluated sensory properties using a 9 point hedonic scale. A score of 7-9 indicated “very good” quality, a score of 4.0-6.9 “good” quality, a score of 1.0-3.9 denoted as “spoiled”. The mean values were calculated to find out the overall quality. All assessments took place in individual booths in a daylight conditions.

Data were treated analysis of variance and ‘complete randomized design’ for statistical evaluation to determine the significance among the storage hours (Düzgüneş et al., 1987).

Results and discussion

The initial histamine content (8.40±2.22 mg/kg) of sardine caught in winter was lower than of sardines caught in summer (14.23±2.15 mg/kg) (Fig. 1). The difference between 0 and 96 h of storage in histamine levels of winter sardine was significant (p<0.05) and also in summer sardine was significant (p<0.01). Histamine concentration of summer sardine rapidly reached to 60.52±3.63 mg/kg at the end of the storage period. Whereas, the increase in histamine
content of winter sardine was slow. Histamine level of winter sardine at 96th hour was even lower than of winter at 24th hour. According to FDA guideline, fish that contain histamine above 50 mg/kg are prohibited from being sold for consumption (FDA, 1996). Histamine levels of summer sardine exceed 50 mg/kg on the second day; however, of winter sardine did not reached to toxic level at the end of the storage period. After this time they became unacceptable from the point of sensory. The interaction between the histamine values of winter and summer was also significant (p<0.01).

Sensory analyses were conducted in order to determine spoilage time and relationship with the results of histamine analysis. Sensory scores of winter and summer sardines significantly (p<0.01) decreased during the storage. The scores of winter sardines were slight lower than of summer sardines. Both sardine caught in winter and summer spoiled after 96 h in refrigerated storage (Fig. 2).

![Fig. 1. Changes in histamine contents of sardine during refrigerated storage](image1)

![Fig. 2. Changes in sensory scores of sardine during refrigerated storage](image2)
There was a good negative correlation between histamine levels and sensory scores for winter ($r=-0.89$) and summer ($r=-0.95$) sardines.

In conclusion, it can be said that catching season of sardine had significant affect on histamine content. High initial histamine content resulted in rapid increase during refrigerated storage. Histamine level can be reached to toxic level depending on initial concentration. Initial concentration may be high depending on the conditions following harvest. For these reason, especially in summer season which air temperature is high the fish should be chilled immediately after harvesting. Because the biogenic amine formation is more related to activity of mesophilic than psychrotrophic bacteria and histamine level can be easily raised to high levels at ambient temperature.

References


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3.2 INCIDENCE OF LISTERIA SPP. IN FISH AND ENVIRONMENT OF FISH MARKETS IN NORTHERN GREECE

Nikolaos Soultos, Amin Abrahim, Konstantinos Papageorgiou and Vasilios Steris

Introduction

Listeria bacteria are widespread and commonly found in soil, sewage, dust and water. A number of surveys have also shown that these organisms are frequently found in raw and processed fish at the retail level (Jinnemal et al., 1999). One particular Listeria species, Listeria monocytogenes, can cause a serious foodborne illness called listeriosis. While several reports indicate that fish and fishery products can be frequently contaminated with L. monocytogenes, no major outbreaks associated with these products have been reported (FAO, 1999). The involvement of seafood in the transmission of listeriosis was suggested by Lennon et al. (1984) who based on epidemiological evidence, proposed that consumption of shellfish and raw fish was responsible for an epidemic of prenatal listeriosis in New Zealand in 1980. Subsequently, sporadic cases of foodborne listeriosis have been reported. Since fish and fishery products may be a vehicle for L. monocytogenes, it is important to have information on the incidence of this pathogen. While most investigators have reported a relatively high incidence of L. monocytogenes on seafood, however, information on the incidence of this pathogen on European and especially on Greek fish is very limited.

Thus, the purpose of the present study was to generate information on the incidence of Listeria species on salt-water fresh edible fish, as well as on environment of fish markets of Thessaloniki in Northern Greece.

Materials and methods

A total of 150 samples were analysed. Ninety fish samples including 30 from mackerel (Scomber scombrus), 30 from bogue (Boops boops) and 30 from horse mackerel (Trachurus trachurus) and sixty environmental and personnel (swabbed) samples including 12 from workers’ hands, 12 from workers’ knives, 12 from work surfaces (wooden board), 12 from containers (wooden boxes) and 12 from floor were collected from local fish markets. All samples were transported to the laboratory inside cold portable insulate boxes and processed with 1h of collection.

Isolation of Listeria spp.

Methodology based on EN ISO 11290 – 1: 1996 (Anon. 1997) was used to isolate Listeria spp. from fish and environmental and personnel samples. 25g of each fish sample (flesh and skin) was homogenized in 225ml of half Fraser broth using a stomacher 400-laboratory blender (Seward Ltd) and incubated (24h, 30°C). For the detection of Listeria spp. on work surfaces (wooden board), containers (wooden boxes) and floor surfaces, an area of 100 cm² was swabbed with 3 sterile cotton swabs, which had been moistened with 1% peptone water containing 0.85% NaCl. Swabbing the surface of the workers’ hands, twelve samples were collected, which included 6 eviscerators and 6 dealers. For workers’ knives the knife blade, from its tip to base was swabbed. Swab samples were directly inoculated into half Frazer broth and incubated (24h, 30°C). A loopful (10ml) was streaked onto Oxford agar and Palcam agar and examined after 24h and 48h (30°C). An aliquot (0.1ml) was transferred to 10ml Fraser broth (48h, 30°C) then a loopful (10ml) was streaked onto Oxford agar and Palcam agar and examined after 24h and 48h (30°C). Five suspect Listeria spp. were streaked to purify on Tryptone Soya agar with yeast extract (24h, 37°C). Pure cultures of Listeria spp. were confirmed to the genus level and subsequent to the species level as described by Rocourt and Cossart (1997) and Encinas et al., (1999).

Results and discussion

In temperate regions, L. monocytogenes and other Listeria species have been isolated from fishery products on a regular basis since the late nineteen eights. Embarek (1994) reviewed the incidence of Listeria in seafood worldwide and found that the prevalence of L. monocytogenes varied from 4-12% in surveys from temperate areas. An overall prevalence of 3% L. monocytogenes was observed in European fish (Davies et al., 2001).
In Ioannina (North-western Greece), Salamoura et al. (2004) isolated *L. monocytogenes* in 2 of 75 samples of fresh and salt-water edible fish. One isolate was detected from a fish of local capture (cephalus) and the other strain was isolated from imported fresh salmon fillet.

In our study, the overall incidence of *Listeria* spp. and *L. monocytogenes* in the fish samples were 4.44% and 1.11% respectively (Table 1). *L. monocytogenes* was detected only from one fish sample (bogue). *L. innocua* was the only other *Listeria* spp., being detected in 3.33% of fish samples. As in other raw foods, fishery products more frequently contain *L. innocua* than *L. monocytogenes*. Since both species share ecological niches, the presence of *L. innocua* is considered as an indicator of possible contamination with *L. monocytogenes* (Jones and Seeliger, 1992; Jinneman et al. 1999). Other *Listeria* species were not isolated from any of the fish samples tested (Table 1).

### Table 1. Incidence of *Listeria* species in fish samples

<table>
<thead>
<tr>
<th>Fish type</th>
<th>Number examined</th>
<th><em>Listeria</em> spp.</th>
<th><em>L. monocytogenes</em></th>
<th><em>L. innocua</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mackerel (<em>Scomber scombrus</em>)</td>
<td>30</td>
<td>1 (3.33)</td>
<td>-</td>
<td>1 (3.33)</td>
</tr>
<tr>
<td>Bogue (<em>Boops boops</em>)</td>
<td>30</td>
<td>3 (10)</td>
<td>1 (3.33)</td>
<td>2 (6.66)</td>
</tr>
<tr>
<td>Horse mackerel (<em>Trachurus trachurus</em>)</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>90</strong></td>
<td><strong>4 (4.44)</strong></td>
<td><strong>1 (1.11)</strong></td>
<td><strong>3 (3.33)</strong></td>
</tr>
</tbody>
</table>

* Percentage of positive *Listeria* spp. samples

As shown in Table 2, *Listeria* species were found in 23.33% of the total personnel and environmental samples. *Listeria* spp. were not detected in workers’ hands and knives. Of the total personnel and environmental samples, 4 (6.66%) were positive for *L. monocytogenes* (Table 2) of which 2 were taken from work surfaces (wooden board), 1 from containers and 1 from floor. *L. innocua* was the most common *Listeria* spp. being isolated from 4 (33.33%) of the 12 work surfaces (wooden board), from 4 (33.33%) of the 12 floor surfaces and from 1 (8.53%) of the 12 containers (wooden boxes). *L. seeligeri* was detected in 1 of the 12 work surfaces (wooden board).

### Table 2. Incidence of *Listeria* species in environmental and personnel samples

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number examined</th>
<th><em>Listeria</em> spp.</th>
<th><em>L. monocytogenes</em></th>
<th><em>L. innocua</em></th>
<th><em>L. seeligeri</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Workers’ hands</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Workers’ knives</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Containers (wooden boxes)</td>
<td>12</td>
<td>2 (16.66)</td>
<td>1 (8.33)</td>
<td>1 (8.33)</td>
<td>-</td>
</tr>
<tr>
<td>Work surfaces (wooden board)</td>
<td>12</td>
<td>7 (58.33)</td>
<td>2 (16.66)</td>
<td>4 (33.33)</td>
<td>1 (8.33)</td>
</tr>
<tr>
<td>Floor surfaces</td>
<td>12</td>
<td>5 (41.66)</td>
<td>1 (8.33)</td>
<td>4 (33.33)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>60</strong></td>
<td><strong>14 (23.33)</strong></td>
<td><strong>4 (6.66)</strong></td>
<td><strong>9 (15)</strong></td>
<td><strong>1 (1.66)</strong></td>
</tr>
</tbody>
</table>

* Percentage of positive *Listeria* spp. samples

The present study shows that *L. monocytogenes* and other *Listeria* species are not commonly found in the samples of salt-water edible fish at the retail level in Thessaloniki, Northern Greece, while the level of contamination of the environment of fish markets is higher. However, certain measures must be taken for the prevention of human infections, such as:

1) avoidance of consumption of raw or insufficiently cooked fish by at-risk population (pregnant women, children and elderly or immunocompromised individuals) and
2) the use of adequate hygienic practices to reduce the potential contamination of fish by *Listeria*.
References


Authors

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Tel: +3031999807 Fax: +3031999833
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3.3 AEROMONAS SPECIES ISOLATED IN FISH AND ENVIRONMENT OF FISH MARKETS IN NORTHERN GREECE

Amin Abrahim, Nikolaos Soultsos, Vasilios Steris and Konstantinos Papageorgiou

Introduction

Some strains of Aeromonas species are considered to be important pathogens to human, amphibian, reptiles and fish (Goodwin et al., 1983; Austin and Allen-Austin, 1985). They have been implicated as the cause of gastroenteritis and most causes of septicemia and meningitis, particularly, in young children, the elderly and immunocompromised patients (Stelma, G.N., 1989; Varnam and Evans, 1991; Palumbo et al., 1992). A. hydrophila has been isolated almost in all food samples such as fish, seafood, red meat, poultry, (Palumbo et al., 1985) raw milk and other milk products (Melas et al., 1999). Aeromonas spp. in foods thought to be associated with the spoilage of refrigerated foods products (Buchanan and Palumbo, 1985) and the organism can grow competitively in foods held at 5°C (Palumbo et al., 1985). The objective of the present study was to investigate the presence of Aeromonas spp. in fresh fish and in personnel and environment of local fish markets of Thessaloniki, Northern Greece.

Materials and methods

A total of 150 samples (fish, personnel and environmental) were collected in retail fish markets of Thessaloniki, Northern Greece and analysed for the presence of Aeromonas spp. These consisted of 90 fish samples representing three types of fish: 30 mackerel (Scomber scombrus), 30 bogue (Boops boops) and 30 horse mackerel (Trachurus trachurus) as well as 60 personnel and environmental samples: 12 workers’ hands, 12 workers’ knives, 12 work surfaces (wooden board), 12 containers (wooden boxes) and 12 floor surfaces. Twenty-five grams of fish samples were homogenized in 225 ml Tryptone Soy broth containing 30 µg/ml of ampicillin using a stomacher (Lab Blender 400, Seward Medical, London, UK). The homogenate was incubated for 24 h at 28°C. For the detection of Aeromonas spp. on work surfaces (wooden board), containers (wooden boxes) and floor surfaces, an area of 100 cm² was swabbed with 3 sterile cotton swabs, which had been moistened with 1% peptone water containing 0.85% NaCl. Swabbing the surface of the workers’ hands, twelve samples were collected, which included 6 eviscerators and 6 dealers. For workers’ knives the knife blade, from its tip to base was swabbed. The swabs were then placed in tubes containing 10 ml Tryptone Soy broth containing 30µg/ml of ampicillin. The tubes were incubated at 28°C for 24 h. After incubation of fish, personnel and environment samples, one loopfull of the enriched culture was streaked on Starch Ampicillin agar containing 30µg/ml of ampicillin and incubated at 28oC for 24 h. Yellow to honey coloured, amylase and oxidase positive colonies were isolated. The presumptive colonies were confirmed biochemically to species level according to Popoff (1984) as modified for A. veronii biotype sobria by Hickman-Brenner et al. (1987) and by using a Microbact MB 24E identification kit.

Results and discussion

As seen from Table I, Aeromonas species was isolated from 62 (68.9%) of the 90 fish samples analysed. Aeromonas species was isolated from 20 (66.66%) of the 30 mackerel (Scomber scombrus), from 24 (80%) of the 30 bogue (Boops boops) and from 18 (60%) of the 30 horse mackerel (Trachurus trachurus). Of the fish samples examined, 26.66%, 28.89% and 11.11% harboured A. hydrophila, A. caviae and A. sobria, respectively (Table 1). In the present study, we observed that A. hydrophila and A. caviae were the dominant species. Radu et al., 2002 reported that 69%, 55%, 11.5% and 2.3% of the fish samples examined harboured Aeromonas spp., A. veronii biovar sobria, A. hydrophila, and A.caviae respectively. As reported by other authors, mesophilic Aeromonads were isolated from 37.3% of finfish and 36.6% of prawn (Thaymanavan et al., 2003), 72% of fish and shrimps (Neyts et al., 2000), 27 (93%) of 29 fish, from 17 (100%) of fish-egg, from 2 (16%) of 12 shrimp samples and from 23 (100%) freshwater samples (Hännienen et al., 1997). A total of 82 strains of Aeromonas spp. were also isolated from 250 samples of frozen fish (Castro-Escarpulli et al., 2002).
Table 1. Incidence of *Aeromonas* species in fish samples

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number examined</th>
<th>No. (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Aeromonas</em> spp.</td>
<td><em>A. hydrophila</em></td>
</tr>
<tr>
<td>Mackerel (<em>Scomber scombrus</em>)</td>
<td>30</td>
<td>20 (66.66)</td>
</tr>
<tr>
<td>Bogue (<em>Boops boops</em>)</td>
<td>30</td>
<td>24 (80)</td>
</tr>
<tr>
<td>Horse mackerel (<em>Trachurus trachurus</em>)</td>
<td>30</td>
<td>18 (60)</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>62 (68.88)</td>
</tr>
</tbody>
</table>

a Percentage of positive *Aeromonas* spp. samples

As seen from Table 2, *Aeromonas* species was isolated from 44 (73.3%) of the 60 personnel and environment samples analysed. *Aeromonas* species was isolated from 5 (41.66%) of the 12 workers’ hands, from 7 (58.33%) of the 12 workers’ knives, from 8 (66.66%) of the 12 containers (wooden boxes), from 12 (100%) of the 12 work surfaces (wooden board) and from 12 (100%) of the 12 floor surfaces. Of the personnel and environmental samples examined, 23.33%, 31.66% and 11.66% harboured *A. hydrophila*, *A. caviae* and *A. sobria*, respectively (Table 2).

Table 2. Incidence of *Aeromonas* species in environmental and personnel samples

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number examined</th>
<th>No. (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Aeromonas</em> spp.</td>
<td><em>A. hydrophila</em></td>
</tr>
<tr>
<td>Workers’ hands</td>
<td>12</td>
<td>5 (41.66)</td>
</tr>
<tr>
<td>Workers’ knives</td>
<td>12</td>
<td>7 (58.33)</td>
</tr>
<tr>
<td>Containers (wooden boxes)</td>
<td>12</td>
<td>8 (66.66)</td>
</tr>
<tr>
<td>Work surfaces (wooden board)</td>
<td>12</td>
<td>12 (100)</td>
</tr>
<tr>
<td>Floor surfaces</td>
<td>12</td>
<td>12 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>44 (73.33)</td>
</tr>
</tbody>
</table>

a Percentage of positive *Aeromonas* spp. samples

The present data confirm that *Aeromonas* species are frequently found in fish, personnel handling fishes and fish markets environment. The results of this study show that *Aeromonas* spp. are frequently found in fresh fish and confirms the finding of Palumbo et al. (1986) showing its presence in almost all foods of animal origin. Our results indicate that the high prevalence of *Aeromonas* spp. in fish samples is well correlated with the high prevalence in the environment of fish markets. The high prevalence of *Aeromonas* spp. in fish and the environment of fish markets support the conclusion that it is of paramount importance the effective cooking of fish and the prevention of the possibility of cross-contamination at the processing and food preparation plants.
References


Authors

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3.4 DISTRIBUTION OF MERCURY AND CADMIUM IN SWORDFISH 
(XIPHIAS GLADIUS)

Erwin Schuirmann

Introduction

The aquatic environment of fish shows naturally occurring heavy metal concentrations as well as contaminations by 
human activities. Especially in large predatory fish species the mercury and cadmium content is partly really high. Also 
lead, tin, and arsenic have to be mentioned (Großklaus 1989). 
Cadmium and lead is concentrated in liver and kidney of the fish (Oehlenschläger, 1994) .
The most prevalent form of mercury in the aquatic environment is methyl mercury . These organic form is fat soluble
and therefore biological membranes are easily penetrated and methyl mercury is accumulated in the fat tissue. It’s known to be neurotoxic to humans as well as to animals.
In the last time the european rapid alert system reported more and more of extended cadmium and mercury contents in
swordfish. The commission regulation 466/2001 EC setting maxi-
mum levels for certain contaminants in foodstuffs.
The maximum level for cadmium in swordfish is set to 0,05 mg/kg and for mercury 1,0 mg/kg. In the working
document Draft Sanco/15/2004 Rev1 a higher cadmium limit of 0,1 mg/kg had been proposed.
The topic of our examinations was to determine the mercury and cadmium content as well as the distribution of these
heavy metals in swordfish.

![Fig. 1. swordfish (Xiphias Gladius)](image)

Material and methods

We tested five swordfish of different length and weight (Table 1).

Table 1. Size of the tested swordfish

<table>
<thead>
<tr>
<th>Swordfish sample</th>
<th>Length (m)</th>
<th>Weight (kg)</th>
<th>Girth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.03</td>
<td>27.92</td>
<td>0.80</td>
</tr>
<tr>
<td>B</td>
<td>1.05</td>
<td>28.62</td>
<td>0.78</td>
</tr>
<tr>
<td>C</td>
<td>1.33</td>
<td>70.67</td>
<td>1.01</td>
</tr>
<tr>
<td>D</td>
<td>1.39</td>
<td>66.50</td>
<td>1.08</td>
</tr>
<tr>
<td>E</td>
<td>1.82</td>
<td>120.97</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Each fish was riped open by four vertical trims into five sections (Fig. 2). The test material was then collected from the
dorsal and the ventral side in four sections (head end; second quarter; third quarter; tail end) (Fig. 3). Each laboratory
sample showed a weight of app. 500 g. This material was homogenized by an ultra turrax to get the analytical sample.
For digestion we use a Microwave Accelerated Reaction System (Mars 5; CEM). So at least eight samples per fish were
tested by atomic absorption spectrometrie (Spectra AA200/GTA 100 Varian); cadmium with graphite tube technique
(ASU § 35 LMBG L00.00-19/2 1993-08) and mercury with cold vapour atomic absorption (ASU § 35 LMBG L00.00-
19/4 1996-02).
Fig. 2. Vertical trims

Fig. 3. Ventral and dorsal side
Results

The mercury and cadmium levels detected in the different swordfish samples are shown in Table 2 and 3 (maximum values in bold).

Table 2. Distribution of mercury in swordfish

<table>
<thead>
<tr>
<th></th>
<th>mercury mg/kg</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sample a</td>
<td>sample b</td>
<td>sample c</td>
<td>sample d</td>
<td>sample e</td>
</tr>
<tr>
<td>Dorsal head end</td>
<td>0.61</td>
<td>0.71</td>
<td>2.20</td>
<td>0.62</td>
<td>1.26</td>
</tr>
<tr>
<td>Dorsal second quarter</td>
<td>0.58</td>
<td>0.74</td>
<td>2.06</td>
<td>0.87</td>
<td>1.25</td>
</tr>
<tr>
<td>Dorsal third quarter</td>
<td>0.53</td>
<td>0.91</td>
<td>2.07</td>
<td>1.19</td>
<td>1.20</td>
</tr>
<tr>
<td>Dorsal tail end</td>
<td><strong>0.75</strong></td>
<td>0.83</td>
<td>2.12</td>
<td>1.05</td>
<td>1.22</td>
</tr>
<tr>
<td>Ventral head end</td>
<td>0.61</td>
<td>0.59</td>
<td>2.46</td>
<td><strong>1.50</strong></td>
<td>1.34</td>
</tr>
<tr>
<td>Ventral second quarter</td>
<td>0.67</td>
<td>0.67</td>
<td>2.18</td>
<td>1.02</td>
<td>1.12</td>
</tr>
<tr>
<td>Ventral third quarter</td>
<td>0.73</td>
<td>0.66</td>
<td>2.73</td>
<td>1.12</td>
<td>1.30</td>
</tr>
<tr>
<td>Ventral tail end</td>
<td>0.72</td>
<td>0.73</td>
<td><strong>2.94</strong></td>
<td>1.09</td>
<td><strong>1.35</strong></td>
</tr>
<tr>
<td>average</td>
<td>0.65</td>
<td>0.73</td>
<td>2.35</td>
<td>1.06</td>
<td>1.26</td>
</tr>
</tbody>
</table>

Table 3. Distribution of cadmium in swordfish

<table>
<thead>
<tr>
<th></th>
<th>cadmium mg/kg</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sample a</td>
<td>sample b</td>
<td>sample c</td>
<td>sample d</td>
<td>sample e</td>
</tr>
<tr>
<td>Dorsal head end</td>
<td>0.042</td>
<td>0.086</td>
<td><strong>0.084</strong></td>
<td>0.100</td>
<td>0.182</td>
</tr>
<tr>
<td>Dorsal second quarter</td>
<td>0.042</td>
<td>0.069</td>
<td>0.081</td>
<td>0.081</td>
<td>0.125</td>
</tr>
<tr>
<td>Dorsal third quarter</td>
<td>0.038</td>
<td>0.055</td>
<td>0.059</td>
<td>0.094</td>
<td>0.144</td>
</tr>
<tr>
<td>Dorsal tail end</td>
<td>0.048</td>
<td>0.065</td>
<td>0.064</td>
<td>0.105</td>
<td>0.214</td>
</tr>
<tr>
<td>Ventral head end</td>
<td><strong>0.068</strong></td>
<td><strong>0.104</strong></td>
<td>0.080</td>
<td><strong>0.138</strong></td>
<td><strong>0.216</strong></td>
</tr>
<tr>
<td>Ventral second quarter</td>
<td>0.049</td>
<td>0.084</td>
<td>0.055</td>
<td>0.103</td>
<td>0.153</td>
</tr>
<tr>
<td>Ventral third quarter</td>
<td>0.043</td>
<td>0.060</td>
<td>0.076</td>
<td>0.100</td>
<td>0.152</td>
</tr>
<tr>
<td>Ventral tail end</td>
<td>0.050</td>
<td>0.057</td>
<td>0.058</td>
<td>0.108</td>
<td>0.176</td>
</tr>
<tr>
<td>average</td>
<td>0.048</td>
<td>0.073</td>
<td>0.070</td>
<td>0.117</td>
<td>0.170</td>
</tr>
</tbody>
</table>

Discussion

The average mercury level of three of the five swordfish was higher than the allowed maximum concentration of 1 mg/kg. The maximum cadmium concentration of 0.05 mg/kg was exceeded by four of the five fishes.

The mercury content does not correlate with the cadmium content.

The mercury content decreased in the following order: c-e-d-b-a

The cadmium content decreased in the following order: e-d-b-c-a

The mercury content of sample c was distinctly higher than the content of the other tested swordfish. Although the samples c and d showed comparable weights, the heavy metal content was distinctly different. The different tested areas of the fish showed frequently comparable concentrations. But some sections of the same fish were distinctly different from others.

The highest cadmium content was detected in four of the five swordfish in the head end of the ventral side. The highest mercury content was detected in different sampling areas in each fish.
Conclusions

The cadmium and mercury content of swordfish doesn’t correspond with the weight of the fish in every case. Only the highest cadmium content was detected at the ventral head end of the fish in most cases. The test of only one sample taken at a specified location of the swordfish doesn’t show the average cadmium or mercury content. If the average level of the cadmium and mercury content of samples taken from different fishes of the same lot, is close to or a bit higher than the maximum allowed level, the sampling area of the fish could be decisive. For example the mercury content of sample d in the dorsal head end and dorsal second quarter was within the allowed range. The following six tested areas of sample d showed an increased mercury content which was higher than the allowed 1,0 mg/kg. A similar coherence we see for the cadmium content of sample a. In these cases we recommend to take more laboratory samples all over the fish and to determine the heavy metal content in a mixed sample.

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3.5 RELATION BETWEEN TOTAL BODY LENGTH AND MERCURY LEVELS IN SOME FISH SPECIES

Lourenço, H. M.; Afonso, C.; Martins, M. F.; Lino, A. R.; Nunes, M. L.

Fish species present higher contents of heavy metals than other foods. Within these compounds mercury has been considered of a major concern due to its possible role in several metabolic pathways. On the other hand it is know that most species biocaccumulate this compound over its life. Thus, the establishment of relations between size and concentrations is very important, since can contribute to give good indication in terms of suitable consumption. The purpose of this work was to establish a relation between the total mercury levels vs. length and/or weight of the most important wild and farmed fish species commercialised in Portugal.

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** Sciences College of Lisbon University, Edificio C8, Campo Grande, Lisbon, Portugal
^Author to whom correspondence should be addressed (e-mail – helena@ipimar.pt)
3.6 INTERLABORATORY STUDY: DETERMINATION OF CHLORAMPHENICOL (CAP) RESIDUES IN SHRIMPS

U. Schröder

Introduction

Chloramphenicol (CAP) is a broad-spectrum antibiotic with very effective antibacterial properties which is totally banned since 1994 within the European Union as veterinary medical product in foodstuffs of animal origin. CAP was placed on the Annex IV of the Council regulation (EEC) 2377/90. It compiles a list of pharmaceutically active substances for which no Maximum Residue Level (MRL) can be fixed. Food products with any CAP residues are not more suitable for human consumption, they have to be destroyed. The reasons why a MRL can not be laid down for CAP are based in its toxicity. The use for medical purposes has been found associated with dose-independent cases of aplastic anaemia, a serious blood disorder. In addition there are concerns related to potential carcinogenicity and genotoxicity of this antibiotic.

Nevertheless some shrimp farmers in Asia repeatedly apply CAP in their ponds for disease control. The detection of CAP residues in imported shrimps confirm the use.

In Germany an official validated method for the determination of CAP-residues with concentrations below 1 µg/kg does not specifically exist for shrimps. Many different analytical methods are now established in the laboratories for the CAP-examination. One step to harmonise the CAP-analysis in Europe was the legal implementation of a „Minimum Required Performance Limit (MRPL)“ for CAP at 0,3 µg/kg in the Decision 2002/657/EC.

The MRPL is a moving target and it is desired that laboratories continue to decline the decision limit with advancing analytical technology. This situation lead to competitive distortions parallel for shellfish producers and importers. In Germany some federal states have rejected containers of shrimps with CAP residues at 0,03 µg/kg whereas another federal state has rejected shrimps products not until to 0,1 µg/kg because of its limited performance in CAP analysis. In some cases the certainty of some methods obviously seemed to be a problem, because different laboratories determined different CAP residues for the same sample.

Implementation

In summer 2002 an interlaboratory study was organized for gaining an overview of the actual performance of the different analytical methods for determination of CAP-residues in shrimps.

15 laboratories took part at this study whereas 5 of them were official institutions.

The test material was shrimps of the species Parapeneaopsis stylifera, a fishery product from the Indian Ocean / Pakistan. The homogenised shrimp material was spiked with an aqueous CAP standard solution after CAP-absence has been confirmed.

The participants of the study received 5 homogeneous samples with CAP concentrations from 0,03 µg/kg to 1,0 µg/kg and one blank sample with no spike in order to carry out the analytical method they normally used for CAP residues in shrimps.

The applied methods of the participants in this study were: 3 laboratories worked with LC-MS/MS, 7 laboratories with GC where one of these carried out ECD and the other 6 MS-detection. 5 laboratories used the screening method Elisa. With the exception of the Elisa, which had nearly the same extraction steps and clean up, the other methods compiled very different analytical steps before detection.

Results and discussion

After application of outlier tests the data from 14 of 15 laboratories could be analysed. In the following chapter the deviations of the mean values from the nominal value, demonstrate as percentage, will be presented. Here, no differentiation between the several analytical methods is made.
Sample with 0.03 µg/kg CAP

The study shows that the analysis of CAP in shrimps at the level 0.03 µg/kg is associated with uncertainty. Only 9 of 14 laboratories could determine a positive result for CAP. The majority (6 labs) of the 9 laboratories, this is equivalent to approximately 70%, determined CAP concentrations with a deviation of more than 50% from the nominal value. A possible explanation for this could be the increased effects of matrix which may disturb the measurement near the LOD (limit of detection).

Samples with 0.1 µg/kg CAP

Because the encoded samples A and E compromised the same CAP concentration of 0.1 µg/kg, it was possible to combine the results. 13 of 14 labs could detect and quantify a CAP-residue in the shrimp material. But only 5 labs (38.5%) determined the CAP-concentration with a deviation up to 30% of the nominal value. 38% of the labs presented results with a deviation above 70% of the nominal value. These results present a slight uncertainty in the CAP-analysis too.

Sample with 0.3 µg/kg

Evident better results are found at the concentration level 0.3 µg/kg. All labs were able to quantify the CAP residue. 57% of the labs (8 labs of 14 labs) determined CAP concentrations with a deviation not higher than 30% from the nominal value. Only 29% of the participants (4 labs of 14 labs) presented results with a deviation above 60% of the nominal value. The analysis of the sample with 1 µg/kg (here without diagram) shows much better results in lower deviations from the nominal value than at the level 0.3 µg/kg.
Sample without spiking

5 from 14 laboratories detected CAP residues in this sample without spiking. 3 of the 5 laboratories, carrying out the Elisa method, determined CAP concentrations from 0.05 µg/kg to 0.27 µg/kg. Because Elisa is a screening method, positive results have to be confirmed with another chromatographic method like GC-MS or LC-MS. This study case shows how important the demand for a confirmation method is. But it is also necessary that the approval method is working quite sure.

Conclusions

For this interlaboratory study it could be established that most of the applied analytical methods show uncertainty in determining CAP concentrations of 0.03 µg/kg in shrimp matrix. The cases, where either no CAP residues are determined or large deviations from the nominal value exist, confirm that the methods work near their limit of performance. At the concentration level 0.1 µg/kg large deviations from the nominal value indicate some uncertainty in the analysis of CAP too. The methods are indeed able to detect CAP residues but they are working with large range in their results. The results of the sample without spiking indicate that the possibility to get false positive results increases when methods are working near their limit of performance. Due to the very serious consequence of a positive result (prohibition, recall and destruction of the product) it is essential to confirm the value with a qualified confirmation method.

At present the results of the 0.3 µg/kg level show that the established MRPL value is the convenient level, where less analytical problems appear. So far as the toxicological point of view allows, it should be better to aim at a common temporary CAP value for prohibition of shrimp products where lower analytical problems for the most of the laboratories appear. Then a higher certainty of CAP-results and higher legal certainty for all concerned persons exists. For the future better results may be expected in quantifying lower CAP concentrations if the analytical methods for CAP residues will be validated at a common criterion like the Decision 2002/657/EC.

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3.7 DIFFERENCES IN STRUCTURAL DECOMPOSITION OF CONNECTIVE TISSUE IN COD (GADUS MORHUA) AND SPOTTED WOLFFISH (ANARHICAS LUPUS).

Ofstad, R¹, Taylor, R², Olsen RL³, Hannesson, KO¹

For fish species in general, texture of fillets changes rapidly postmortem. Loss of texture hardness has been related to changes in the connective tissue such as loss of myofibre-myofibre attachment, breaks in the connective tissue and myofibre detachment from the myocommata. The connective tissue contains a collagenous network embedded in a matrix consisting of proteoglycans and glycoproteins. The proteoglycans contribute to the structural integrity and mechanical properties of the tissue by interactions with collagen fibres and cell surface components linking the collagen fibres, and different cell types such as fibroblast, phagocytes and adipocytes.

Gapping is a phenomenon in which the connective tissue fails to hold the fish fillet together resulting in slits and tears at the myofibre-myocommata attachments and/or between myofibres. Some species like codfish are very prone to gapping while fillets of wolffish much more rarely show gapping. The purpose of this work was to compare the ultrastructure of myocommata in cod and wolffish and to study the post mortem degradation to determine which structures are related to the myofibre-myocommata detachments. The structural changes in the samples were examined by both light and transmission electron microscopy. The structural differences were quantified by the use of stereological methods.

Both the composition and the structure of the myocommata differed between these fishes. Wolffish contained more sulphated glycosaminoglycans than cod and in the wolffish the collagen network was denser and the collagen fibre diameter was smaller than that of the the cod. Myofibre-myofibre detachment occurred during 2 days of storage for both fish species and was related to disintegration of the endomysial layer. Myotomy to myocommata detachment (gaping) occurred more rapidly and to a larger extend in cod than in wolffish and were mainly related to breaks in the connective tissue. The majority of the breaks occurred in the matrix between the collagen layers and between the collagen and the cells probably due to degradation of proteoglycans and/or glycoproteins crosslinking the collagen network. In both fish species the number of these breaks increased during storage. Only a minority of the breaks occurred intracellular between the myofibrils and the sarcolemma or extracellular between the sarcolemma and the matrix. These results imply that the gapping is caused mainly by degradation of structures maintaining the spatial arrangement of the collagen network in the myocommata and that the stability of these structures is species specific.

Authors

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3. Ragnar L. Olsen, Norwegian College of Fishery science, University of Tromsoe
4. Kirsten O. Hannesson, Matforsk, Norway
3.8 COMPOSITIONAL CHARACTERISTICS OF CLAM (RUDITAPES DECUSTATUS., L.) AND WARTY VENUS (VENUS VERRUCOSA., L.)

Sukran Cakli, Asli Cadun, Tolga Dincer, Emre Caglak, Latif Taskaya

Introduction

Total aquaculture production in the world is 37 851 356 million tons, of which 370 631 mt from mussels, 4 207 818mt from oysters, 1 219 127mt from scallops and pectens and 3 109 024mt from clams. Capture fisheries production is 92 356 034mt of which 257 015mt from oyster and scallops, 702 525mt from pectens, 808 945mt from clams (FAO, 2001).

Total fisheries production in Turkey is 624 847 ton of which 5000 ton from mussels, 10 000ton from clams, 70 ton from oysters and also 2-ton mussels from aquaculture production (ANON, 2002). Bivalve export began in the year of 1970 with clam in Turkey.

In Turkey, bivalve mollusks are not well known and they are not consumed prevalent as food. Coastal cities consume especially mussel Mytillus galloprovincialis. Mussels are consumed as fried or dolma (filled with rice). The most important species exported to foreign countries from Turkey are Mytilus galloprovincialis, Tapes decussates, Venus verrucosa, Ostrea edulis, Venus gallina and Arca sp. There are very few studies about bivalve mollusks in Turkey. Studies on bivalve mollusks in Turkey are usually about their population and aqua culture.

Clam and warty venus have been consumed in many countries for hundreds of years and they are commercially valuable species in the world. These species are particularly appreciated in Mediterranean countries like Spain, Portuguese, France, Italy and Greece.

The aim of the study was to determine proximate composition (moisture, crude fat, crude protein, fatty acids), biometric and colour measurements of clam and warty venus during 8 months.

Material and method

Material

Ruditapes decussates and Venus verrucosa collected by divers from Aegean region (Ayvalık) in Turkey were used as materials. The samples were collected at 10m dept on a sandy bottom, from July (2003) to January (2004) with monthly frequency. Production area coordinates of clams and warty venus were given in Table 1.

Table 1. Production area coordinates of clams and warty venus

<table>
<thead>
<tr>
<th>Name of the production area</th>
<th>Production area</th>
<th>Coordinates of the production area</th>
<th>Coordinates of sample taken</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayvalık, Turkey</td>
<td>No 19</td>
<td>I.39°19’00”N</td>
<td>I.26°38’08”E</td>
<td>Clam</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II.39°18’50”N</td>
<td>II.26°38’12”E</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>III.39°19’14”N</td>
<td>III.26°38’37”E</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV.39°19’18”N</td>
<td>IV.26°38’30”E</td>
<td></td>
</tr>
<tr>
<td>Ayvalık, Turkey</td>
<td>No 65</td>
<td>I.39°19’25”N</td>
<td>I.26°39’45”E</td>
<td>Warty venus</td>
</tr>
<tr>
<td>Tavuk Island area</td>
<td></td>
<td>II.39°19’25”N</td>
<td>II.26°39’10”E</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>III.39°19’50”N</td>
<td>III.26°39’10”E</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV.39°19’55”N</td>
<td>IV.26°39’55”E</td>
<td></td>
</tr>
</tbody>
</table>
Methods

Biometrical measurements

Length, width, total weight and flesh weight of clams and warty venus were determined during 8 months. And also condition factors were determined according to formula: \( (CF) = \frac{W}{L^3} \times 100 \)

Proximate Analysis

Moisture (Ludorff and Meyer, 1973), crude fat (Bligh and Dyer, 1959), crude protein (AOAC, 1984) and fatty acid were performed as proximate composition analysis of clams and warty venus were determined during 8 months.

Analysis of fatty acid compositions

Total lipid(TL) was extracted and purified according to Bligh and Dyer (1959), and TL content was determined gravimetrically. The lipids were saponified and esterified for fatty acid analysis by the method of IUPAC II D19. Separation of fatty acid methyl esters was achieved on a SP-2330 Fused Silica Capillary Column (30 m x 0.25 mm i.d.,0.20µm). The oven temperature was 120 °C for 5 min, programmed to 180 °C at 10°C/min, then programmed to 220 °C at 20°C/ min and then held there for 20 min. The injector and detector temperatures were maintained at 240 and 250°C, respectively. The carrier gas a high purity helium with a linear flow rate 0.5 ml/min and split ratio of 1/150. Fatty acid methyl esters were identified using marine lipid methyl esters as standards( Sigma : 189-19 lipid standard).

Colour measurement

The colour measurement on calm and warty venus samples trials were carried out with the spectral colour meter Spectro- pen ® (Dr. Lange, Dusseldorf, Germany). The colour was measured on homogenates prepared from clams and wart venus separately. The homogenate was placed in plastic petri dishes and the colour measurement was repeated ten times. In the CIE Lab system L denotes lightness on a 0 to 100 scale from black to white; a, (+) red or (-) green; and b, (+) yellow or (-) blue (Schubring, 2002).

Statistical analysis

The results were statistically evaluated by SPSS 9.05(Kruskall wallis).

Results and discussion

Biometrical measurements were given in Table 2.

Table 2. Biometrical measurements* of warty venus and clam during 8 months

<table>
<thead>
<tr>
<th>Period (month)</th>
<th>Species</th>
<th>Length</th>
<th>width</th>
<th>Total weight</th>
<th>Flesh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.month</td>
<td>Venus</td>
<td>48.58±2.46</td>
<td>43.21±2.32</td>
<td>45.36±7.24</td>
<td>9.88±1.54</td>
</tr>
<tr>
<td></td>
<td>Clam</td>
<td>41.60±4.13</td>
<td>30.35±3.56</td>
<td>13.77±3.22</td>
<td>4.62±1.54</td>
</tr>
<tr>
<td>2.month</td>
<td>Venus</td>
<td>39.24±3.7</td>
<td>34.9±4.21</td>
<td>21.8±8.40</td>
<td>4.91±1.86</td>
</tr>
<tr>
<td></td>
<td>Clam</td>
<td>35.93±4.1</td>
<td>26.28±2.81</td>
<td>9.47±3.68</td>
<td>3.06±1.35</td>
</tr>
<tr>
<td>3.month</td>
<td>Venus</td>
<td>49.51±2.64</td>
<td>43.33±23.79</td>
<td>44.55±11.15</td>
<td>11.03±1.40</td>
</tr>
<tr>
<td></td>
<td>Clam</td>
<td>44.33±3.47</td>
<td>31.05±2.20</td>
<td>14.39±4.03</td>
<td>6.48±1.92</td>
</tr>
<tr>
<td>4.month</td>
<td>Venus</td>
<td>49.29±3.40</td>
<td>41.67±4.50</td>
<td>46.10±11.31</td>
<td>9.57±2.97</td>
</tr>
<tr>
<td></td>
<td>Clam</td>
<td>36.43±0.94</td>
<td>25.98±0.87</td>
<td>9.21±0.81</td>
<td>3.52±0.32</td>
</tr>
<tr>
<td>5.month</td>
<td>Venus</td>
<td>48.04±3.33</td>
<td>41.61±3.54</td>
<td>43.23±10.5</td>
<td>8.89±2.46</td>
</tr>
<tr>
<td></td>
<td>Clam</td>
<td>36.05±1.22</td>
<td>26.33±0.75</td>
<td>8.97±1.30</td>
<td>3.14±0.55</td>
</tr>
<tr>
<td>6.month</td>
<td>Venus</td>
<td>49.88±1.48</td>
<td>42.10±1.85</td>
<td>43.60±3.61</td>
<td>10.35±1.39</td>
</tr>
<tr>
<td></td>
<td>Clam</td>
<td>35.10±2.21</td>
<td>30.22±1.36</td>
<td>7.65±1.85</td>
<td>3.04±1.05</td>
</tr>
<tr>
<td>7.month</td>
<td>Venus</td>
<td>40.52±4.38</td>
<td>35.21±3.42</td>
<td>37.10±3.20</td>
<td>6.54±3.10</td>
</tr>
<tr>
<td></td>
<td>Clam</td>
<td>33.08±2.30</td>
<td>24.62±1.30</td>
<td>7.22±1.85</td>
<td>3.10±1.20</td>
</tr>
<tr>
<td>8.month</td>
<td>Venus</td>
<td>38.66±5.48</td>
<td>33.62±5.29</td>
<td>20.27±9.04</td>
<td>3.89±2.02</td>
</tr>
<tr>
<td></td>
<td>Clam</td>
<td>31.20±3.78</td>
<td>22.87±5.42</td>
<td>6.54±2.58</td>
<td>2.48±1.28</td>
</tr>
</tbody>
</table>

*n:30 (Arithmetical mean ± standard deviation)
Colour measurements were given in Table 3.

Table 3. Colour measurements* of warty venus and clam during 8 months

<table>
<thead>
<tr>
<th></th>
<th>Warty venus</th>
<th></th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>Clam</th>
<th></th>
<th>L</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st month</td>
<td>34.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd month</td>
<td>35.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd month</td>
<td>36.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th month</td>
<td>36.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5th month</td>
<td>40.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6th month</td>
<td>40.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th month</td>
<td>42.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8th month</td>
<td>42.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*<i>n:10</i> (Arithmetical mean ± standard deviation), different superscripts between columns characterize significant differences (p<0.05)

Proximate compositions were given in Table 4 and 5.

Table 4. Proximate composition* of clam during 8 months

<table>
<thead>
<tr>
<th>Months</th>
<th>Moisture(%)</th>
<th>Fat(%)</th>
<th>Protein(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>86.01±1.28&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.88±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.66±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>August</td>
<td>81.81±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.10±0.35&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>September</td>
<td>86.01±1.28&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.88±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.66±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>October</td>
<td>83.99±1.47&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.90±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.61±0.27&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>November</td>
<td>88.16±1.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.49±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.02±0.22&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>December</td>
<td>84.55±0.71&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.60±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.28±0.48&lt;sup&gt;ce&lt;/sup&gt;</td>
</tr>
<tr>
<td>January</td>
<td>86.72±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.12±0.35&lt;sup&gt;be&lt;/sup&gt;</td>
</tr>
<tr>
<td>February</td>
<td>85.15±0.08&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>0.76±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.10±0.26&lt;sup&gt;be&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*<i>n:3</i> (Arithmetical mean ± standard deviation), different superscripts between columns characterize significant differences (p<0.05)

Table 5. Proximate composition of warty venus during 8 months

<table>
<thead>
<tr>
<th>Months</th>
<th>Moisture(%)</th>
<th>Fat(%)</th>
<th>Protein(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>85.91±1.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.78±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.71±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>August</td>
<td>88.18±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.74±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.26±1.25&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>September</td>
<td>85.81±1.43&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.68±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.71±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>October</td>
<td>87.10±2.68&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.61±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.26±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>November</td>
<td>87.88±1.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.15±0.13&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>December</td>
<td>83.82±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.26±0.05&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>January</td>
<td>87.41±0.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.78±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.38±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>February</td>
<td>85.42±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.52±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*<i>n:3</i> (Arithmetical mean ± standard deviation), different superscripts between columns characterize significant differences (p<0.05)
Conclusion

Protein content of warty venus was higher than the protein value in clam (p<0,05) and no significant differences in fat and moisture contents between clam and warty venus were determined (p>0,05 ). No significant differences in fat rates according to months were determined (p>0, 05). However, significant differences in protein and moisture rates and colour measurements were determined according to months (p<0,05).In the flesh of clam and warty venus with different rates, C14 : 0, C15 : 0, C16 : 0, C17 : 0, C18 : 0, C23 : 0, C24 : 0 were determined as saturated fatty acid. C16 :1, C18 :1 n-9, C20 :1 n-9, C22 :1 and C24 :1 n-9 were determined as mono unsaturated fatty acids. C18 : 2, C19 : 3 n-3, C22 : 2 were determined as polyunsaturated fatty acids and C20 : 5 n-3 and C22: 6n-3 were determined as HUFA fatty acids.

References


Sukran CAKLI, Aslı CADUN, Tolga DINCER, Emre CAGLAK, Latif TASKAYA cakli@mail.ege.edu.tr, cadun@mail.ege.edu.tr Ege University Faculty of Fisheries Department of processing Technology, 35100 Bornova-Izmir/TÜRKİYE
3.9 DESALTED COD PRODUCTS PRESERVATION: EFFECT OF DIFFERENT MICROBIAL LOADS IN RAW MATERIALS

Sónia Pedro, Carla Pestana, Irineu Batista and Maria Leonor Nunes

Introduction

In Portugal dried salted cod is a very popular food, which is generally consumed cooked after being desalted for more than 24 hours. The increasing consumer demands towards easy or ready to use products have led to the introduction in the market of desalted cod products. However, some presentations have had limited success, due to the short shelf life, safety problems and variability in the product’s sensory quality (Pedro et al., 2002). Moreover, commercial heavy salted cod products are not uniform, in particular with reference to microbial content. Thus, there is a wide range of potential spoilage microorganisms among different samples. Such fact, leads to unexpected difficulties on the preservation of desalted products, being necessary the combination of preservation treatments in order to handle the different spoilage flora that may be present in the raw material. Furthermore, due to this variation, the preservation methods need to be tested on raw materials presenting different microbiological contamination levels. The objective of this work was to evaluate the efficacy of the addition of citric acid and potassium sorbate to the desalting water on the preservation of desalted cod containing different microbial loads.

Materials and methods

Raw material and treatment

Portions of dried salted cod (Gadus morhua) from the same batch with 1 cm wide were submitted to several treatments and storage conditions (Table 1). With the aim of having different bacterial loads in the material an inoculum was added to some samples during desalting in order to obtain a higher bacterial content.

Table 1. Raw material treatments and storage conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage at 4 ºC</th>
<th>Samples code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desalting in a solution with 0.13 % citric acid and 0.13 % potassium sorbate, at 4 ºC for 48 h. Ratio fish:solution 1:9</td>
<td>Vacuum packed</td>
<td>V+P</td>
</tr>
<tr>
<td>Desalting in tap water at 4 ºC for 48 h. Ratio fish:solution 1:9</td>
<td>Vacuum packed</td>
<td>V</td>
</tr>
<tr>
<td>Desalting in a solution with 0.13 % citric acid and 0.13 % potassium sorbate with inoculum(*) added, at 4 ºC for 48 h. Ratio fish:solution 1:9.</td>
<td>Vacuum packed</td>
<td>Vi+Pi</td>
</tr>
<tr>
<td>Desalting in tap water with inoculum(*) added, at 4 ºC for 48 h. Ratio fish:solution 1:9</td>
<td>Vacuum packed</td>
<td>Vi</td>
</tr>
</tbody>
</table>

(*) Inoculum prepared from desalted cod, aerobically stored at 2-4 ºC for 7 days, homogenised in physiological saline solution (1 part fish and 9 parts solution). Inoculum/dried salted cod ratio - 15 ml/100 g.

Analytical methods

Physical (pH, weight), chemical (moisture, NaCl, TVB-N, citric acid and potassium sorbate), sensory and microbiological analyses (halotolerant viable count) were performed weekly, during 28 days of storage at 4 ºC either in vacuum or air packages.

The pH was measured in 1:10 fish/water suspensions using the pH Meter Methrom 691. Moisture content was measured by determination of water loss after drying in an oven. The determination of TVB-N (total volatile basic nitrogen) was done by distillation (IPQ, 1988). For the citric acid determination the kit from Boehringer, Cat. no 139076 was used. The quantification of potassium sorbate was done according to AOAC (1997).
During the trial, 5 judges performed sensory analyses and the Quality Index Method was used to score raw samples. The smell and appearance was the sole attribute considered in the sensory evaluation of cod samples. It was also asked to the panellists the preferred product and if they reject the product and the reason for that rejection. The product was considered unacceptable when more than 50 % of the panellists rejected the sample. The microbiological analyses were performed by the spread plate method in PCA added with 3 % NaCl for quantification of halotolerant viable counts.

Results and discussion

The moisture content of desalted cod samples ranged between 74.3 % and 76.1 %. The citric acid and potassium sorbate levels detected in treated samples were approximately 0.07 % and 0.11 %, respectively. A considerable increase in the TVB-N level after the 21st day of storage was recorded in air packed samples (A) (Fig. 1). On the other hand, the TVB-N values of samples V and V+P samples remained constant at a low level during all the storage period. These results put into evidence the importance of oxygen in the bacterial growth as well as the inhibitory effect of the potassium sorbate/citric acid mixture on the bacteria responsible for the production of volatile bases. A regular increase of TVB-N values occurred in inoculated samples kept in air packages (Ai) (Fig. 2) whereas only a slight increase was recorded in Vi samples. In Vi+Pi samples no changes of this index were noticed during the storage period. These results suggest that the mixture of preservatives used was very effective.

The evolution of pH (Fig. 3) in non-inoculated samples was similar to that recorded for TVB-N in the case of air packed sample (A). The increase of pH noticed only after the 21st day of storage was possibly a result of the high concentration of TVB-N. The low pH value of the V+P sample is due to the presence of citric acid. The evolution of pH (Fig. 4) in the inoculated samples was also similar to that recorded for TVB-N index in these samples.
In what concerns sensory analysis V and V+P samples were preferred by more than 40% of the panellists after 28 days of storage whereas all the panellists rejected A sample after this storage period. In the case of inoculated samples, the smell of Ai and Vi samples was very disagreeable after 7 days of storage and were rejected by all panellists. However, Vi+Pi samples were considered sensory acceptable after the storage period of 28 days.

In non-inoculated samples, the halotolerant counts of V+P-samples were quite low and always close to the detection levels. Those of V samples were kept below the acceptance level of $10^5-10^6$ cfu/g until the twelfth day, whereas in A samples this level was attained on day 7 (Fig. 5).

The treatment of inoculated samples (Fig. 6) with preservatives together with vacuum packaging (Vi+Pi samples) was able to keep the microbial growth close to the acceptance level of $10^5-10^6$ cfu/g until the end of the trial, in spite of the high initial microbial load.

Conclusions

The results achieved in this work indicate that desalting cod in a potassium sorbate/citric acid solution (0.13 % w/v) followed by chilled storage in vacuum packaging was very effective even for highly contaminated samples.

The shelf life of non-inoculated samples treated with the preservatives followed by vacuum packaging and chilled storage was estimated in 28 days. For inoculated samples submitted to the same preservative treatment and storage conditions the shelf life was of approximately 15 days, which can be considered quite reasonable.

Acknowledgements

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References


Authors

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3.10 CHANGES IN LIPIDS AND PROTEINS DURING STORAGE OF MINCED MACKEREL (*SCOMBER SCOMBRUS*) AT –2°C AND –10°C TEMPERATURE

Revilija Mozuraityte, Ivar Storrø and Turid Rustad

**Introduction**

Lipid oxidation is an important factor for quality loss in refrigerated and frozen storage of fish. Fish lipids are highly susceptible to oxidation because they contain high levels of polyunsaturated fatty acids. The rate of lipid oxidation can be influenced by several factors: processing operations, storage temperature, oxygen availability, content of antioxidant etc (Flick et al., 1992, Frankel, 1998a and Erickson, 1998). Temperature is the most important factor for the oxidative stability of unsaturated fats. The activation energy of lipid oxidation is higher in the presence of antioxidants than in their absence, because antioxidants lower the rate of oxidation by increasing the energy of activation (Frankel, 1998b). Mechanical processing causes cellular damage and oxygen incorporation, and so induces oxidation (Beltran et al., 2003, Schaich, 1980, Pastoriza et al., 1994, Erickson, 1998). Mechanical disruption of the tissue also induces membrane lipids to form smaller vesicles, and the increased surface area accelerates their degradation (Erickson, 1998).

Oxidised unsaturated lipids and oxidation products react with proteins, causing crosslinking and modification of protein functional properties (Srinivasan and Hultin, 1997). As a result of the contact of lipid hydroperoxides with protein, protein-centered radicals can be formed, which ultimately result in various types of damage: denaturation, polymerisation etc. (Gardner, 1979, Schaich, 1980). Contact between lipid and protein components is critical for radical transfer (Schaich and Karel, 1975, Schaich, 1980). During frozen storage, low temperature prevents or minimises microbial growth, but some chemical reactions, which affect product quality, still occur. Protein changes such as denaturation, formation of aggregates, decrease in solubility, could also be caused by frozen storage (Srikar and Reddy, 1991, Badii and Howell, 2001, Badii and Howell, 2002a,b). By cooling just below the freezing point, rancidity development is accelerated in fish by a complicated process involving the removal of free water by crystallisation (Fennema, 1985). Higher protein and lipid concentration as well as increase in oxidation increased the rate of the free radical transfer reaction (Funes et al., 1982).

The aim of this work was to study lipid oxidation and lipid protein interaction at –2°C and –10°C temperature, study the effect of oxidised lipids and temperature on the denaturation and the functional properties of proteins and also examine if degree of cellular disruption influence the reactions. Mackerel has a high content of highly unsaturated lipids and was chosen as a model system for the studies.

**Materials and methods**

The mackerel used in the experiment was received frozen in October 2003. 28 fish were used, the length of the fish was in the range 34 - 41 cm; and the weight between 580 -750g. Minced (M) light muscle samples were prepared with a food mincer (diameter 0,5cm) and homogenised (H) mackerel light muscle samples were prepared using a food processor, homogenised for 2 min. with knives as cutting elements. 80g of the prepared sample was placed in polyethylene bags, pressed by hand to remove air bubbles by making small blocks (9x6x2 cm) and stored at –2°C and –10°C. Minced (MA) and homogenised (HA) samples, with 200ppm BHT (butylated hydroxytoluene) antioxidant to inhibit lipid oxidation, were used as control samples.

The chemical composition of the samples was as follows: 23% fat, 57% water, protein 19% and ash 1,2%.

Moisture was determined gravimetrically after drying at 104°C for 24 hours. Ash content was estimated by charring in a crucible at 600°C until the ash had a white appearance (AOAC, 1990). The total N was determined by CHN-S/N elemental analyser 1106 (Carlo Erba Instruments S.p.A., Milan, Italy) and crude protein was estimated by multiplying total N by the factor 6.25. The extraction of total lipids from the samples was performed according to the method of Bligh and Dyer (1959).

Oxidation of lipids was determined as amount of 2-thiobarbituric acid reactive substances (TBARS) and peroxide values (PV). TBARS was determined as described by Ke and Wooyewoda (1979) using 1,1,3,3- tetraethoxypropane as standard. PV was analysed as described by International Dairy Federation (Anon., 1991) with modifications of Ueda, Hayashi, and Namiki (1986) and Undeland, Stading and Lingnert (1998).
Formation of interaction compounds was measured at 393/463 and 327/415 nm excitation/emission maxima using Perkin-Elmer 3000 fluorimeter according to Aubourg et al. (1997). Relative fluorescence value was calculated as $F_\text{rel} = F/F_\text{std}$ where: $F_\text{rel}$ – sample, $F_\text{std}$ – standard solution (quinine sulphate 1μg/ml) fluorescence at the corresponding wavelength. The fluorescence shift $\Delta F = F_{\text{393nm}}/F_{\text{327nm}}$ was determined for the aqueous and organic phase resulting from lipid extraction (Bligh and Dyer, 1959).

Proteins were extracted by a modification of the method of Anderson and Ravesi (1968) and Licciardello et al. (1982). Water soluble proteins were extracted with phosphate buffer (0.05M phosphate, pH 7.0) using Ultra Turrax. After centrifugation (20 minutes at 8000 × g, 4°C), the salt soluble protein were extracted by homogenising the precipitate for 10s in phosphate buffer with KCl (0.05M phosphate, 0.6M KCl, pH 7.0) and centrifuged as above. Amount of protein in the extracts was determined with the BioRad protein assay using bovine serum albumin as a standard (Bradford, 1976). Heat-set gels were made by placing 8 ± 0.5 g of sample in a mould (3 x 3 x 0.8 cm), covered with aluminium foil, and held at 40 ± 3°C temperature for 3 hours. The gels were stored for 18 hours at 5°C temperature. Gels strength was measured with a TA.XT2 Texture Analyser (Stable Micro Systems, UK). Force resistance was measured using a 3mm-compression test. A flat-ended cylindrical plunger with diameter 50mm was used.

Liquid leakage was measured by using filter paper press method, a modification of the method of Grau and Hamm (1953). 1g of sample was placed on a nylon material and known weight of filter paper (Schleicher & Schuell No 5891, Dassel, Germany) covered by aluminium foil. The sample was pressed with 1 kg for 5 min. Removing the nylon material with the sample, the filter paper with aluminium foil was weighed and the liquid leakage calculated.

The statistical program Guideline (CAMO ASA, Oslo, Norway) and Microsoft Excel were employed for data processing and statistical analysis. Significance level was set at 95%.

Results and discussion

The lipid oxidation rate was influenced by the storage temperature, how the fillets were processed and antioxidant addition. During storage lower PV and TBARS values were obtained in the samples stored at -10°C than at -2°C. Addition of 200ppm BHT inhibited lipid oxidation in both control samples. At both storage temperatures higher PV and TBARS values were found in the homogenised samples (H) than in the minced ones (M).

Fluorescence measurements were used to measure formation of interaction compounds between lipid and protein. The fluorescence shift of the water phase increased with storage time. Aubourg et al. (1998) obtained similar results for sardine storage with increasing storage time and temperature, fluorescent compounds became progressively more soluble in the aqueous phase. In the homogenised samples (H), a significant increase was obtained after 34 days of storage at -2°C and after 5 months at −10°C. Better contact between protein and lipid in the homogenised samples could accelerate rate of interaction compound formation.

Comparing water phase fluorescence shift after one month of storage at both storage temperatures, no significant difference was observed, but higher TBARS and PV results was however found at -2°C than at −10°C temperature. In their studies Aubourg (1999) and Aubourg et al. (1998) also found significant increase in fluorescence shift of the water phase in the latest stage of storage. Buttkus (1967) observed that decreasing the temperature below 0°C, increased the reaction rate between myosin and malonaldehyde and this could be caused by closer association of the molecules in the reaction mixture due to freezing. The explanation for the similar water phase fluorescence shift values at -2°C and –10°C could be that at −10°C part of water was frozen and removed as ice. The high rate of interaction could be due to increased concentration of reactants resulting in the formation of a similar amount of water soluble fluorescent compound formation as at -2°C.

Protein extractability decreased during storage. After one month of storage the amount of water soluble protein in the samples stored at -2°C was lower than at -10°C temperature (62 and 91% respectively). Anderson and Ravesi (1970) explained the decrease in sarcoplasmic protein extractability as the result of increased resistance of the muscle to homogenisation. Salt soluble protein extractability decreased with storage time and at -10°C it decreased faster in homogenised samples than in the minced ones. In the studies of the effect of mincing and frozen storage on functional properties of Ray muscle, Potoriza et al. (1994) found a relationship between protein extractability and particle size, the smaller the particles (higher degree of cellular disruption), the lower protein extractability was measured during frozen storage.

No significant difference was found for liquid leakage during storage.

Both changes in the gelling ability of fish proteins and increase in hardness of fish muscle during frozen storage could influence the force resistance of heat-set gels. The rate of denaturation and aggregation has been associated with gel matrix properties (Ferry, 1948). In some studies a relationship between gel forming ability and protein solubility has been observed, but the solubility of proteins is not the only factor dictating differences in gel strength. Xiong and Brekke (1989) obtained stronger gels from samples with lower protein solubility. In our studies higher gel strength were obtained from fish muscles stored at -2°C than at -10°C. Gao et al. (1999) observed that fish lost gelling ability after...
frozen storage. The loss of gel forming ability for samples stored at -10°C could be due to frozen storage. At -2°C the gel strength increased until the 34th day of storage and this could be the result of increased hardness of fish muscle during storage. Lipid-protein complexes formed by reaction with oxidised lipids could also account for the increase in toughness (Castell, 1971). Hsieh and Regenstein (1989) studied texture changes of frozen cod and perch minces and found that hardness increased with storage time, this increase was higher at higher temperature.

The control samples (HA and MA) stored at -2°C, gave higher gel strength than H and M. Higher hardness of aseptically processed low-fat beef gels with antioxidants was found in the studies by Butler and Laric (1993). In their studies, Tunhun et al (2002) and Decker et al. (1993) obtained lower gel forming ability of oxidised meat. Higher force resistance of heat set gels made from control samples could be the result of better gel forming ability and increased hardness of frozen stored fish muscles. The decrease in gel strength, observed after 40 days of storage, could be due to formation of some water soluble lipid oxidation substances. Murakawa et al. (2003) found that the thermal gelation properties of surimi were markedly reduced by incorporation of water soluble substances from oxidised cod liver oil.

Conclusions

Rate of lipid oxidation is higher when the muscle tissues are more disrupted. Amount of oxidation products, interaction time and contact area between reactive substances influence the formation of lipid and protein interaction compounds. Freezing reduces rate of lipid oxidation, but at the same time increased contact between reactive substances and proteins can influence changes in proteins.

During frozen storage, protein extractability is influenced by muscle disruption, as higher disruption reduced extractability.

The increase in the force resistance of heat set gels, made from samples, stored at –2°C, could be due to protein denaturation and toughness increase during frozen storage of the sample and interaction between oxidised lipid and protein. It is difficult to separate the effect of freeze denaturation from the effect of lipid-protein interaction.

References


AOAC, Official Methods of Analysis, Association of Analytic Chemists, Washington, DC, USA


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3.11 SOUS VIDE TECHNOLOGY FOR UNDERUTILISED FISH SPECIES

J.D. Fagan and T.R. Gormley

Introduction

Current fishing practices in Ireland, and the imposition of stringent fishing quotas in Europe and around the world, have significantly decreased the supply of commercial fish species for processing (Brennan and Gormley, 1999). This shortfall has created a demand for high quality fillets in addition to seafood products. Many underutilised fish species yield high quality fillets, which are comparable to those from commercial species. Sous vide technology has considerable potential as a method for processing value-added seafood products. The minimum recommended thermal process for sous vide products is 90 °C for 10 minutes, or its time-temperature equivalent (Sous vide advisory council, 1991). Sous vide/freezing technology has proved successful for cod and salmon portions as potential ready-meal components (Gormley et al., 2003). However, virtually no research has been conducted on the sous vide cooking of underutilised fish species. The objective of the current trials was to study the effect of sous vide cooking on selected quality parameters of seven underutilised fish species with and without 12 savory sauces.

Materials and methods

Seven underutilised fish species; orange roughy (Hoplostethus atlanticus), albacore tuna (Thunnus alalunga), cardinal fish (Epigonus telescopus), deepwater redfish (Sebastes mentella), roundnose grenadier (Coryphaenoides rupestris), blue ling (Molva dypterygia) and Greenland halibut (Reinhardtius hippoglossoides) were sourced during fishing trials by the Irish Sea Fisheries Board (BIM). The samples (on ice) were filleted by a local seafood company and delivered (on ice) to The National Food Centre within 24 h of landing. The fillets were vacuum packed, blast-frozen at –35 °C for 2.5 h, and were stored at –20 °C until required (up to 5 weeks) for sous vide processing.

Eleven oil-based and one water-based sauces were sourced from commercial companies. The sauces were: tomato+pesto, szechwan, cajun, arrabbiata, bearnaise, hollandaise, mushroom, toskana, tomato+basil, Italian, rosemary+garlic and tikka (water-based). The sauces were ready-to-use and were added to the fish at a 1:1 ratio.

Fillets were thawed overnight at 2-4 °C, portioned (circa 200 g) and vacuum packed (+/- sauce) in 200 x 250mm 15/45 antifog vacuum bags (200 x 250; 15/45). (Millerpack Ltd, Dublin, Ireland). Trial 1 (three time-temperature combinations—see Table 1) was conducted without sauce and Trials 2 (sensory acceptability of sauces—see Table 2) and 3 (sensory acceptability of selected species and sauces—see Table 3) with sauce using a 1:1 fish:sauce ratio. The samples were cooked in a Barriquand Steriflow retort and the time to achieve a process temperature equivalent to 90 °C for 10 min was determined (Ellab TM9608 temperature recording system). The cooked samples were blast frozen (-35 °C) and stored at –20 °C for 1 week before testing. A range of physico-chemical and sensory tests was conducted as described by Fagan et al. (2003) and these were used as required in the different trials. A 4-person taste panel familiar with fish tasting was used in all but one of the sensory tests (samples reheated by microwaving). The panelists were asked to score on a 6 cm line and the responses were converted to values by measuring the marked point with a ruler. In tests to determine ideal product texture the line had end points of 0 (too soft) to 6 (too firm) with 3 indicating ideal texture. In acceptability tests, the line had end-points of 0 (unacceptable) and 6 (very acceptable). In the final acceptability test 25 tasters were used. The results were tested by analysis of variance (ANOVA) using SAS (Version 6.12, SAS Institute Inc., Cary, NC, USA).

Results and discussion

Effect of cooking time/temperature and post-cook freezing/chilling (Trial 1)

The cooking times equivalent to 10 min at 90 °C (P_a>10) used in these trials in the Barriquand retort system were 40 min at 85 °C, 20 min at 90 °C and 15 min at 95 °C. Process time/temperature did not influence product texture with mean sensory texture scores of 2.76 (40 min/85 °C), 2.89 (20 min/90 °C) and 2.82 (15 min/95 °C). However, there were
large differences (P< 0.001) between species with albacore tuna having an over-firm texture, and roundnose grenadier and Greenland halibut a soft texture. Sous vide-cooked orange roughy, cardinal fish, redfish and, to a lesser extent, blue ling had texture scores in the ideal range, i.e. circa 3. The different time/temperature process treatments had no effect on cooking loss, shear values, colour, centrifugal drip and moisture of the fish portions, and so fish species data are only presented for the 20 min/90 °C (P90>10) treatment. This process was also used for trials 2 and 3. There was a wide range (P< 0.001) in centrifugal drip and in moisture content between the seven species (Table 1). Albacore tuna had the lowest centrifugal drip value (4.1 %) and Greenland halibut the highest (18.9%). Albacore tuna had by far the lowest moisture content (Table 1). Freezing versus chilling (i.e. not freezing) post sous vide cooking had no effect on cooking loss or on shear values.

Table 1. Centrifugal drip and moisture contents of sous vide cooked samples of seven fish species

<table>
<thead>
<tr>
<th>Species</th>
<th>Centrifugal drip (%)</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange roughy</td>
<td>11.6</td>
<td>70.5</td>
</tr>
<tr>
<td>Albacore tuna</td>
<td>4.1</td>
<td>63.9</td>
</tr>
<tr>
<td>Cardinal fish</td>
<td>11.5</td>
<td>78.2</td>
</tr>
<tr>
<td>Redfish</td>
<td>12.9</td>
<td>77.7</td>
</tr>
<tr>
<td>Roundnose grenadier</td>
<td>16.2</td>
<td>80.3</td>
</tr>
<tr>
<td>Blue ling</td>
<td>11.7</td>
<td>75.1</td>
</tr>
<tr>
<td>Greenland halibut</td>
<td>18.9</td>
<td>72.4</td>
</tr>
</tbody>
</table>

F-test P< 0.01 P< 0.001
LSD 5.41 4.07

* 20 min/90 °C (P90 > 10 min)

The above differences between species are a reflection of fish composition and other factors. For example, lipid and water comprise up to 80% of fish muscle (Lee, 1992) and fluctuations in water, protein and lipid content of fish flesh are influenced by spawning, age and the feeding pattern of the fish. Shrinkage of the myofibrils at 45 to 50 °C corresponds to a loss of water as they shorten and denature (Ofstad et al., 1993). This expels moisture and fat from the fish and toughening of the muscle occurs as a result. Liquid holding capacity is influenced by structural changes in the proteins, fibril swelling, contraction and the distribution of fluid between intra and extracellular locations (Fennema, 1990). Ofstad et al. (1993) suggested that liquid loss and structural changes during heating of fish products are affected by intrinsic and external factors. Liquid loss is fairly constant between 5 and 20 °C and increases rapidly, and to a maximum, when the fish is heated to 50 °C. The process (P90>10 min) used in the current trials was sufficient to inactivate C. botulinum, but not proteolytic C. botulinum spores which have a D121-value of 12 seconds and z-value of 10 °C. However, growth can be prevented by controlled chilled storage (Juenja, 1998). The Chilled Foods Association (2003) suggest that cooked products be cooled quickly through the temperature range 63 to 5 °C (or lower) to minimise risk of spore germination. Such cooling times vary from product to product, but should be no longer than 4 hours. In the current trials, product core temperature was reduced from 90 to 20 °C within 20 min and products were then blast-frozen at –35 °C for 2.5 h and reached a core temperature of –20 °C. These conditions were not conducive to the growth of C. botulinum spores. All samples were reheated from frozen (-20 °C) in a microwave oven at 850W for 6.5 min to a core temperature of > 70 °C for 2 min. This combined with the sous vide cooking was sufficient to destroy vegetative pathogens and spores of psychrotrophic Clostridium botulinum (Hatae et al., 1984).

Sensory acceptability of sauces (Trial 2)

Sauce colour lightened on sous vide cooking as indicated by a rise in L/b mean values from 1.79 to 2.03. However, this had little impact on overall product appearance with all sauce+fish products receiving good appearance scores (values ranged from 4.29 to 4.97 on a 6 cm line scale) with the exception of hollandaise (2.97) which the panel deemed to have an artificial colour. Flavour acceptability scores were also favourable and seven sauces received panel scores of 4 or above with tomato+pesto, arrabbiata, and hollandaise the best liked (Table 2). Rosemary+garlic had the lowest panel score (2.88). All the sauces were heat and freeze-thaw stable This is an essential requirement in the current application.
Table 2. Taste panel acceptability scores\textsuperscript{a} for sous vide cooked\textsuperscript{b} sauce portions\textsuperscript{c}

<table>
<thead>
<tr>
<th>Sauce type</th>
<th>Score</th>
<th>Sauce type</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tikka</td>
<td>4.37</td>
<td>Hollandaise</td>
<td>4.56</td>
</tr>
<tr>
<td>Cajun</td>
<td>4.19</td>
<td>Béarnaise</td>
<td>3.41</td>
</tr>
<tr>
<td>Toskana</td>
<td>3.98</td>
<td>Mushroom</td>
<td>4.31</td>
</tr>
<tr>
<td>Italian</td>
<td>3.98</td>
<td>Scechuan</td>
<td>4.23</td>
</tr>
<tr>
<td>Tomato + basil</td>
<td>4.00</td>
<td>Arrabbiata</td>
<td>4.64</td>
</tr>
<tr>
<td>Rosemary + garlic</td>
<td>2.88</td>
<td>Tomato + pesto</td>
<td>4.83</td>
</tr>
</tbody>
</table>

\textbf{F-test for sauce acceptability}: P< 0.001; LSD 0.74

\textsuperscript{a} 6 cm line with end-points of 0 (unacceptable) and 6 (very acceptable)
\textsuperscript{b} 20 min/90°C (P90 > 10 min)
\textsuperscript{c} Data averaged over 5 species (see Table 1)

The pH values for the 12 sauces before sous vide cooking were in the range 3.96 (cajun) to 5.42 (bearnaise) with a mean of 4.66. pH values were typically reduced by about 0.2 to 0.3 pH units after sous vide cooking and the overall mean was 4.38. The acidic nature of the sauces is beneficial and acts as a microbial hurdle in the fish/sauce packs. The addition of sauces may soften fish texture during sous vide cooking due to their acidity and the presence of sauce water in the pack. This was the case in the current study and taste panel scores for the samples with sauce were lower (indicating softer texture) than those sous vide cooked without sauce with the exception of blue ling, which retained its texture.

Sensory acceptability of selected fish species and sauces (Trial 3)

Three fish species (albacore tuna, cardinal fish, and blue ling) sous vide cooked in four sauces (tikka, hollandaise, arrabbiata and tomato+pesto) were selected for further sensory tests (25 tasters). All three species received good acceptability scores (Table 3) while tikka and tomato+pesto were the preferred sauces. All samples received scores well above the mid-point of the 6 cm scale and all mean values were above 4. There was no statistically significant interaction between fish species and sauce type.

Table 3. Taste panel acceptability scores\textsuperscript{a} for sous vide cooked\textsuperscript{b} fish with sauces

<table>
<thead>
<tr>
<th>Species</th>
<th>Sauce type</th>
<th>Score</th>
<th>Sauce type</th>
<th>Score</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tikka</td>
<td>4.20</td>
<td>Hollandaise</td>
<td>4.20</td>
<td>4.31</td>
</tr>
<tr>
<td>Albacore tuna</td>
<td></td>
<td></td>
<td>Arrabbiata</td>
<td>4.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.73</td>
<td></td>
<td>3.77</td>
<td>4.94</td>
</tr>
<tr>
<td>Cardinal fish</td>
<td></td>
<td>4.73</td>
<td></td>
<td>3.77</td>
<td>4.94</td>
</tr>
<tr>
<td>Blue ling</td>
<td></td>
<td>4.73</td>
<td></td>
<td>3.77</td>
<td>4.94</td>
</tr>
<tr>
<td>Mean</td>
<td>4.47</td>
<td>3.98</td>
<td>4.07</td>
<td>4.64</td>
<td></td>
</tr>
</tbody>
</table>

\textbf{F-test for acceptability}: Species (NS; LSD = 0.31)
Sauces (P< 0.001; LSD = 0.36)
Interaction (NS; LSD = 0.62)

\textsuperscript{a} 6 cm line with end-points of 0 (unacceptable) and 6 (very acceptable)
\textsuperscript{b} 20 min/90°C (P90 > 10 min)

Conclusions

The outcomes from these trials show that a number of underutilised fish species are suitable for sous vide cooking in a range of savoury sauces and have a high level of acceptability. Freezing post-sous vide cooking did not reduce product quality and was beneficial in terms of an extended shelf life and increased product safety. The results from these trials have been disseminated to seafood companies and scale-up tests are in progress.
Acknowledgements

Thanks are extended to Enterprise Ireland for funding this study under their Advanced Technologies Research Programme; Mr Tony Hegarty of Teagasc for the statistical analyses; the Irish Sea Fisheries Board (BIM) for procuring the fish samples and the ingredient suppliers for the sauce samples.

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http://www.chilledfood.org/fdsafepr.htm#process

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3.12 TECHNOLOGICAL IMPLICATIONS OF ADDITION OF GRAPE FIBRE TO RESTRUCTURED FISHERY PRODUCTS

Sánchez-Alonso, Isabel and Borderías, A. Javier

Introduction

The American Association of Cereal Chemists (Prosky, 2001) define dietary fibre as “an edible part of plants or analoge carbohydrates resistant to digestion and absorption in the small intestine and that ferments totally or partially in the large intestine.” Fibre recommendations for adults, following the American Dietetic Association’s indications, are 25 - 30 g/day, and the insoluble/soluble ratio should be 3:1. In Europe, consumption is around 20 g/person/day. There are very few references on the addition of fibre as an ingredient to seafood products and there are also very few products in the market that contain fibre (or at least that mention fibre on the label). On the other hand, there are many other products in the market such as dairy, meat, or bakery products, which include fibre in their formulations. From a nutritional point of view, the inclusion of fibre in seafood products appears interesting and marketable.

Dietary fibre has two fractions: soluble and insoluble. A part of its technological properties will be determined by the percentage of these fractions. Fibres that are most commonly used in seafood products for a technological purpose are soluble and are mainly from seaweeds, such as the case of alginates and carrageen.

Most dietary fibres for food use come from cereals, but also from rice, peas, bamboo and fruit. Fruit fibres have an interesting equilibrium between soluble and insoluble fractions. Some of these fruit fibres have antioxidant properties; such is the case of grape fibre. This antioxidant characteristic has a twofold effect: it prevents product rancidity, which is important for fish muscle containing a high proportion of unsaturated lipids and also makes the product nutraceutical for the consumer. Fibre can be added to fish by injecting it into the whole muscle or directly adding it into restructured products made from surimi, minced fish, or small pieces of fish.

Dietary fibre from red grape (Saura and Larraury, 1997) has been used in this work. The effect of different proportions of this fibre on the technological functional characteristics of frozen minced muscle of horse mackerel (Trachurus trachurus) was checked over six months of storage.

Material and methods

The restructured products were made from minced muscle of horse mackerel (Trachurus trachurus). Red grape fibre (20.8% soluble fibre and 51.04% insoluble fibre) was extracted following the method used by Saura F. and Larrauri J.A. (1997). Red grape fibre has antioxidant properties and was added in two different proportions (2% and 4%). Samples were frozen, stored for 6 months at -20ºC and were analysed monthly.

Samples were prepared by obtaining minced muscle from an average-size fresh horse mackerel in a debonair machine and mixing it with 2 and 4 % of fibre, and adjusting the amount of water so that all the samples had the same moisture levels. Aluminium foil containers of 21.5 x 15 x 3.5 cm were filled with samples and then frozen in a plate freezer at -40 ºC until the thermic core reached -20ºC. The samples were next cut into 1.5 cm slices with a saw and placed in plastic bags sealed at atmospheric pressure. All the samples were stored at -20 ºC.

All the analyses were performed monthly in triplicate. The analyses were:

- Proximate analyses: Moisture and ashes (AOAC, 1995). Protein content was measured by a Nitrogen determinator LECO FP_2000 (Leco Corporation, St Joseph, MI).
- Protein solubility (Ironside and Love, 1985).
- Water Binding Capacity: The frozen sample cut in small pieces was placed in a centrifuge tube along with enough filter paper. Centrifugation took place after thawing the muscle in the tube. A Jouan MR1812 centrifuge (Saint Nazaire, France) was used: 5000 rpm for 10 min at room temperature. Water Binding Capacity (WBC) was expressed as percent water retained per 100 g water present in the muscle prior to centrifuging.
- Water Holding Capacity: Paralellepipedic 7 x 3 x 1,5 cm frozen pieces of sample were cut from mince blocks and placed in a plastic bag in which small holes had been made for the drip to drain. This bag with the sample inside was put into another bag and hung with the holes at the bottom at a constant temperature of 8ºC. The samples were in these conditions overnight and the drip was measured. Then the samples were cooked in the same way in an oven (Rational Combi-Master CM6) at 100 ºC for 15 min; then the oven was set at room temperature and the drip collected was measured.
Mechanical properties:
- TPA (Bourne, 1978) using a TA-XT2 Texture Analyser (Texture Technologies Corp., Scarsdale, NY). Eight probes (diam=2cm, height=1.5cm) of cooked (microwaved) samples were axially compressed to 40% of their original height. Force-time deformation curves were derived with a 250 N load cell applied at a crosshead speed of 0.8 mm/sec.
- Kramer test: With the same texturometer adapting a Kramer cell with five blades. Crosshead speed was 2.0 mm/seg. Samples were cut with a knife in paralepipeds of 5.5 x 1.5 x 2.5 cm and cooked in a microwave. The breaking strength was measured at least in quadruplicate. Force in N was divided by g of sample (N/g).

Lipid oxidation:
- Conjugated hydroperoxides (dienes and trienes) (AOCS, 1989).
- TBA index (Vyncke, 1970)
In both instances, the percentage of inhibition of oxidation (%I) (Frankel, 1998) was used.

Statistical analyses: Tukey’s test was used to determine where the differences were in the mean values (ANOVA- one way) and the different lots throughout frozen storage (ANOVA- two way). The Statgraphics Plus 2.1 program was used for this.

Results and discussion

Protein solubility

As shown in Fig. 1, fibre does not protect protein aggregation in frozen storage. Generally, the evolution in the three samples was quite constant. Two way-variance analysis indicates that the evolution of individual samples is significantly different and the relation between soluble protein and total is higher when the amount of fibre is lower. Ponte et al. (1985, 1987) report stabilization of frozen fish muscle when some fibres such as xanthan and i-carrageenan are added, but they do not analyse protein aggregation.

Fig. 1. Protein solubility in 5 % NaCl

Water binding capacity

Strongly bonded water was directly proportional to the amount of added fibre (Fig. 2) Differences throughout frozen storage were significant (P< 0.05).
No reference was found in the bibliography on the addition of fruit fibre to a fish product. For other kind of fibres (carrageenan, xanthan, carboxilmethilcelulose), Ponte et al (1985, 1987) report the water retaining capacity of frozen minced products when they are used as ingredients.

Water holding ability

The total drip release after thawing and cooking is called the water holding ability. Differences in the lots (Fig. 2) were significant throughout frozen storage (P<0.05). Just as for the water binding capacity, the water retained was in proportion to the added fibre (we should remember that when fibre was added, water was also added to maintain the same level of moisture). Inasmuch, the addition of grape fibre is a good method for preventing the breadcrumb coating from breaking because of the excessive drip release.
Mechanical properties

**Hardness**: Two measurements of hardness were determined (Fig. 3). The Kramer test gives an idea of the hardness of the particle, and the hardness measured with the TPA method gives an idea of the hardness of the bigger pieces composed of many particles. In both instances, the hardness was inversely proportional to the amount of added fibre (fibre is added to meat products to give the sensation of a fatty texture (Nelson, 2001)). In both instances, the average measurements for each lot were significantly different (P<0.05). However, the measurements for the two lots with 2% and 4% of fibre were very similar, but quite different from the control lot.

**Springiness and cohesiveness**: Both properties diminished with the addition of fibre (Fig. 4), but they did not change throughout frozen storage. Differences in springiness between the two lots containing fibre were not significant (P<0.05), but these differences were higher than the control lot and also significant. As regards cohesiveness, the averages of different analyses throughout frozen storage were significant for the three lots, but the values were higher in the samples without fibre. The loss of cohesiveness causes in the interior of the fish portions with fibre some cracking, thus giving a poor image of the product.

**Conjugated hydroperoxides**: The samples with fibre exhibited lower values than the control lot until 90 days of frozen storage. The percentage of oxidation inhibition when the control increments were the highest (at 30 days of storage) was 26.42% for the lot with 2% of fibre and 62.34% for the lot with 4% (Table 1). From these values it would seem that grape fibre protects the initial oxidation compound formation at 30 days of frozen storage.
TBA-index: TBA values were lower in the samples with fibre for most of the storage period. The percentage of inhibition at 90 days of frozen storage (higher control values of TBA-index) was 57.28% for the samples with 2% of added fibre and 54.13% for samples with 4% of added grape fibre (Table 1). The antioxidant action is therefore the same for the two levels of fibre used.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dienes Day 30</th>
<th>Trienes Day 30</th>
<th>i-TBA Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00±8.96a</td>
<td>0.00±6.32a</td>
<td>0.00±4.49a</td>
</tr>
<tr>
<td>2% Added Fibre</td>
<td>26.42±0.08b</td>
<td>32.16±4.25b</td>
<td>57.28±9.17b</td>
</tr>
<tr>
<td>4% Added Fibre</td>
<td>62.34±1.02c</td>
<td>89.43±7.79c</td>
<td>54.13±12.16b</td>
</tr>
</tbody>
</table>

1 Different letters in the same column indicate significant differences (P<0.05)

Conclusions

The primary technological advantages of adding antioxidant fibre to minced horse mackerel muscle for frozen storage are:

- The aspect and flavour of the samples with added fibre were very similar to the control lots.
- Water retention is greater when mince with added fibre and water is subjected to a intense force, such as centrifuging and probably also chewing.
- There is less drip upon thawing and cooking when fibre and water are added to mince.
- The addition of red grape fibre considerably inhibits oxidation during the first three months of frozen storage. The reason for this can either be the chelant action of fibre on some prooxidant metals or the action of polyphenols associated with fibre (Bravo et al. 1994).

References


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3.13 EVALUATION OF THE QUALITY OF HAKE PRODUCTS DURING FROZEN STORAGE

Martins, A.; Bronze, M. R.; Batista, I.; Nunes, M. L.

Introduction

Freezing is widely used as a preservation method to prevent microbial spoilage and slow down the chemical and enzymatic reactions in fish muscle. Nevertheless, it is not completely effective because myofibrillar proteins suffer denaturation/aggregation and lipid oxidation occurs. Moreover, different species exhibit particular changes during frozen storage. Thus, taking into account the importance of frozen hake products in commercial terms and consumer’s actual demand, the objective of this work was to study the effect of storage time and temperature of storage on the quality of two hake products.

Materials and methods

Hake fillets (Merluccius capensis) and headed and gutted hake (Merluccius australis) processed and frozen on board and kept at -20 ºC were received at IPIMAR. The batches of fillets and headed and gutted hake were divided and stored respectively at -10 ºC and -20 ºC and -10 ºC, -20 ºC and -30 ºC. Regularly, at intervals of one and two months, depending on storage temperature, 5 individual headed and gutted fish or fillets were collected and analysed. The changes taking place during storage were followed by chemical: proximate composition (AOAC, 1990), free formaldehyde (Nash, 1953), dimethylamine (Dyer and Mounsey, 1945), soluble protein (Ironside and Love, 1958) and peroxide value (Shanta and Decker, 1994); physical: pH (Vyncke, 1981), texture-Kramer shear cell (Hsieh and Regenstein, 1989) and sensory methods (Kent et al., in press).

Results and discussion

Fillets

As expected, the levels of the main four constituents were not affected either by time or storage temperature, being the differences observed certainly due to the variability among individuals. pH values were almost constant, with a short range between 6.9 and 7.1. In what concerns protein solubility, a significant decreased was seen (0.08 and 0.07 % per day when storing respectively at -10 and -20 ºC), denoting an accentuated protein denaturation of this product over storage period. Both dimethylamine nitrogen (DMA) and formaldehyde (FA) showed steep slopes at -10 ºC which reflect the higher activity of OTMAase at this temperature, although post-spawned hake had been used as raw material. Rancidity, measured by peroxide value, was quite evident, since increased from about 3 up to 13 meq/kg oil (-10 ºC) and up to 18 meq/kg oil (-20 ºC). In spite of the similarity of texture measurements by the Kramer cell in raw and cooked material, fillets kept at –10ºC were considered tougher than those stored at –20 ºC by panellists, which is in accordance to the high formaldehyde level registered for fillets stored at the highest temperature. The results of the sensory analysis (total of demerit points) of raw fillets stored at the different temperatures showed noticeable changes during storage. Within the considered parameters for sensory evaluation general appearance seemed to be the attribute that better translates the main changes perceived by the panellists. The evolution of sensory evaluation indicated a more evident loss of quality of the product stored at -10 ºC.

In order to have a global idea of quality evolution, trends for protein solubility, formaldehyde, peroxide value and sensory analysis were considered, and the following scheme accordingly proposed.

<table>
<thead>
<tr>
<th>Quality of hake fillets (Merluccius capensis) during frozen storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>-20ºC</td>
</tr>
<tr>
<td>Good</td>
</tr>
<tr>
<td>Acceptable</td>
</tr>
<tr>
<td>151 days</td>
</tr>
</tbody>
</table>
Headed and gutted hake

The moisture content of all samples was almost constant over the three storage temperatures. Small differences in the fat content were found, but they were ascribed to differences between specimens. pH values ranged between 7.2 – 7.4. Like in fillets, the most evident loss of protein solubility was verified at -10 ºC and between -20 ºC and -30 ºC the differences were minor. DMA and FA were produced during storage at the three temperatures; however the production rate was quite influenced by the storage temperature, since at -30 ºC the slopes were smaller. Thus, the formation rates at -10, -20 and -30 ºC were, respectively 60, 6 and 13 µg N/100g per day and 60, 20 and 6 µg/100g per day, for DMA and FA. The storage temperature did not have a marked influence on the development of rancidity as it had already been observed with the fillets. Texture values both in raw and cooked hake were significantly reduced during the storage period; however such changes were not completely perceptive for panellists. On the other hand, panellists noticed some loss of quality both in raw and cooked samples over the frozen storage, and samples at -10 ºC were assigned as showing a faster degradation. However, the total of demerit points (11) was not attained in any case. The global analysis of some results, mainly sensory analysis in raw and cooked, formaldehyde, dimethylamine, peroxide value and protein solubility enables drawing the following scheme.

![Quality of headed and gutted hake (Merluccius australis) during frozen storage](image)

As a conclusion it can be said that these products showed different behaviour over frozen storage. Moreover, protein solubility, dimethylamine, formaldehyde and sensory values, when appreciated jointly, constitute a good tool to assess changes and discriminate quality levels.

Acknowledgements

This study has been carried out with financial support from the Commission of the European Communities, Fifth Framework Programme, specific RTD programme Quality of Life and Management of Living Resources, project QLK1-2001-01643, ‘A New Method For Measurement Of The Quality Of Seafood’. It does not necessarily reflect the Commission’s views and in no way anticipates its future policy in this area.

References

Authors
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3.14 EFFECT OF MODIFIED ATMOSPHERE ON THE SHELF LIFE OF COMMON OCTOPUS (OCTOPUS VULGARIS)

Amparo Gonçalves and Maria Leonor Nunes

Introduction

Common octopus (Octopus vulgaris) is a species with high commercial value and much appreciated by Portuguese consumers. The shelf life (based on sensory criteria) reported for this species is 6-8 days, at a temperature range of 0-2.5°C (Hurtado et al., 1999; Barbosa and Vaz-Pires, 2004; Vaz-Pires and Barbosa, 2004). Since modified atmosphere packaging (MAP) could extend the shelf life and improve safety during the distribution chain and considering the actual consumer demand for fresh convenient products, the purpose of the present study was assess the effectiveness of MAP on the extension of the shelf life of whole, gutted common octopus.

Materials and methods

Raw material and experiments

Common octopus was from artisanal fishing and was purchased in the auction market of Matosinhos (North of Portugal). Octopus were kept in ice and transported to laboratory within 16 h. At the laboratory octopus were gutted, washed and allow to drainage the water. Then, one octopus was packed in a polystyrene tray (25.5x15.6x6 cm), which was placed inside a polyamide/polyethylene gas barrier bag (85µm of thickness; transmission rates of 5.5 for O₂; 13.4 for CO₂ and 1.9 for N₂, cc/m²/24h, at 75% RH and 23°C - Vaessen-Schoemaker, Portugal). Three batches were constituted: batch A - packed in 40%CO₂/32%O₂/28%N₂; batch B – packed in 64%CO₂/33%O₂/3%N₂ and batch C – packed only in air (control). All packages were sealed by a Multivac A 300/52 machine (Multivac Sepp Haggenmüller KG, Wolfertschwenden, Germany) and stored at 1.0±0.9°C.

Methods

At each sampling day three packages from each batch were taken for sensory, microbial and chemical analysis. The gas composition inside the packages was measured with a gas analyser ABISSPRINT (Abiss, Chatillon, France). From each octopus only tentacles were used for analyses. For total microbial counts 25 g of muscle with skin was homogenized, diluted nine fold in tryptone salt solution (0.85%) and poured onto Plate Count Agar (Merck, Darmstadt, Germany). The plates were incubated at 30°C for 72 h, under aerobic and anaerobic conditions. The pH was measured directly on octopus mince, using a surface electrode Metrohm 744 pH meter. Total volatile basic nitrogen (TVB-N) was determined in extracts, from 25 g of octopus mince homogenised with 50 ml 10% trichloracetic acid, by a microdiffusion method, according to Cobb et al. (1973). Sensory evaluation was done by five panellists, in raw and after steam cooking (anterior parts of tentacles, during 60 min. at 100°C). Assessment of organoleptic properties (appearance, odour, flesh texture and taste) was done by a descriptive technique, using a category scale from 0 to 10 points (Meilgaard et al., 1999). An overall classification was defined based on the average of odour, colour (pigmentation/brightness) and taste scores: high quality (8 - 10 points); acceptable quality (4 – 7 points) and unacceptable (0 - 3 points).

Results and discussion

The main changes in the packages gas composition occurred within the first two days of storage, when CO₂ decreased to a concentration of 20% and 31%, respectively in batches A and B, mainly due to the CO₂ dissolution into the product. In regard to O₂, the levels were nearly 23%, 25% and 16% respectively for batch A, B and C over the storage period.

Octopus packed in air (control) became sensory unacceptable after 13 days of storage, at pH value of 6.54 and 62 mg/100g of TVB-N (table 1). Both MAP batches were considered unacceptable only at the 16th day and pH values of 6.67, 6.50 and 42, 51 mg/100g TVB-N were reported, respectively for batches A (packed in 40%CO₂/32%O₂/28%N₂) and B (packed under 64%CO₂/33%O₂/3%N₂). Sensory rejection was mainly due to raw odour (acid, intense) and bitter taste, which was more pronounced in the octopus packed in gas mixtures. Ruiz-Capillas et al. (2002) reported similar results for other octopus species stored under controlled atmosphere.
Table 1. Changes in pH value, total volatile basic nitrogen (TVB-N) and sensory quality during the storage of packed common octopus at 1.0 ±0.9°C

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Batch</th>
<th>Days of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>pH value</td>
<td>A</td>
<td>6.50±0.08</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6.44±0.28</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>6.06±0.03</td>
</tr>
<tr>
<td>TVB-N (mg/100g)</td>
<td>A</td>
<td>61±9</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>41±22</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>9±4</td>
</tr>
<tr>
<td>Sensory quality</td>
<td>A</td>
<td>Acceptable</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Acceptable</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>High</td>
</tr>
</tbody>
</table>

1 A - Packed in 40%CO2/32%O2/28%N2; B - Packed in 64%CO2/33%O2/3%N2; C - Packed in air (control). Physicochemical values are the mean±standard deviations (n=3).

Microbial counts increased from 1.5x10⁴ cfu/g (10³ under anaerobic conditions) to 10⁵ cfu/g (10⁴ under anaerobic conditions) at the rejection point in the three batches. These results are in accordance with Vaz-Pires and Barbosa (2004) who reported 10⁵-10⁶ cfu/cm² at sensory rejection of iced common octopus (after 8 days). Other authors found low microbial counts during storage of cephalopod species and stated that deterioration is mainly autolytic for a longer period (Ohashi et al., 1991; Hurtado et al. 1999).

Conclusion

Despite some results variability, conventional packaging (in air) was effective in the preservation of whole, gutted common octopus quality, contributing to the extension of shelf life (defined based on sensory criteria) to 13 days, compared with ice storage (6-8 days). The inclusion of gas mixtures had a positive effect, in particular the mixture containing 64%CO2/33%O2/3%N2, which provides the lowest physicochemical results. Therefore, modified atmosphere packaging could increase the shelf life of octopus to 16 days, but more studies are needed to demonstrate its effectiveness.

Acknowledgements

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3.15 EFFECT OF DIFFERENT PREVIOUS ICING CONDITIONS ON SENSORY, PHYSICAL AND CHEMICAL QUALITY OF CANNED HORSE MACKEREL (TRACHURUS TRACHURUS)

Vanesa Losada, Ines Lehmann, Reinhard Schubring, Santiago P. Aubourg

Introduction

The fish industry is suffering from dwindling stocks of traditional species as a result of drastic changes in their availability, so that fish technologists and fish traders have turned their attention to some unconventional sources of raw material. One of such species is horse mackerel (Trachurus trachurus), a medium-fat fish abundant in the Northeast Atlantic.

Efforts have been done to utilize it in the manufacture of several fish products such as smoked, canned, chilled, frozen or restructured.

During processing and storage, fish quality may decline as a result of several factors. The most employed on board pre-canning method has shown to be chilling. During chilled storage of fish, important changes are known to take place concerning the lipid fraction, so that significant losses of the sensory and nutritional values have been detected.

The present work aims to study the effect of a previous chilled storage on the quality of canned horse mackerel.

Slurry ice has recently been reported as a promising chilling technique for the preservation of aquatic food products as a result of several advantages compared with flake ice such as lower temperature, faster chilling, lower physical damage to product and better exchange power. Fish traders have widely employed chilled storage as a previous step to the technological treatments. The effect of previous chilling conditions (storage time, fish-ice ratio, room storage temperature) on the quality of frozen and canned fish has been demonstrated. In the present work, traditional flake ice and slurry ice conditions were applied to horse mackerel prior to canning. Qualities of the resulting canned products were compared by means of sensory, physical and chemical determinations.

Materials and methods

Fresh horse mackerel (Trachurus trachurus) were obtained from Southeast Atlantic. Upon arrival in the laboratory, horse mackerel was kept chilled under flake ice and slurry ice in an isothermal room at 2°C.

The composition of the slurry ice binary mixture was 40 % ice and 60 % water, prepared from filtered seawater. Samples were taken for analysis on the starting day and at days 5, 8 and 12 of both chilling conditions. Then, the fish was cooked placed in cans and sterilised. After three months of storage in cans under room temperature, the cans were opened and the fish muscle was examined for sensory (odour, taste and colour), physical (colour) and chemical (volatile amines and lipid hydrolysis and oxidation) analyses.

Chemical measurements

Free fatty acids (FFA) content was determined by the Lowry and Tinsley (1976) method based on complex formation with cupric acetate-pyridine. Results are expressed as g FFA/100 g lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). Results are expressed as mg malondialdehyde/kg fish sample.

Formation of fluorescent compounds was determined with a Perkin Elmer LS 3B fluorimeter by measurements at 393/463 nm and 327/415 nm (Aubourg et al., 1997).

Browning development was measured from the lipid extract at 450 nm and 400 nm. The 450/400 absorbance ratio was studied according to Hassan et al. (1999).

Physical investigations

Colour measurements were taken using a MINOLTA chroma meter CR 300 on the muscle tissue of horse mackerel after removing excess moisture by allowing the fish to drain for 2 min and the muscle tissue has been homogenated (Krups3Mix8008, 1 min) according to Schubring and Meyer (2002). Using the measured CIELab results for lightness
(L*), redness (a*) and yellowness (b*) the overall colour difference between the different samples was assessed by using the equation \( \Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \).

Sensory analysis

Sensory analysis was conducted by a trained panel consisting of eight experienced judges. For each sample four equal cans were opened. The samples were pooled so that each person of the sensory panel tested pieces from each of the cans. Odour and taste were then evaluated for freshness, rancidity, sour, metallic, and bitter taste. The texture was valued by solidity and juicyness. The intensity of the attributes were valued on a scale from 0 to 100.

Results

Values of free fatty acid formation and thiobarbituric acid reactive substances as well as of fluorescence and browning assessments increased with prolonged chilling storage time. However, no significant (p<0.05) differences were obtained when comparing both (flake and slurry) icing conditions. Rancid and fresh odour showed very small differences after evaluation by the trained panel. After 12 days storage in ice freshness was lost, for both chilling conditions (Fig 1). Metallic, bitter and sour odour increased slowly in all kinds of samples. However there are only very small differences between the two different kinds of canned fish. Storage conditions under slurry ice seemed to keep the fish muscle softer and more juicy compared to that stored under traditional flake ice (Fig. 2). However the differences are very small too. No differences in lightness could be observed by sensory assessment.

![Figure 1: Taste fresh and rancid](image-url)
A remarkable colour difference of approximately $\Delta E = 20$ was found between homogenised fresh muscle of horse mackerel and the homogenated canned muscle tissue. On the other hand, when the influence of chilled storage prior to canning was observed it became obvious that colour differences were slightly more pronounced when flake ice was used for chilling. A clear tendency concerning the influence of storage time was not visible. Differences in colour between cans processed by using horse mackerel chilled in flake ice and those chilled in slurry ice were also small and decrease with increasing storage time as to be seen in Table 1.

**Table 1: Colour differences between cans prepared using differently chilled raw material**

<table>
<thead>
<tr>
<th>$\Delta E$ between flake and slurry ice samples</th>
<th>after days of chilling</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6</td>
<td>5</td>
</tr>
<tr>
<td>2.6</td>
<td>8</td>
</tr>
<tr>
<td>0.5</td>
<td>12</td>
</tr>
</tbody>
</table>

**Conclusions**

It can be summarised that style and time of pre-processing (chilling in flake or slurry ice) do not significantly influence the quality parameters of canned muscle tissue from horse mackerel.

**References**


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3.16 INCREASE IN FILLETING YIELD AND BY-PRODUCTS FROM COD IN FACTORY TRAWLERS

Helgi Nolsøe

Introduction

According to “Code of Conduct for Responsible Fisheries”, the aim has to be, that the waste from the fishery has to be as small as possible and the environment has to be protected. “Code of Conduct for responsible Fisheries” is an agreement and a declaration of intent from FAO with rules and procedures for fishery and fish breeding.

In a previous work, Hjáframleiðslur, it was demonstrated that the utilization of the quotas for the factory trawlers, not was according to the principal rules in “Code of Conduct for responsible Fisheries”.

It was estimated that good possibilities are for a substantially improved filleting yield for the factory trawlers. Also the possibilities for utilization of different by-products were pointed out.

Regularly by-products are in focus in the fishery sector. The occasions can be limitations in supply of raw-materials or partly that the by-products have been a waste problem. Also the possibilities for a better utilization of the by products has been the point of interest.

Based on the fact that the fish resources are limited and have to be utilized in the best way, an investigation of the Faroese utilization of the by-products was performed in 1999-2000. The investigation covered the by-products from both sea based and land based production.

The purpose of the investigation was to get information about the amounts of by-products for the different parts of the fishing industry, and to uncover possibilities for utilizations not utilized.

The investigation showed that in certain areas it is possible to get a better utilization. Some raw materials are not utilized at all or only partly utilized.

For the factory trawlers the investigation showed a relatively low utilization. This is because most of the catch is filleted on board while only a small part is sold as eviscerated or headed and eviscerated fish. Only a small part of the by-products, the roes, have been utilized.

The investigation showed that about 64% of the produced amount of fish material, was not utilized, but thrown over board after the processing.

This low utilization is connected to certain factors limiting the utilization. The production facilities on board the trawlers are not aiming against a high yield and utilization of by-products.

In the arrangement of the production and selection of filleting machines, the aim has not been to get a high filleting yield and a high utilization of the raw material, but to get a high filleting capacity of produced raw material per hour.

The filleting machines used on board the factory trawlers for cod, haddock and Saithe are developed on the assumption of free fishery with no limiting quotas. These conditions are not present any longer. It has to be realized that the fish resources are limited. Everything has to be utilized in the best way. The fishery has to be sustainable. The fish resources from this part of the fishing fleet have also to be utilized in the best way.

In future fishery higher requirements to utilization have to be expected, both in international agreements and from interest groups in the fishery.

Investigation of the filleting yield on board a factory trawler

Based on some preliminary investigations it was decided to test a Baader 252 filleting machine in comparison to Baader 190. Bader 252 is a saddle machine developed by Baader in Iceland for factory trawlers. The machine was tested on board the factory trawler M/t Sundaberg.

Materials and methods

An indication of the filleting yield was investigated in the preliminary investigations of the filleting yield on a land based fish factory, with experience in working fish from the same area, as the factory trawlers catch their fish. This investigation was performed by investigating processing data for frozen fish caught in the Baring Sea.

After these preliminary investigations the following tests were performed comparing the yields from two different filleting machines.

The tests were performed as parallel tests where selected fish of the same size, from the same haul was sorted out in two lots before the filleting, one lot on each machine. The fish was weighed before the filleting and the fillets were weighed after the filleting. The operations were performed by experienced operators. The fish was Cod, Gadus morhua caught in Norwegian waters in January 2001.
In the last test filleting yield after trimming was measured. That means the fish was weighed before filleting and the fillets were weighed after filleting, trimming and packing.

Results

There were made 4 tests. The three first were tests of machine filleting yield while the last one was filleting yield after trimming.

Table 1. Filleting yields with Baader 190 and Baader 252

<table>
<thead>
<tr>
<th>Machine</th>
<th>Size beheaded Fish</th>
<th>Fish number</th>
<th>Weight after beheading</th>
<th>Weight after filleting</th>
<th>Yield %</th>
<th>% Increase with Baader 252</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Test</td>
<td>Machine yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 252</td>
<td>40-50 cm</td>
<td>12</td>
<td>16.2</td>
<td>12.72</td>
<td>78.52</td>
<td></td>
</tr>
<tr>
<td>B 190</td>
<td>40-50 cm</td>
<td>12</td>
<td>15.6</td>
<td>10.52</td>
<td>67.44</td>
<td></td>
</tr>
<tr>
<td>Increase %</td>
<td>(11.08/67.44)100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16.43</td>
</tr>
<tr>
<td>2. Test</td>
<td>Machine Yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 252</td>
<td>50-60 cm</td>
<td>8</td>
<td>17.14</td>
<td>13.44</td>
<td>78.41</td>
<td></td>
</tr>
<tr>
<td>B 190</td>
<td>50-60 cm</td>
<td>8</td>
<td>16.98</td>
<td>11.58</td>
<td>68.2</td>
<td></td>
</tr>
<tr>
<td>Increase %</td>
<td>(10.21/68.20)100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.97</td>
</tr>
<tr>
<td>3. Test</td>
<td>Machine Yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 252</td>
<td>50-60 cm</td>
<td></td>
<td>90</td>
<td>71.72</td>
<td>79.69</td>
<td></td>
</tr>
<tr>
<td>B 190</td>
<td>50-60 cm</td>
<td></td>
<td>89.72</td>
<td>60.1</td>
<td>66.99</td>
<td></td>
</tr>
<tr>
<td>Increase %</td>
<td>(12.7/66.99)100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18.96</td>
</tr>
<tr>
<td>4. Test</td>
<td>Net Yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B 252</td>
<td>50-60 cm</td>
<td></td>
<td>533.06</td>
<td>399.42</td>
<td>74.93</td>
<td></td>
</tr>
<tr>
<td>B 190</td>
<td>50-60 cm</td>
<td></td>
<td>543.06</td>
<td>344.50</td>
<td>63.44</td>
<td></td>
</tr>
<tr>
<td>Increase %</td>
<td>(11.49/63.44)100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18.11</td>
</tr>
</tbody>
</table>

Discussion on the yield tests

The tests show that the amount of fillets can be increased by 15-19% by using Baader 252 in stead of Baader 190 for the tested fish sizes. The tests were performed with beheaded fish. If the amounts used in the fourth test are converted to round fish the yield for Baader 190 can be calculated to 40.9% and the yield for Baader 252 can be calculated to 48.3%. The difference in yield is by this 7.4% based on round fish. The conversion factor between beheaded and round fish is 1.55. That means the beheaded fish has to be multiplied by 1.55 to get round fish. The products in the tests were fillets with skin on, pin bone in. The conversion factor for this product is 2.6 which give about 38.46% yield. In this case the actual yields are not so important, because the figures are calculated from beheaded fish. What is more important is the difference in the yields for the two filleting machines. The prerequisite for the factory trawlers to get full advantage of the improved yields is that the conversion factors are changed according the changes in filleting yield if they change to other machines giving a higher yield. This is a political question that has to be solved in political negotiations. The tests are limited to these few tests with limited amounts of fish and the involved fish sizes, but the tests show that replacing the Baader 190 with Baader 252 can improve the yield substantially. The tests are based on beheaded fish. That means that the improvement in yield is only based on the filleting part of the processing. It is estimated that the filleting yield can be increased about 2-3% more by using heading machines giving a higher yield than Baader 424 and similarly. The conversion factors ought to be directly connected with the machines used for beheading and filleting. Changes in the production procedures with other machines require changes in the production lay-out. This can at the same time give opportunities to create better possibilities for utilization of a bigger part of the fish.
Conclusion

Present production system on the factory trawlers is a limiting factor for utilizing the fish for food. Higher demands to the utilization have to be expected in the future. FAO declaration of intent instructs to aim for as little waste as possible.

The aim has to be for higher yields and better utilization. The by-products have mainly to be used as food products. Tests of the filleting yield for certain fish sizes show that the yield can be increased by more than 7% of the whole fish, just for the filleting part. The tests show that the amount of fillets can be increased by 15-19% just by using filleting machines giving a higher yield.

It can be expected to increase the yield from the beheading by a further 2-3% by using better heading machines. The possibilities for substantially increasing the value of the factory trawlers quotas in the Baring Sea can only be achieved by political negotiations about changing the conversion factors. The conversion factors have to be directly tied to the machines used for beheading and filleting.

References


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3.17 PROCESSING FORECAST OF COD

Sveinn Margeirsson, Gudmundur R. Jonsson, Sigurjon Arason, Gudjon Thorkelsson

Abstract

The objective of this research was to find out what variables in catching and processing of cod affected fillet yield, gaping, parasites and bruises. Data was collected in a fish processing plant in N-Iceland in a period of over 2 years. Conditions by catch and transportation were registered. Time of year and location of the catch was important for almost all the variables under examination. The time from catch until the cod was measured (age of the raw material) affected both gaping and bruises. Fillet yield and condition factor were tightly correlated.

Introduction

Many different variables influence the return from cod processing. The objective of this research was to explore and map a few of these variables, variables such as fillet yield, gaping, parasites and bruises in fish caught in different catching grounds around Iceland and find out what variables affected them. Knowing how these factors behave, e.g. in different catching grounds and at different times of the year, might help controlling fisheries more effectively, by taking the needs of the fish processing plants into consideration when organizing the fisheries. It might also result in better production management, due to more understanding of the cod as a raw material, better production plans and supply management (Nahmias, 2000). An example of the possibilities of better production management in an onshore production plant is that if the production manager knows what kind of cod (e.g. caught in a specific fishing ground) has been caught, he is better capable of predisposing the catch and organizing the production. This might include allocating cod that is expected to have much gaping to products that are less sensitive to gaping, insuring that there are enough workers to pluck out parasites if the cod is expected to be rich of parasites and so on. Former results indicate that the return of Icelandic fisheries and processing can be increased considerably by managing the fisheries and the processing as a whole. Advantages of such linkage and use of prior knowledge in controlling the fisheries and processing might e.g. be easier planning for fisheries and production managers (Bjarnason, 1997). A big catch results in most cases in a longer waiting time until bleeding of the cod. A longer waiting time results in less valuable products (Rikhardsson and Birgisson, 1996). It is considered safe to keep cod in ice for about 10-12 days (Magnusson and Martinsdottir 1995). The quality of the cod does though start to fall much earlier and a rule of thumb is that the older the cod is the less valuable it is (Wendel, 1995). Keeping cod in ice for about 6-7 days can result in 8-10% less value of the cod (Rikhardsson and Birgisson, 1996). From those examples it is evident that the interests of the fisheries and the processing are not always parallel (it is better for the ships to get big catches rather than small catches, but it may result in a fish of less quality) and therefore important to manage the fisheries and the processing as a whole.

Materials and methods

Data was collected over a 30 months period in cooperation with a large fisheries and fish processing company in N-Iceland. The time and location of the catch was registered, along with the length (minutes) and size (kg) of the catch. After the catch had been unloaded, weight (kg) in tubs, length (cm) and weight (kg) of the cod and weight (kg) of the head were measured and registered. Samples of four cods were taken from all tubs that were reweighed (reweighing a certain proportion of tubs is obligatory for all companies processing fish in Iceland). The fish was headed and weighed again and thereafter filleted. The fillets were weighed and all visual parasites counted. Gaping and bruises were measured by putting a transparent plastic card with a grid on the fillets (Fig. 1). The grids were 4x4cm. If a bruise was as big as one grid, it counted as one bruise. The same measurement was used for gaping. After the measurements had been done, the fish was processed as any other fish.
Results and discussion

Regression analysis was used to find a functional relationship between the response variables and the independent variables after a thorough outlier detection of all variables. Time of year, catching ground and length of the catch affected fillet yield. The fillet yield was also affected by the weight and the length of the cod (condition factor) as well as by its head proportion. A regression model was made and used for forecasting (only variables with p-values lower than 0.05 were used in the model). Fig. 2 shows the forecast from the model in addition to the actual measurements of the fillet yield and the 95% upper and lower confidence limits. The forecast applies to cod caught in August 2003, but the measurements for these samples were left out while making the model. Fig. 3 shows how the fillet yield varied from one catching ground to another. The figure shows the results for all months and can be treated as an indication of the real situation. It is though dangerous to conclude much from the figure, since time of year and more factors do affect the fillet yield. The difference between catching grounds is in many ways uniform to the results of Eyjolfsson (2001), but a precise comparison is not possible since he used other partition of catching grounds.

Catching ground and time of year were the variables that mostly affected number of parasites. The number of parasites per fish and the geological distribution of fish containing parasites were similar to Dagbjartsson (1973). The size of the cod also had significant effect on number of parasites. The bigger the cod was the more parasites it contained. This is uniform with Birgisson (1995).

Time of year, age of the raw material (the time from catching to measurement) and the size of the catch affected bruises. The longer time that passes from catch until the fish is measured the longer time do the natural brake down processes of the fish have to spoil it. The size of the catch must correlate strongly to the waiting time until bleeding. It is therefore
not surprising that the size of the catch correlates to bruises. It is not evident why the time of year does affect bruises. Possible explanations are e.g. nutritional status of the fish and the temperature of the sea.

Catching ground, time of year and age of the raw material were the variables that mostly affected gaping. The size of the cod did not affect gaping significantly. The correlation between gaping and the size of the cod seems to be rather unclear. Birgisson (1995) found a positive correlation between the size of the fish and gaping, while Love (1975) said that gaping in small cod was more than in large cod. Since the method of measuring gaping in cod is not standardized, such comparison may though be questionable.

Conclusions

The results indicate that organizing and controlling the fisheries and processing as a whole can increase the return of the catch-processing chain considerably. The project, which was an M.Sc. project, has been continued and expanded into a Ph.D. project. In the last step of the Ph.D. project it is the intention to use the results as well as data on price of oil, product prices, wages and more to make an optimization model. The purpose of this model is to help fisheries and production managers to take decision on where to send their trawlers and what to with the catch after unloading it.

References


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3.18 EFFECTS OF STORAGE IN OZONISED SLURRY ICE ON THE SENSORY AND MICROBIAL QUALITY OF SARDINE (SARDINA PILCHARDUS)

Carmen A. Campos, Óscar Rodríguez, Vanesa Losada, Santiago P. Aubourg and Jorge Barros-Velázquez

Introduction

Slurry represents a relatively novel refrigeration system and consists of an ice-water suspension at a subzero temperature. Among its main advantages, two should be highlighted: (i) its faster chilling rate –due to a more rapid heat exchange–, and (ii) the reduced physical damage caused to seafood products by its spherical microscopic particles, as compared with conventional flake ice, which tend to be aciculate. From a technical point of view slurry ice can be combined with other additives for different purposes; i.e., ozone- to achieve a better microbial control of the fish catch or melanosis inhibitors, to minimise browning reactions in crustaceans (Huidobro et al., 2002).

The objective of the present study was to evaluate the effect of slurry ice, either alone or combined with ozone on the evolution of the sensory and microbial quality of sardines throughout storage.

Materials and methods

Slurry ice was prepared using a FLO-ICE prototype (Kinarca S.A.U., Vigo, Spain). Its composition was 40% ice and 60% seawater. The injection of ozone in the slurry ice mixture was accomplished with a prototype provided by Cosemar Ozono (Madrid, Spain), the redox potential being adjusted to 660 mV (0.17 mg ozone/l). Flake ice was prepared with an Icematic F100 Compact device (Castelmac SPA, Castelfranco, Italy). The fish specimens were surrounded by either ozonised slurry ice, slurry ice, or flake ice at a fish:ice ratio of 1:1, and stored for up to 22 days in a refrigerated room at 2°C.

Sardine (Sardina pilchardus) specimens were caught in the day and kept in ice as they arrived at our laboratory. The fish specimens were neither headed nor gutted. Three different batches, one for each refrigeration system, were used and studied separately along the whole experimental period. Samples were taken from each batch on days 0, 2, 5, 8, 12, 15, 19, and 22. All analyses were performed in triplicate.

Sensory analyses were conducted in whole fish by a panel consisting of five experienced judges, according to official guidelines concerning fresh and refrigerated fish (DOCE, 1989).

Samples of 5 g of fish muscle were dissected aseptically, mixed with peptone water, and homogenized in a stomacher (Seward Medical, London, UK). Total aerobic and psychrotrophic bacteria were investigated in Plate Count Agar (PCA, Oxoid) after incubation at 30°C for 48 h or at 7-8°C for 10 days, respectively. Anaerobes were also monitored. Lactose-fermenting Enterobacteriaceae (coliforms) were investigated in Violet Red Bile Agar (VRBA medium, Merck, Darmstadt, Germany). The proteolytic phenotype was investigated in casein-agar medium. Microorganisms exhibiting a lipolytic phenotype were detected in tributyrine-agar medium.

Results and discussion

The results from the sensory analyses are shown in Table 1. It can be seen that sardines refrigerated by the combined used of slurry ice and ozone retained a good quality (E and A categories) up to day 8 and were acceptable up to day 19. However, when slurry ice was used alone, the good quality was only retained up to day 5 and the product was acceptable up to day 15.

It should be stressed that the use of slurry ice –either alone or in combination with ozone– produced a significant increase in sardine shelf life, this trend being enhanced when ozone was present. In previous studies, it was demonstrated that the use of slurry ice extends the shelf life of non-fat fish species –such as farmed sea bream (Huidobro et al., 2001), and European hake (Losada et al., 2004) – and shrimp (Huidobro et al., 2002). Moreover, the
The evolution of microbial growth in sardine muscle along refrigerated storage in the slurry ice, ozonised-slurry ice and flake ice batches is shown in Figure 1 (panels A to F). As can be seen for all bacterial groups investigated, the use of flake ice allowed notable increases in the microbial populations, the counts of mesophiles, psychrotrophic bacteria, and of both proteolytic and lipolytic microorganisms reaching figures of approximately $10^6$ CFU/g after 12 days of storage (Fig. 1, panels A, D, E, and F). By contrast, microbial growth was significantly slower in the slurry ice batch, the average differences in the counts of mesophiles, psychrotrophic bacteria, and both lipolytic and proteolytic microorganisms after 12 days of storage being in the range of 1.5-2.5 log units below those determined for the flake ice batch (Fig. 1, panels A, D, E, and F). By this time, according to sensory analysis the samples stored in flake ice were unacceptable, while slurry ice samples still had an acceptable quality (Table 1). According to sensory analyses, the less intense bacterial growth in sardine muscle when slurry ice was employed coincided with an extended shelf life of this batch. This trend is in agreement with the results of a previous study that reported significantly lower bacterial counts and an extended shelf of shrimp stored in slurry ice, as compared with conventional flake ice (Huidobro et al., 2002).

The combined use of ozone and slurry ice produced an additional reduction in the counts of the anaerobes, psychrotrophic bacteria, and of both proteolytic and lipolytic microorganisms along storage (Fig. 1, panels B, D, E, and F). A similar trend was observed for the evolution of mesophilic bacteria, but in this case the beneficial effect of ozone was only observed after 15 days of storage (Fig. 1, panel A). It should also be highlighted that the use of ozone combined with slurry ice induced a decline in the growth of mesophiles and lipolytic bacteria that was so important that their counts were similar to those determined at the beginning of the storage period (3.16 log CFU/g for mesophiles, and 2.17 CFU/g for lipolytic microorganisms).

Conclusions

Storage of sardine in slurry ice –alone or in combination with ozone– improves the sensory, and microbiological quality of sardine as compared with storage in conventional flake ice, a result that implies a significant extension of the shelf life of this fish species. Of special relevance are the significant reductions of the psychrotrophic bacteria –both in muscle and– as well as of proteolytic and lipolytic bacteria. On the basis of the results obtained, the use of slurry ice – either alone or combined with ozone– for the refrigerated storage of sardine is advisable.

References


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Table 1. Comparative sensory acceptability of sardine batches

<table>
<thead>
<tr>
<th></th>
<th>Ozonised slurry ice</th>
<th>Slurry ice</th>
<th>Flake ice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(days of storage)</td>
<td>(days of storage)</td>
<td>(days of storage)</td>
</tr>
<tr>
<td>0</td>
<td>2 5 8 12 15 19 22</td>
<td>2 5 8 12 15 19 22</td>
<td>2 5 8 12 15 19 22</td>
</tr>
<tr>
<td>Skin aspect</td>
<td>E E A B B B B E E A B B B B A A B C C C C C C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External odor</td>
<td>E E A A B B B C E A A B B C C A A C C C C C C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gills</td>
<td>E E A A A B C C E A A B C C C E A B B C C C C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyes</td>
<td>E E A A B B C C E A B B C C C A B C C C C C C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consistency</td>
<td>E E E A A A B C E E A A B B C E A B B C C C C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flesh odor</td>
<td>E E A A B B B C E A A B B C C A A C C C C C C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend:
- E: Excellent
- A: Acceptable
- B: Borderline
- C: Critical
Fig. 1. Evolution of microbial growth in sardine muscle along refrigerated storage using flake ice (○), slurry ice (■) and ozonised slurry ice (△). Panel A: mesophiles; panel B: anaerobes; panel C: coliforms; panel D: psychrotrophic bacteria; panel E: lipolytic bacteria; panel F: proteolytic bacteria.
3.19 LIPID CHANGES RELATED TO QUALITY DURING SARIDNE (SARDINA PILCHARDUS) CHILLED STORAGE: EFFECT OF OZONISED SLURRY ICE

Vanesa Losada, Carmen Piñeiro, Marcos Trigo, José M. Antonio, Jorge Barros-Velázquez and Santiago P. Aubourg

Introduction

Slurry ice has been reported to be a promising technique for the preservation of aquatic food products in an ice-water suspension at subzero temperature (Chapman, 1990; Harada, 1991). Ozone is a powerful antimicrobial agent that is suitable for application in food in the gaseous and aqueous states leading to significant increases in sensory quality and shelf-life of fish. Molecular ozone or its decomposition products inactivate microorganisms rapidly by reacting with intracellular enzymes, nucleic material and other components. Slurry ice applications (Price, 1991; Huidobro, 2002) have shown damage inhibition concerning sensory assessment, microbiological activity, nucleotide degradation and volatile amine formation. However, the effect of slurry ice on lipid matter has hardly been elucidated till now.

The present work focuses on the evolution of lipid damage (hydrolysis and oxidation) as affected by storage in slurry ice, either alone or combined with ozone. For it, a fatty fish species (sardine, Sardina pilchardus) was chosen and stored up to 22 days. Results were compared with traditional flake icing. Lipid damage assessment is complemented by sensory analysis.

Materials and methods

Slurry ice was prepared using a FLO-ICE prototype (Kinarca S.A.U., Vigo, Spain). The composition of the slurry ice binary mixture was 40% ice and 60% water, prepared from filtered seawater (salinity: 3.3%). The temperature of the slurry ice mixture was -1.5°C. The injection of ozone in the slurry ice mixture was accomplished with a prototype provided by Cosemar Ozono (Madrid, Spain), the redox potential being adjusted to 660 mV (0.17 mg ozone/L). Flake ice was prepared with an Icematic F100 Compact device (Castelmac SPA, Castelfranco, Italy).

The fish specimens were surrounded by either ozonised slurry ice, slurry ice, or flake ice at a fish:ice ratio of 1:1, and stored for up to 22 days in a refrigerated room at 2°C. When required, the ice mixtures were renewed.

Fresh sardine (Sardina pilchardus) specimens were caught (November, 2003) near the Galician Atlantic coast and transported to the laboratory ten hours after catching. The fish specimens were not headed nor gutted and were directly placed in ozonised slurry ice, slurry ice or flake ice in an isothermal room at 2°C. The length of the specimens was in the 16–21 cm range and average weight was 150 g. Three different groups were used for each icing treatment and studied separately along the whole experimental period. Samples were taken for analysis on days 0, 2, 5, 8, 12, 15, 19 and 22. Once fish specimens had been subjected to sensory analyses, the white muscle was separated and employed for biochemical analyses. All analyses were performed in triplicate.

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to guidelines concerning fresh and refrigerated fish (Council Regulation, 1990).

Lipids were extracted by the Bligh and Dyer (1959) method. Quantification results are expressed as g lipid/100 g muscle.

NaCl content in fish muscle was calculated from the amount of chlorine by boiling in HNO₃ with excess of AgNO₃, followed by titration with NH₄SCN (AOAC, 1990). Results are expressed as g NaCl/100 g muscle.

Free fatty acid (FFA) content was determined by the Lowry and Tinsley method (1976). Results are expressed as g FFA/100 g lipids.

The peroxide value (PV) was determined according to the ferric thiocyanate method (Chapman and McKay, 1949). Results are expressed as milliequivalents of oxygen/kg lipids.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). Results are expressed as mg malondialdehyde/kg fish sample.

Formation of fluorescent compounds was determined with a Perkin Elmer LS 3B fluorimeter by measurements at 393/463 nm and 327/415 nm as previously described (Aubourg et al., 1997; Aubourg and Medina, 1999). Biochemical data corresponding to the three chilling methods were subjected to one-way analysis of variance to assess significant
(p<0.05) differences among treatments (Statsoft, 1994). The SPSS 11.5 software for Windows (SPSS Inc., Chicago, IL, USA) was also used to explore the statistical significance of the results obtained.

Results and discussion

Lipid hydrolysis was determined according to the FFA assessment (Table 1). Evolution of FFA content along storage in the three conditions did not provide good correlation values with time (Table 1). In the present experiment, no significant differences were obtained among the three treatments during the 0-15 days period. Then, a higher FFA content was observed for flake icing when compared to both slurry ice conditions. For this period, an inhibitory effect of slurry ice on lipid hydrolysis was concluded. No significant differences were obtained between both slurry ice conditions, so that no effect of ozone on lipid hydrolysis was denoted. The formation of FFA itself does not lead to nutritional losses.

Primary lipid oxidation was followed by the PV (Table 1). Its assessment in both slurry ice conditions showed an increasing tendency with time, that was specially sharp at the end of the experiment (day 22). A different behaviour was observed for flake ice treatment, since an increase pattern was observed till day 19, that was followed by a sharp decrease. This decrease can be explained as a result of peroxide breakdown. Comparison between flake and slurry icing conditions showed a higher PV for flake ice at days 5, 8 and 19. Ozonised slurry ice treatment did not show significant differences when compared to slurry ice, although higher mean values were obtained for ozonised slurry ice in most cases.

Secondary lipid oxidation was followed by the TBA-i (Table 1). Its assessment in all three conditions showed an increasing pattern, with some exceptions. Differences between flake and both slurry icing conditions were obtained at day 8, being higher for the flake ice. Comparison of both slurry ice conditions showed a higher oxidation level at days 15 and 22 for the ozonised samples; a sharp increase of thiobarbituric acid reactive substance content was obtained at day 22 for fish samples treated under ozonised slurry ice.

Interaction compound formation, also called tertiary oxidation compounds, produced during the chilled storage was studied by means of the fluorescence ratio (Aubourg et al., 1997; Aubourg and Medina, 1999). Till day 12, no differences were observed for the three treatments when compared to the raw material. After that time, a gradual increase was observed for flake ice fish, which showed higher FR values than both slurry ice treatments. Along the whole storage time, no differences (p>0.05) were obtained as a result of the ozone presence. The sharp increase found in flake ice samples for the 19-22 days period agrees with the no-variation period (days 15-22) observed for the TBA-i and with the sharp decrease obtained at day 22 for the PV.

Sardine specimens stored in flake ice, maintained good quality (categories E and A) until day 2 (Table 2). After this time, sensory quality decreased and the batch exhibited unacceptable quality on day 8. In this batch, the limiting factors were the gills and the flesh odour. Sardine fish stored in slurry ice maintained good quality up to day 5 (Table 2). After this time, sensory quality decreased and on day 15 this batch was no longer acceptable. The appearance of the gills and eyes were the first parameters that limited fish acceptability. This enlargement of shelf-life found for the slurry ice treatment agrees with previous research on lean fish (Losada et al., 2004) and crustacean (Huidobro et al., 2001) species. Finally, fish treated under ozonised slurry ice maintained good quality up to day 8. After this time, sensory quality decreased and on day 19 this batch was no more acceptable. The appearance of the gills and eyes were again the first parameters that limited fish acceptability.

The presence of NaCl in the chilling medium has lead in both slurry ice treatments to a progressive increase of NaCl content in fish white muscle. This increase was stronger in the presence of ozone than without it (p<0.05). It is concluded that ozone renders fish samples to be more permeable to NaCl diffusion. Both slurry ice treatments provided a good correlation value between NaCl content and chilled time (r² = 0.98 in both cases) (Table 3). Fish samples treated under traditional flake ice did not show differences (p>0.05) in NaCl content during the experiment.
Table 1. Lipid damage assessment* during sardine storage under different chilled treatments**

<table>
<thead>
<tr>
<th>Storage time (days)</th>
<th>FI (flake ice)</th>
<th>SI (slurry ice)</th>
<th>OSI (ozonised slurry ice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.36 ± 0.05</td>
<td>0.34 ± 0.05</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>0.49 ± 0.12</td>
<td>0.38 ± 0.02</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>8</td>
<td>0.41 ± 0.17</td>
<td>0.55 ± 0.15</td>
<td>0.54 ± 0.09</td>
</tr>
<tr>
<td>12</td>
<td>0.49 ± 0.04</td>
<td>0.53 ± 0.01</td>
<td>0.56 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>0.40 ± 0.06</td>
<td>0.49 ± 0.01</td>
<td>0.56 ± 0.04</td>
</tr>
<tr>
<td>19</td>
<td>0.89 ± 0.12</td>
<td>0.40 ± 0.17</td>
<td>0.64 ± 0.17</td>
</tr>
<tr>
<td>22</td>
<td>1.70 ± 0.17</td>
<td>0.40 ± 0.17</td>
<td>0.64 ± 0.06</td>
</tr>
</tbody>
</table>

* Lipid damage abbreviations: FFA (free fatty acids), PV (peroxide value), TBA-i (thiobarbituric acid index) and FR (fluorescence ratio)

For each damage index, mean values (n = 3) in the same row followed by different letters are significantly different (p<0.05). Standard deviations are indicated in parentheses. Raw values: 0.24 ± 0.08 (FFA); 1.51 ± 0.88 (PV); 0.30 ± 0.02 (TBA-i); 0.18± 0.01 (FR).

** Chilled treatments: FI (flake ice), SI (slurry ice) and OSI (ozonised slurry ice).

Table 2. Sensory acceptance during sardine storage under different chilled conditions

<table>
<thead>
<tr>
<th>Storage Time (days)</th>
<th>Flake Ice</th>
<th>Slurry Ice</th>
<th>Ozonised Slurry Ice</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>A</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>8</td>
<td>C</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>12</td>
<td>C</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>15</td>
<td>C</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td>19</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>22</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

*Freshness categories: E (excellent), A (good), B (fair) and C (unacceptable). Raw fish was category E.

Table 3: Correlation values* between the storage time and NaCl measured during sardine chilled storage under different conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Flake Ice</th>
<th>Slurry Ice</th>
<th>Ozonised Slurry Ice</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.40</td>
<td>0.98</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* Linear correlations are expressed. Non-linear fittings logarithmic are expressed in brackets when the coefficients are higher than the linear ones.
Conclusions

The employment of slurry ice as a chilling technology has produced an inhibitory effect on lipid damage (hydrolysis and oxidation) related to quality loss during the chilled storage of a fatty fish species. These results agreed with the sensory assessment that led to longer shelf life times and good quality periods. Ozonised slurry ice employment has provided an increase in shelf-life time and good quality period when compared to slurry ice alone. These increases are interesting and, since a pro-oxidant effect of ozone on lipid matter is not concluded, its employment is found beneficial for quality retention during a fatty fish species chilling. Fish traders have employed chilled storage as a previous step to other technological treatments. The effect of previous chilling conditions (storage time, fish-ice ratio, room storage temperature) on the quality of frozen (Undeland and Lingnert, 1999; Aubourg et al., 2002) and canned (Slabyj and True, 1978; Aubourg and Medina, 1997) fish has been demonstrated. In this sense, according to the shelf life times obtained for the three treatments, the employment of slurry ice or ozonised slurry ice can be recommended as profitable for a fatty fish species.

References

Statsoft (1994) Statistica for Macintosh. Statsoft and its licensors, Tulsa, Oklahoma (USA)

Authors

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3.20 LIPID DAMAGE ASSESSMENT DURING COHO SALMON
\textit{(Oncorhynchus kisutch)} CHILLED STORAGE

Vanesa Losada, Julio Gómez, Liliana Maier, Má Elisa Marín, Julia Vinagre, 
Má Angélica Larraín, Vilma Quitral, Alicia Rodríguez and Santiago P. Aubourg

Introduction

Marine foods have attracted a great attention from consumer as a source of high amounts of important nutritional components to the human diet (Simopoulos, 1997). However, in recent years the fishing sector has suffered from dwindling stocks of traditional species as a result of dramatic changes in their availability. This has prompted fish technologists and the fish trade to pay more attention to aquaculture techniques as a source of fish and other seafood products (Josupeit et al., 2001).

In recent years, Coho salmon (\textit{Oncorhynchus kisutch}), also called silver salmon, has acquired a great attention because of its increasing production in Chilli, reaching values round 76,000 mt, 93,000 mt and 137,000 mt for years 1999, 2000 and 2001, respectively (FAO Inform, 2003). In spite of this commercial interest, previous research related to this salmon species only concerns the cholesterol content (Romero et al., 1996) and the fatty acid distribution in fresh (Gruger et al., 1964; Braddock and Dugan, 1969), frozen (Braddock and Dugan, 1972) and canned (Romero et al., 1996) products.

Wild and farmed fish species are known to deteriorate rapidly after death due to the action of different mechanisms (Cheftel and Cheftel, 1976). Marine lipids are constituted by highly unsaturated fatty acids that are known to be very prone to lipid oxidation (Harris and Tall, 1994). During chilled storage of fatty fish species, a strong effect of lipid damage has been detected on fish quality loss (Hwang and Regenstein, 1996; Undeland et al., 1999) that leads to a negative effect on the commercial value.

The present work concerns Coho salmon and its commercialisation as a chilled product. As a fatty fish species, the study is focused on the lipid fraction damage. For it, lipid hydrolysis and oxidation assessments were carried out during a 24 day storage period. Traditional lipid damage indices (free fatty acids, conjugated dienes, peroxides, thiobarbituric acid reactive substances, fluorescent compounds and browning development) and lipid composition (astaxanthin and polyenes) changes were checked and compared to sensory acceptance.

Materials and methods

Farmed Coho salmon (\textit{Oncorhynchus kisutch}) specimens were obtained from EWOS Innovation Research (Colaco, Puerto Montt, Chilli) in December 2003. Fish specimens (weight range: 2.5-3.0 kg) were sacrificed by a sharp blow to the head, the gills cut, bled in a water-ice mixture, headed, gutted and kept in ice for 24 h until they arrived at our laboratory. The fish specimens were then stored on ice in an isothermal room at 2°C. Samples were taken for analysis on days 0, 3, 6, 10, 12, 17, 19 and 24. Five different individuals were analysed by day (n=5) and studied separately to achieve the statistical analysis. Analyses were carried out on the white muscle.

Lipids were extracted by the Bligh and Dyer (1959) method.

Free fatty acid (FFA) content was determined by the Lowry and Tinsley (1976) method. Results are expressed as g FFA/100 g lipids. Conjugated diene (CD) formation was measured at 233 nm (Kim and Labella, 1987).

The peroxide value (PV), expressed as meq oxygen /kg lipid, was determined by the ferric thiocyanate method (Chapman and McKay, 1949). The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). Results are expressed as mg malondialdehyde/ kg fish sample. Formation of fluorescent compounds was determined with a Perkin Elmer LS 3B fluorimeter by measurements at 393/463 nm and 327/415 nm, as previously described (Aubourg et al., 1997). Browning development was measured from the lipid extract at 450 nm and 400 nm. The 450/400 absorbance ratio was studied according to Hassan et al. (1999). Lipid extracts were converted into fatty acid methyl esters and analysed by gas chromatography according to the method of Lepage and Roy (1986). The polyene index (PI) was calculated as the following fatty acid ratio: C 20:5 + C 22:6 / C 16:0 (Lubis and Buckle, 1990).

Astaxanthin content was measured according to the Sheehan et al. (1998) method. Results are expressed as mg all-E-astaxanthin /kg fish muscle.

Sensory assessment was carried out according to Howgate (1992). Data from the different lipid analyses were subjected to one-way analysis of variance (p<0.05); comparison of means was performed using a least-squares difference (LSD) method (Statsoft, 1994).
Results and discussion

Lipid hydrolysis was studied according to the FFA assessment. Mean values of FFA content provided a slow and progressive increasing trend in salmon muscle during chilled storage (Table 1). Compared to raw material, a significant increase was only observed at day 17. FFA values obtained at the end of the experiment were below 1.5g FFA/ 100g lipids, that can be considered a low value when compared to other fatty fish species treated under similar conditions (Aubourg et al., 1997; Aubourg, 2001). Different and complementary lipid oxidation indices were assessed to evaluate the rancidity development in the present experiment. The conjugated diene detection did not show differences during the chilled time. Ratio between diene formation and diene breakdown was almost the same during the storage period. This index did not show to be sensitive in the present experiment for showing quality changes with time. Peroxide formation was low along the whole experiment, although a continuous increase was observed with time (Table 1). Compared to raw material, a significant increase was observed at day 6. However, values obtained were in all cases under a PV = 4.0, which means a low formation of primary lipid oxidation compounds along the storage time. No formation of thiobarbituric acid reactive substances could be observed in the 0-17 day period (Table 1). However, at days 19 and then 24, significant content increases could be observed. Again, as in the case of the peroxide detection, a relatively low oxidation development can be concluded when compared to values concerning other fatty fish species under the same chilled conditions (Aubourg et al., 1997; Aubourg, 2001). Compounds produced as a result of interaction between lipid oxidation products and nucleophilic compounds (protein-type namely) were measured by fluorescence and browning. Since primary (PV) and secondary (TBA-i) lipid oxidation was low, little changes in fluorescence and browning could be detected, so that both indices did not provide interesting differences. Fatty acid analysis of the raw material led to the following proportions (%): 5.92 (C 14:0), 20.71 (C 16:0), 7.69 (C 16:1 ω9), 4.34 (C 18:0), 19.33 (C 18:1 ω9), 3.55 (C 18:1 ω7), 6.11 (C 18:2 ω6), 1.38 (C 18:4 ω3), 2.37 (C 20:1 ω9), 1.18 (C 20:4 ω6), 1.38 (C 20:4 ω3), 7.10 (C 20:5 ω3), 4.14 (C 22:5 ω6) and 14.79 (C 22:6 ω3). From a nutritional point of view, interesting and profitable polyunsaturated and ω3-polyunsaturated fatty acid contents were obtained. Little differences along the storage period could be observed for the PI (Table 1). Accordingly, this index did not show to be accurate for following the lipid damage. The lowest mean value was obtained at day 19. Astaxanthin content (Table 1) did not provide a decreasing tendency during the chilled storage, so that relatively high contents were present in the white muscle till the end of the experiment.

Table 1. Lipid changes* in Coho salmon white muscle during chilled storage**

<table>
<thead>
<tr>
<th>Chilled Time (days)</th>
<th>FFA</th>
<th>PV</th>
<th>TBA-i</th>
<th>PI</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.50</td>
<td>1.17</td>
<td>0.02</td>
<td>1.05</td>
<td>8.70</td>
</tr>
<tr>
<td>3</td>
<td>0.49</td>
<td>1.59</td>
<td>0.05</td>
<td>1.14</td>
<td>9.86</td>
</tr>
<tr>
<td>6</td>
<td>0.69</td>
<td>2.32</td>
<td>0.02</td>
<td>1.03</td>
<td>9.22</td>
</tr>
<tr>
<td>10</td>
<td>0.78</td>
<td>2.35</td>
<td>0.05</td>
<td>1.19</td>
<td>8.54</td>
</tr>
<tr>
<td>12</td>
<td>0.84</td>
<td>2.50</td>
<td>0.07</td>
<td>0.94</td>
<td>8.48</td>
</tr>
<tr>
<td>17</td>
<td>1.36</td>
<td>2.69</td>
<td>0.07</td>
<td>1.10</td>
<td>9.15</td>
</tr>
<tr>
<td>19</td>
<td>1.40</td>
<td>3.00</td>
<td>0.21</td>
<td>0.73</td>
<td>9.80</td>
</tr>
<tr>
<td>24</td>
<td>1.45</td>
<td>3.80</td>
<td>0.36</td>
<td>1.90</td>
<td>9.28</td>
</tr>
</tbody>
</table>

* Abbreviations: FFA (free fatty acids), PV (peroxide value), TBA-i (thiobarbituric acid index), PI (polyene index) and AST (astaxanthin).

** For each column, mean values (n=5) followed by different letters are significantly (p<0.05) different.

Conclusions

According to FFA, PV and TBA-i values, it is concluded that Coho salmon lipids have been relatively stable during the chilled storage when compared to other fatty fishes (Aubourg et al., 1997; Aubourg, 2001). Previous research has shown that endogenous astaxanthin could act as an active antioxidant during processing and storage of fatty fish species (Jensen et al., 1998). In the present study, an important astaxanthin content was present in the white muscle till the end of the experiment that could explain the relative stability of lipid matter. Sensory analysis showed a shelf-life of 17 days. Then, the fish was no more acceptable for consumption. Most lipid damage indices have led to an important increase at days 19 and 24. The shelf-life is high when compared to small size.
fatty fish species such as sardine and mackerel (El Marrakchi et al., 1990; Bennour et al., 1991) and agrees with previous results concerning other salmon species (Sveinsdottir et al., 2002; Fletcher et al., 2003).

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References


Authors

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3.21 INFLUENCE OF STORAGE METHOD AND FRESHNESS ON MASS TRANSFER PHENOMENA DURING SALMON (SALMO SALAR L.) SALTING

Lorena Gallart Jornet, Turid Rustad, Isabel Escriche, José Manuel Barat and Pedro Fito

Introduction

The main quality parameters for fresh salmon are fat, colour, texture and freshness (Sigurgisladóttir et al, 1997). Among them, freshness is one of the most important parameters of fish quality and can vary in most markets (Olafsdóttir et al, 1997). The effects of this wide variation are not clearly defined during the salting step. Freshness in salmon is not as important for the farming industry as for wild industries (Nielsen et al, 2002), however consumers prefer products that are perceived as fresh (Elvevoll et al, 1996). Traditionally the way of storing and distributing fresh fish is either in crushed ice in sealed boxes or frozen, which is a good alternative to extend the fish shelf life. Super-chilling has been used industrially with a few fish species to extend freshness-preservation up to 2 months (Kato et al, 1974). Salmon stored in the different ways mentioned above can be used for direct consumption or as raw material to make processed fish products such as marinated and smoked salmon. Salting of salmon is the first step in the production of smoked salmon.

In order to investigate the advantages and disadvantages of varying raw material freshness and the storage methods, the aim of this work was to analyze the influence on salmon fillets at different freshness and storage methods, on the mass transfer phenomena (measured as weight, salt and water changes) during salmon salting (12 hours of dry-salting without drainage).

Materials and methods

Fish material

A land-based salmon farm in Mid-Norway supplied farmed gutted salmon with an average weight of (3.35 ± 0.3) kg. The fish samples were stored on ice in packed boxes of 25 kg. After rigor mortis was accomplished for 2 days, it was kept under (iced storage at 4ºC during 0 days (raw material 1), 7 days (raw material 2) and 14 days (raw material 3), frozen storage at –40ºC during 30 days (raw material 4), super chilled storage at –1ºC during 7 days (raw material 5) and 14 days (raw material 6)). After the storage treatment, each salmon was filleted, tagged and dried salted with ordinary refined salt for 12 hours, in plastic containers without drainage at 4ºC. 8 fillets were weighed periodically and used to determine the evolution in weight change according to equation 2.

Analytical determinations

Samples for physico-chemical analysis {NaCl (Volhard (AOAC 937.09,1990)) and moisture was determined gravimetrically after drying at 104ºC for 24 hours) were taken from two different fillets randomly and each fillet was analysed twice at 0, 4, 8 and 12 hours of the salting process. Lightly salted salmon was considered upto 12 hours of salting as the raw material for the smoking industry. Salt concentration referring to the fish liquid phase (zNaCl) was estimated from water (xw) and sodium chloride (xNaCl) weight fractions determinations according to equation 1 (Fito and Chiralt, 1996; Barat, et al 2002).

\[
Z_{NaCl} = \left( \frac{x_{NaCl}}{x_w + x_{NaCl}} \right) \quad (1)
\]

The total, water and NaCl salmon weight changes (∆M°w, ∆M°, and ∆M°NaCl, respectively), determined by means of equations 2, 3 and 4 (being M° the salmon weight at the sampling time t and 0, xw, and xNaCl the salmon water weight fractions, xNaCl and xNaCl, the salmon NaCl weight fraction at time t and 0 respectively), along the salting process as a function of the raw material used were:

\[
\Delta M_1^o = \frac{M_{1}^o - M_{0}^o}{M_{0}^o} \quad (2)
\]
\[ \Delta M^w_t = \left( M^w_0 \cdot x^w_t - M^w_0 \cdot x^w_0 \right) \]
\[ \Delta M^{NaCl}_t = \left( M^{NaCl}_0 \cdot x^{NaCl}_t - M^{NaCl}_0 \cdot x^{NaCl}_0 \right) \]

**Results and discussion**

Fig. 1 shows the evolution in weight and NaCl concentration in the salmon liquid phase changes throughout 24 hours of salting. As regards the \( z^{NaCl} \) value, it is observed that during the first hours, the higher values corresponded for salmon stored under 1 and 2 weeks under refrigeration (batch 2 and 3), while the fresh and thawed salmon (batch 1 and 4) reached lower values for the same processing time. Regarding the weight changes, they are higher for the salmon chilled stored for 2 weeks (batch 3-DMt), while the weight of the salmon chilled stored 1 week (batch 2-DMt) was the lower. It seems that the mass transfer phenomena is intercepted when the salmon muscle structure is well kept.

![Graph showing weight changes and NaCl concentration throughout salting time](image)

Fig. 1. Total salmon weight changes and salt concentration referred to the fish liquid phase (\( z^{NaCl} \)) throughout the salting time.

Regarding to salt concentration referred to the liquid phase (\( z^{NaCl} \)), to calculate the salting time required for each raw material for reaching a referenced value of \( z^{NaCl} \) (considered as 0.05 salt concentration in the liquid phase for this product before the smoking step), a linear adjust between the salt concentration in the liquid phase and the root square time was made (Fig. 2). As it can be observed, the results follow a linear correlation with a good correlation coefficient. Moreover, there are two groups: on the one hand the salmon ice-stored for 1 and 2 weeks (raw material 2 and 3), on the other, thawed salmon (raw material 4) and fresh salmon (raw material 1) which also justify the same tendency explained in the Fig. 1. This is to say the fresher raw material (1) and the frozen (4) are the ones, which need a longer salting time 6.5 and 5.9 hours respectively to reach the 5% NaCl concentration in the liquid phase compared to the raw material 2 and 3, that only need about 3.7 hours of salting to reach the same level. This fact indicates that an increase in the chilled storage time for salmon implies a marked decrease in the salting time needed to obtain a smoked salmon product.
Fig. 2. Salt concentration referred to the fish liquid phase ($z_{\text{NaCl}}$) versus $t^{0.5}$

The total, water and NaCl salmon weight changes ($\Delta M_{12}$, $\Delta M_{12w}$ and $\Delta M_{12\text{NaCl}}$) after 12 hours of salting is shown in the following Fig. 3. It can be seen that the total weight changes are quite small for all the batches. Concerning weight yield, a positive weight increase and therefore better yield during salting was observed in batches stored for 0 days and 7 days chilled and 7 days superchilled (1, 2 and 5), while batches 3, 4 and 6 lost weight.

Fig. 3. Total, water and NaCl salmon weight changes after 12 hours of salting depending on the batches 1, 2, 3, 4, 5 and 6.

Once more, it is observed that the highest weight increase was for the batch 2 (1 week ice-stored). Regarding to water weight changes, the highest loss corresponded to the freshest and frozen raw material (batch 1 and 4), while the lower loss was for salmon batches 2 and 4 (1 week chilled and superchilled), which justifies better yield in
the salting step when using 1 week old independently of the storage method. Observing the salt uptake weight changes, the lower value was for batch 1 (the freshest raw material), it could be probably due to better kept tissue structure, which could limit the mechanism of salt uptake. Regarding to the thawed batch (4), the behaviour is similar to the fresh batch (1). Although batch 4 has been frozen, it seems that there is not a markedly variation in the tissue structure, either due to the use of very low freezing temperatures (-40°C), or the fatty tissue nature of the salmon may tolerate brusque changes, or a combination of both. Superchilled storage, seems to have an intermediate behaviour between the freshest batch and the oldest refrigerated batch.

Conclusions

In conclusion, from a mass transfer phenomena and an industrial point of view, the optimum raw material is the use of 7 days old ice-stored salmon for salting as a first step of the smoking industry which seems to have better salt uptake reaching the referenced salt concentration in the liquid phase with less time and a markedly decrease of water loss which implicates better yield within the salting process.

References


Acknowledgements

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4.1 COMPOSITIONAL ANALYSES OF COD (GADUS MORHUA) AND ATLANTIC SALMON (SALMO SALAR) BY HIGH RESOLUTION 1H MR: APPLICATION TO AUTHENTICATION ANALYSES

Martinez, I., Bathen, T., Standal I.B., Halvorsen, J, Aursand, M, & Gribbestad, I.S.

Introduction

Aquaculture opens up possibilities for controlling the eating quality and nutritional value of seafood. The texture and flavour of fatty fish are greatly influenced by the fat content and its distribution in the muscle [Mohr, 1987] and its positive effect on human health is due to their high content in PUFAs, in particular EPA (eicosapentaeanoic acid, 20:5, n-3) and DHA (docosahexaenoic acid, 22:6, n-3) [Vanschoonbeek et al., 2003] and its content in vitamins, minerals and some small molecules (osmolytes, metabolites) whose main function in vivo is to stabilise the structure of proteins [Arakawa and Timasheff, 1985; Konosu and Yamaguchi, 1982], protect cells against osmotic stresses [Burg et al., 1997] and prevent oxidative damage [Hou et al., 2003; Undeland et al., 2002]. Molecules of known positive effects include anserine and carnosine [Candlish and Das, 1996; Hou et al., 2003; Kang et al., 2002], taurine [Schaffer et al., 2000a,b and references therein], choline and betaine [Chambers, 1995]. Lipids and osmolytes are also of relevance in determining the quality and authenticity of Atlantic salmon products. The fatty acid (FA) profile of fish muscle reflects the FA composition of the feed and therefore may be used to discriminate farmed from wild fish [Aursand and Axelson, 2001]. Osmolytes have a potential to serve as markers to trace back the history of the fish since the type and amount of metabolites is affected by physiological factors, stress prior to death, time elapsed post mortem (freshness) and processing parameters, in particular cooking and freeze/thawing (the latter due to the drip-loss occurring after thawing). Metabolite profiling by high-resolution MR spectroscopic techniques has indeed been used for species identification and for the identification of single components in complex mixtures [Fan et al., 1993, Gribbestad et al., 1994]. Consequently, it would be of interest to standardise analytical methods that permit the analysis of lipids and metabolites in fillet samples and in vivo. The latter would permit the possibility to examine the effect of the diet and breeding conditions on the fish composition and also to classify the fish for different purposes according to their biochemical composition. After death, similar analyses would be of relevance to authenticate the product and, again, to determine its biochemical composition and nutritional value.

Magnetic Resonance (MR) spectroscopy is a multicomponent detection technique that offers the opportunity to detect most of the mentioned molecules and study biological tissue non-invasively and non-destructively both in vivo and in vitro. Changes in post mortem metabolism such as the glycogen levels, lactate, creatine and phosphocreatine content, intracellular pH, and K-value have been followed using MR in carcasses after slaughter [Lundberg et al., 1986] and in extracts of Atlantic halibut (Hippoglossus hippoglossus) [Sitter et al., 1999] and Atlantic salmon [Aursand et al., 1995]. In order to interpret spectra of intact tissue it is important to interpret first high-resolution spectra of lipid and acid extracts. Localised in vivo 1H spectroscopy based on MR images combines the information about anatomical structures and biochemical processes from the same area, and it is established as a diagnostic method for certain human pathologies [Ross and Michaelis 1994; Kvistad et al., 1999]. This technique may be a valuable tool in basic fish research as well for the study of biochemical changes in vivo during growth under different conditions.

The primary aim of work was to interpret the HR 1H MR spectra of Atlantic salmon and cod. In addition, we examine the possibility to study metabolic profiles intact Atlantic salmon and, in cod, we conducted to some analyses to evaluate the effect of freezing and thawing on the metabolite profile.

Materials and methods

Analyses of Atlantic salmon.

Farmed Atlantic salmon (Salmo salar) of about 3-4 kg of weight, that had been eviscerated and ice stored for about 3 days were ordered from a local retailer. In vitro and in vivo spectroscopy was performed on fillets and on whole fish respectively. Muscle samples under the dorsal fin, used for the preparation of extracts were immediately minced and frozen stored at –80°C. The lipid and perchloric acid (PCA) extractions were performed according to Bligh and Dyer [1959] and [Gribbestad et al., 1994] respectively. 1H spectra of lipid and PCA extracts and in vitro 1H MR spectroscopy of intact muscle tissue were obtained on a DRX 500 Bruker instrument (Bruker, Karlsruhe, Germany) at 500.13 MHz; and volume selective proton spectroscopy (in vivo 1H MR
spectroscopy) of Atlantic salmon on a Gyroscan S15 (Philips Medical Systems, Eindhoven, The Netherlands) whole body system working at 1.5 Tesla, as described by [Gribbestad et al, 2004].

Analyses of cod

Fresh cod had been caught the same day that was delivered to the lab. Muscle samples under the dorsal fin of about 5 g were excised and frozen at -80°C, prior to freeze drying. Frozen cod was purchased at a local retailer shop and thawed in ice. The fish had been frozen for 5 months. Samples of the thawed muscle and of the drip water were frozen at -80°C prior to freeze drying. The freeze-dried powder was extracted with PCA as described below. PCA extraction of cod was a modification of that applied to salmon: 1.5 ml of 2.5% PCA was added to about 15 mg of the freeze-dried cod samples, left on the bench for 5 min and then continuously stirred for 15 min prior to centrifugation for 4 min at 2,000 g. 1.2 ml of the supernatant were taken out and mixed with 675 µl of 0.36M K₂CO₃. Additional insoluble material was allowed to precipitate for 10 min at room temperature. The tubes were centrifuged (4 min at 2,000 g.) and 1.2 ml were taken to a new Eppendorf tube, freeze dried and frozen stored at -80°C. Prior to MR analysis, the lyophilized samples were dissolved in 0.7 ml of 5mM TSP (trimethylsilyl propionate) in D₂O, and the pH adjusted to 7.5 with addition of 0.5M NaOD. The sample was transferred to 5mm MR tubes. High resolution ¹H MR spectra were recorded at ambient temperature on Bruker Avance DRX500 spectrometer operating at 500.13 MHz. Chemical shift referencing was performed relative to the methyl groups of trimethylsilyl propionate (TSP) at 0.00 ppm.

Identification of chemical components was performed by (1) comparison with those previously published by us and other authors, (2) analyzing pure compounds (betaine, taurine, choline, anserine and creatine) dissolved in 0,5 ml 5mM TSP in D₂O and (3) spiking some samples with a small amount of pure compounds to ensure correct identification.

Results and discussion

Atlantic salmon

From the ¹H MR spectrum of extracted lipids from Atlantic salmon it was possible to estimate the total amount of omega-3 fatty acids and the content of DHA and the values estimated by this method were comparable to those obtained by gas chromatography or ¹³C MR, the analysis can be carried out with a high degree of automation and with short acquisition times (2-5 min), but additional relevant information such as the identification and quantification of individual fatty acids, total saturated, mono- and polyunsaturated fatty acids, omega-3 and omega-6 and the preferential positional distribution of 20:5, 22:5 and 22:6 in triacylglycerols does require ¹³C MR analyses [Aursand et al., 1994].

The ¹H MR spectra of perchloric acid extracted metabolites from Atlantic salmon reported for the first time in this work were dominated by signals from lactate, amino acids (leu, ile, val, lys, ala, glu, gln, and gly), creatine/phosphocreatine and anserine; taurine, choline and formate could also be assigned.

Generally, the skeletal muscle of fish contains large amounts of histidine and histidine-derived dipeptides (anserine) [Crush 1970]. Although the physiological function of the dipeptides is not yet clear it has been shown that anserine contributes to the intracellular buffering of the fish muscle [Abe and Okuma 1991, Abe et al., 1985] and that it has an antioxidant function by chelating ions and scavenging free radicals [Boldyrev et al., 1988]. The dipeptides seem more important in muscles that are used mostly for burst activity and rely on glycolytic metabolism with its lactic acid production [Cameron 1989]. The signals from amino acids identified in the spectra stem most likely from single free amino acids although there might be contributions from peptides. The low field portion of the PCA spectrum is dominated by signals from formate and from the histidine moiety in the anserine molecule. Also hypoxanthine is identified. It has been shown by Sitter et al., [1999] that integration of the high resolution ¹H MR spectra permits to quantify single components, which these authors used to estimate the freshness of Atlantic halibut based on its K-value. Hypoxanthine is not a good freshness indicator in Atlantic salmon [Kennish and Kramer, 1986] its levels may be affected by stress at death [Erikson et al., 1997 and references therein] and, obviously, by the storage temperature [Sigholt et al., 1997].

¹H MR spectra of intact fish muscle permitted the identification of fatty acids, anserine, lactate, acetate, creatine/phosphocreatin, choline and choline containing compounds, ala, gly, and hypoxanthine and the using volume localised ¹H spectroscopy it was possible to identify fatty acids, creatine/phosphocreatin, anserine/choline, glycercyl and the histidine moiety in anserine (Fig.1).
Cod

The $^1$H MR spectra of perchloric acid extracted metabolites from cod, also reported for the first time in this work was, as expected, clearly dominated by TMAO, followed by creatine, anserine, amino acids (gly, ala glu, gln, leu, ß-ala, val, leu, ileu), taurine, choline TMA, hypoxanthine, lactate acetate, formate and betaine. DMA was detectable in all the samples that had been frozen. Although the analysis as performed was not quantitative, it seemed that the drip loss had a higher content per gram freeze dried matter of TMAO, taurine, creatine and choline than either the fresh or the frozen fish, indicating that it is preferentially lost during thawing. Also there seemed to be an increase in the amount of detectable peaks in the thawed samples and specially in their corresponding drip loss. This is an indication of increasing amount of smaller molecules, which may be due to degradation of bigger molecules or to their "liberation" from forms or compartments where they may have been bound. Figure 2 illustrates the results of three samples in the region of 2.5 to 4.5 ppm.

Conclusions

The techniques presented here made it possible to identify single chemical components, such as hypoxanthine, some amino acids, taurine, anserine, lactate and some fatty acids, in extracts, in whole muscle and in whole fish and may have therefore direct practical application in the i) the selection of live specimens for breeding, ii) the classification of both live specimens and fillets according to their qualitative and quantitative content of lipids and small molecules, which is of relevance for the nutritional value of fish and in iii) authentication analyses of seafoods, since the profiles are not only species-specific but also permit the discrimination of frozen and fresh fish in the case of cod and species in which DMA is formed under freezing.
Figure 2. - HR $^1$H MR spectra of PCA extracts from cod: fresh (upper), frozen and thawed (middle) and the drip loss of the frozen samples shown in the middle panel (lower panel). Only the region between 2.5 and 4.6 ppm is shown.

Acknowledgments

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References


Authors

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4.2 RELATIVE QUANTITATIVE TAQMAN™ REAL TIME POLYMERASE CHAIN REACTION SYSTEM FOR THE IDENTIFICATION AND QUANTIFICATION OF THE MOST VALUABLE CANNED TUNA FISH SAMPLES

Miguel Angel Pardo

Introduction

Nowadays, the authentication of canned tuna products by analysing genetic markers amplified by PCR is extensively used (Quinteiro, et al., 1998; Mackie, et al., 1999; Ram, et al., 1996; Rehbein, et al., 1999; Pardo and Pérez-Villareal., 2004). Taking into account that the cans are frequently presented in different chunks or even completely mixed, a trustworthy identification method was much more difficult to develop. To resolve this problem it was necessary to analyse every chunk what is more expensive and time demanding. Moreover, when the product was highly mixed the genetic identification carried out with molecular techniques such as PCR-RFLP or PCR-FINS was completely impossible to deal with.

Real Time PCR technology has been widely applied to quantify the presence of a specific gene in food (i.e. detection of transgenic genes in corn). Absolute quantification requires a feasible DNA extraction and the assumption of similar number of genome per mass of muscle tissue for different species. Taking into account that the number of mitochondrial genome per cell is variable depending of the tissue and species (Battersby and Moyes 1998), nuclear genes are used rather than mitochondrial genes in absolute quantification studies. However, in the case of Scombroidei species most of the DNA sequence information is focused on mitochondrial genome. For that reason, it was necessary to devise a relative quantification methodology which allowed us to estimate the number of mitochondrial gene copy through the development of a consensus system. This way it was possible to estimate the relative presence of a particular mitochondrial gene target with the consensus gene. Brodmann and Moor (2003) devised a system to quantify beef content using a mammalian system as housekeeping gene.

The methodology based on TaqMan™ presented here is a rapid analytical method to detect the presence of $T. \text{alalunga}$ and $T. \text{albacares}$ in seafood samples. Actually it is the first one-step protocol developed to identify and quantify tuna species, by far.

Methodology

The Scombroidei specimens were obtained from a local market or provided for the tissue collection belongs to the Seafood Biochemistry Group from IIM-CSIC (Instituto de Investigaciones Marinas, Spain). All individuals were morphologically identified attending to external characters. In the Table 1, are listed 40 individuals of species belong to the Scombroidei suborden.

Table 1. List of Scombroidei species collected during this study. N number of individuals.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Code</th>
<th>Common name</th>
<th>Source</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthynnus alleteratus</td>
<td>LTA</td>
<td>Little tunny</td>
<td>IIM-CSIC</td>
<td>3</td>
</tr>
<tr>
<td>Katsuwonus pelamis</td>
<td>SKJ</td>
<td>Skipjack</td>
<td>Local market</td>
<td>5</td>
</tr>
<tr>
<td>Sarda sarda</td>
<td>BON</td>
<td>Atlantic bonito</td>
<td>IIM-CSIC</td>
<td>3</td>
</tr>
<tr>
<td>Scomber japonicus</td>
<td>MAS</td>
<td>Chub mackerel</td>
<td>IIM-CSIC</td>
<td>3</td>
</tr>
<tr>
<td>Thunnus alalunga</td>
<td>ALB</td>
<td>Albacre</td>
<td>Local market</td>
<td>5</td>
</tr>
<tr>
<td>Thunnus albacares</td>
<td>YFT</td>
<td>Yellowfin tuna</td>
<td>Local market</td>
<td>5</td>
</tr>
<tr>
<td>Thunnus obesus</td>
<td>BET</td>
<td>Bigeye tuna</td>
<td>Local market</td>
<td>5</td>
</tr>
<tr>
<td>Thunnus thynnus</td>
<td>BFT</td>
<td>Bluefin tuna</td>
<td>Local market</td>
<td>1</td>
</tr>
</tbody>
</table>

Tuna mixed samples were prepared homogenizing the white muscle with a blender. To validate the quantitative techniques developed in this study, different commercial canned tunas were purchased at the
local market. These tunas had been treated with vegetable and olive oil and brine. DNA extraction was
carried out as the method described in Pardo and Pérez-Villareal (2004).
All the primers and fluorogenic probes were designed using the Primer Express™ v2.0 software (Applied
Biosystems). Primers and TaqMan™ probes were chosen based on the alignment of mitochondrial
sequences collected from the GenBank and by Pardo and Pérez-Villareal (2004). All the primers and
probes were purchased to Applied Biosystems. Every multiple alignments were carried out using the
Clustal X program (Thompson et al., 1997).
Amplification was performed in a MicroAmp Optical 96-well reaction plate from Applied Biosystems.
TaqMan™ reactions were carried out with TaqMan™ Universal Master Mix (Applied Biosystems)
containing the primers and probes designed and 10-100 ng of DNA. Reactions were run on the ABI
Prism™ 7000 sequence detection system (Applied Biosystems) with the following thermal conditions:
50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for a min.
The relative quantification method does not use a known amount of standard but compares the relative
amount of the target sequence to any of the reference values. The target and endogenous control
amplifications were carry out in separate tubes. At the end of each reaction, a C_t variation (ΔC_t = C_t
Target – C_t Reference) for each target was calculated from the C_t values. In the end, each ΔC_t value were transformed
to a percentage (%Target= 2^{-ΔCt} x 100).

Results

In this work TaqMan™ technology was applied to develop a novel relative quantification method based
on the comparison of a target gene (mitochondrial citochrome b gene) with an endogenous control gene
(conserved region in mitochondrial 16S rRNA gene). Published mitochondrial DNA sequences of 16S
rRNA gene of 14 species belonging to the Scombroidei suborden (Table 2.) were aligned with the
purpose of finding out a consensus region. Two conserved primers and a specific probe were selected in a
high conserved region of 130 basepairs. Moreover, two pairs of primers and specific probes were
developed in order to identify albacore and yellowfin by scrutinising mitochondrial cytochrome
b sequences (61 sequences were analysed) previously described in the bibliography (Pardo and Pérez-
Villareal, 2004). In a similar way, Brodmann and Moor (2003) devised a system to quantify beef content
using a mammalian system as housekeeping gene.

Table 2. List of GenBank mitochondrial DNA sequences of 16S rRNA of Scombroidei species.

<table>
<thead>
<tr>
<th>Scombroidei species</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auxis rochei</td>
<td>NC_005313, AB105165, AB103467 and AB103468</td>
</tr>
<tr>
<td>Euthynnus alleteratus</td>
<td>AB099716 and NC_004530</td>
</tr>
<tr>
<td>Katsuwonus pelamis</td>
<td>NC_005316 and AB101290</td>
</tr>
<tr>
<td>Lepidopus caudatus</td>
<td>AF100917, AF100918, AF100919 and AF100920</td>
</tr>
<tr>
<td>Leptura canthus sp</td>
<td>AB125749</td>
</tr>
<tr>
<td>Rexea solandri</td>
<td>AF221898</td>
</tr>
<tr>
<td>Scomber australasicus</td>
<td>AB032522</td>
</tr>
<tr>
<td>Scomber japonicus</td>
<td>AB032521</td>
</tr>
<tr>
<td>Scomber scombrus</td>
<td>AF055615 and AY048303</td>
</tr>
<tr>
<td>Scomberomoraras tritor</td>
<td>AF231539</td>
</tr>
<tr>
<td>Thunnus alalunga</td>
<td>NC_005317 and AB101291</td>
</tr>
<tr>
<td>Thunnus thynnus</td>
<td>NC_004901, AY302574 and AB097669</td>
</tr>
<tr>
<td>Trichiurus japonicus</td>
<td>AB126630</td>
</tr>
<tr>
<td>Trichiurus lepturus</td>
<td>AY216492, AY216493 and AY216494</td>
</tr>
</tbody>
</table>

Three detection systems were tested for their selectivity and cross-reactions with those Scombroidei
species listed in the Table 1. The Scombroidei system used as endogenous gene was applicable to every
species used in this work, whereby the detected C_t values were reasonable similar. Neither T. alalunga
system nor T. albacares system has been detected cross reactivity with other related species. We
concluded that the systems are specific to their targets.

In order to apply the relative quantification equations described in the Methodology, both relative
methods were optimised by means of testing the linearity and efficiency. Linearity tests of both methods
were closed to the theorical value of -3.32. As a result a PCR efficiency nearly 100% was achieved
(Figure 1.). Furthermore, the slopes from the target and endogenous gene of two detection methods were
identical, so it was not necessary applying for normalization. Similar results were obtained with DNA extracted from canned tuna.

Figure 1. (A). Linearity test of the ALB and 16SCOM specific TaqMan™ systems belonging to the T. alalunga specific quantification method, (B). Linearity test of the YFT and 16SCOM specific TaqMan™ systems belonging to the T. albacares specific quantification method. Ct values are plotted versus the logarithm of the DNA concentration.

Mixed frozen tuna samples were assayed with the methodology developed, obtaining trustworthy results (Figure 2.). In relation to canned samples, preliminary results indicated that the methodology devised is suitable to quantify albacore and yellowfin in canned samples. However, further studies will be carried out in order to validate the methodology with mixed canned tuna samples.

Figure 2. Relative quantification of several mixture of ALB and YFT
References


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4.3 NEW ISSUES ABOUT AN OLD STORY: AUTHENTICATION OF TUNA CANS

Chapela Garrido M.J., Sotelo C.G*, Pérez-Martín R.I., Pardo M.A., Pérez-Villarreal B., Gilardi P.

Objective

To study the influence on DNA extraction efficiency and quality of strain liquid or sauce in tuna cans. To evaluate the identification resolution of different mitochondrial fragments using FINS (Forensically Informative Nucleotide Sequencing).

Methodology

Several types of yellowfin tuna cans were selected to study different extraction methodologies. Tuna cans contained the same species, yellowfin tuna, and different type of presentations: brine, oil, vinegar and tomato sauce. Extraction methods were selected considering the type of DNA separation methodology employed (i.e. membrane silica, salts, binding resin, etc…). DNA recovery efficiency was evaluated using UV measurement and DNA quality by PCR. Fragment size of extracted DNA was evaluated by using a set of primers aiming amplification of different size fragments. Cytochrome b was fully sequenced in 9 tuna species (Thunnus alalunga, T. albacares, T. thynnus, T. orientalis, T. atlanticus, T. obesus, E. alleteratus, Sarda chilensis, S. sarda) in order to evaluate the identification ability of different published methodologies. The method used was genetic distance measurement and phylogenetic tree reconstruction.

Results

DNA was extracted from tuna cans using SDS-Proteinase K digestion followed by DNA recovery using WIZARD DNA Clean-up system, NucleoSpin (Clontech), GenomicPrep (Amersham Pharmacia Biotech) and CTAB. The highest DNA recovery was obtained in cans with oil filling, whereas tomato sauce and vinegar produced the lowest DNA contents. Also the average size of DNA fragments were higher in the oil and brine samples.

Cytochrome b sequences from 9 tuna species were studied to identify diagnostic positions for the most important tuna species. Also DNA sequences of the 9 tuna species were analysed for different published diagnostic fragments. It was found that most published diagnostic fragments were not able to provide full identification of some of the nine considered species. A new diagnostic fragment is proposed which permits the identification of the 9 tuna species considered.

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4.4 TREATMENT OF TUNA PRODUCTS WITH CARBON MONOXIDE; PRINCIPLES OF ASSESSMENT AND ACTUAL ANALYTICAL ASPECTS

Frerk Feldhusen, Hartmut Rehbein, Reinhard Kruse

Introduction

The commercial fish industry has to maintain the colour characteristics of aquatic foods during processing, transport, storage and display and at the same time assure safety (Ross 2000). Dark muscle fish species get an important quality attribute from the oxygenated and reduced form of the heme proteins myoglobin and hemoglobin (Mb/Hb-Fe$^{2+}$-O$_2$). Dark muscle is very susceptible to discoloration after it is cut and also on freezing, yielding a brown colour due to the oxidation of the heme proteins to give metHb and metMb (Mb/Hb-Fe$^{3+}$) (Livingston and Brown, 1981). This discoloration is highly undesirably as the product becomes less appealing to the consumer, thus leading to a lower price than a bright coloured cut. To avoid or to minimize discoloration some processors/importers introduced the use of carbon monoxide (CO) and “tasteless smoke” or “clear smoke” containing CO to stabilize the colour of red muscle (Kristinsson et al. 2003, Feldhusen 2003). This stabilization is due to the strong binding of CO to the heme in hemoglobin and myoglobin, making it highly resistant to autoxidation and discoloration (Sorheim et al. 1997).

The use of CO applied either as a single gas or a component in “tasteless smoke” is increasing in both domestic and international fish commerce in the United States. Use of filtered smoke to concentrate the favourable components and CO were patented in the 1990’s (Yamaoka et al. 1996), in terms of fish application the use of “tasteless smoke” in frozen seafood was patented (Kowalski 1999). Today CO is being applied to seafood as a single gas, as a component in filtered “tasteless smoke” and more recently as so-called artificially-filtered smoke based on gas blends to exemplify “tasteless smoke” (Otwell et al. 2003). A controversial decision by the U.S. Food and Drug Administration suggested recognition as a generally recognized as safe (GRAS) procedure with required labelling to distinguish treated products. “Clearsmoke” is a system of which after filtering the remaining smoke particles are removed by usage of ozone in the final processing.

The Standing Committee on the Food Chain and Animal Health of the EU, Section on Toxicological Safety of the Food Chain officially ascertained on the use of carbon monoxide in tuna fish: The process has an effect on the colour of the fish by maintaining a bright red appearance. Consumers can therefore be misled as to the freshness of the product, as the colour remains even when the fish deteriorates. The Netherlands had refused the use of carbon monoxide as this gas is not in the list of authorised food additives. The producers then switched to so-called “cold smoking” or “clear smoking”. However, the technique does not impart a smoky flavour or the typical colour which results from smoking, but is nevertheless considered as a smoking process by the Courts in the Netherlands. The Committee expressed concern that consumers were being misled as to the freshness of the product. Carbon monoxide would fall under the definition of a food additive and was thus not authorised. The Committee also agreed that if a product was labelled as “smoked”, it must have a smoky flavour. Finally, reference was made to Directive 91/493/EEC on fishery products, which requires that treatments applied to inhibit the development of pathogenic micro-organisms or constituting an important element in the preservation of the product must be scientifically recognised or formally approved.

Because of the illegality of use of CO for fresh and frozen fish in EU methods for detection of CO are needed. SANCO of the EU 2003 published results of investigations from the Netherlands (Jonker et al. 2001). The method used makes it possible to determine carbon monoxide in tuna with sufficient accuracy. Carbon monoxide is formed naturally in the tuna tissue. Although it is not expected that this basal level will be higher than 20 to 50 µg/kg, the action level was set to 200 µg/kg in conformity with Japanese regulations. The following investigation shows results of carbon monoxide determination in correlation to the CO-heme protein content and the colour from tuna samples.
Material and Methods:

Material

Samples were taken from the north German market and originated from different levels of commerce. The type of storage prior to arriving at the institutes was either the deep frozen status or samples were cooled at 0 °C using water ice. After entering the laboratories they were normally analysed as soon as possible. In several cases however a further intermediate deep frozen storage became necessary.

Methods

1: Detection of Carbon Monoxide Treatment of Tuna:
The relative amount of CO-heme protein (CO-Hp), as percentage of total heme protein, was determined by the method of Beutler & West (1984) in the following adaptation to analysis of fish muscle:

About 20 grams of fish muscle were cut into small pieces and centrifuged at high speed (Sorvall RC 5B, rotor SS34, 18 000 rpm, 5 °C, 30 min) yielding 2-5 ml of muscle press juice to be used for determination CO-Hp.

A volume of 0.1 ml of press juice was mixed with 0.1 ml of 10 mM potassium phosphate pH 6.85 and incubated for 5 min at ambient temperature. Then 0.1 ml of the mixture was pipetted into a cuvette containing 1.15 ml of reducing solution (25 mg of sodium dithionite/20 ml 0.1 M potassium phosphate pH 6.85), and mixed by inverting gently several times.

The absorbance at 420 nm and 432 nm was read against a cuvette containing water. The fraction of CO-Hp was calculated according to the equation given by Beutler & West using the constants determined for reduced and CO-hemoglobin. The results are given as % CO-Hp.

Method 2:

Colour measurement (CIE L*a*b*) of intact tuna muscle was performed using a spectral colour meter Spectro-pen ® (Dr. Lange, Düsseldorf, Germany). In this system L* denotes lightness on a 0 to100 scale from black to white; a*, (+) red or (-) green; and b* (+) yellow or (-) blue. Measurements were repeated 5-fold at least. Comparative measurements were performed using a tristimulus colorimeter CR 300 (MINOLTA, Ahrensburg, Germany).

Method 3:

Determination of Carbon Monoxide in Tuna by Headspace GC FID.
The specified procedure of head-space gas chromatography, presented in “SANCO-2003-02727-00-00TRA-00(NL),” (2001) is actually the chemical-analytical test-method of choice. This method has recently also been installed in the Veterinary Institute for fish and fishery products Cuxhaven. We have elaborated different suitable steps to make it available for standard conditions and usual equipment in laboratories engaged in food control.

GC Equipment (major components): Split injector or transfer capillary injector, wide bore column (molecular sieve, 5 A), FID, nickel catalyst

Calibration standards: Gas mix containing 100 vpm CO in synthetic air (commercially available)

Sample Handling and Measurement

1. Take care for keeping the sample cooled down to no more than 5 °C.
2. Prepare a representative subsample of at least 50 g, avoid temperature raising, cut manually into small pieces.
3. Add 20 ccm precooled water (< 5°C) to 10 g sample material in a 50 ml centrifugation tube.
4. Apply Ultra Turrax homogenisation, work quickly (< 1 min.), avoid temperature raising.
5. Centrifuge at 5 °C for 7 min.
6. Transfer a supernatent of 10 ml to a 20 ml head space vial.
7. Add 6 µl n-octanole.
8. Tilting the vial add carefully 4 ml sulphuric acid (w = 20 %).
9. Immediately close the vial.
10. Shake for 1 min.
11. After 10 min, repeat shaking.
12. Inject an aliquote of the head space into the GC.
13. For calibration purpose: Inject different amounts of gas mix, using unique injection volumes for sample and calibration standards measurements.

Results and Discussion

Spectrophotometric Measurement of CO-Myoglobin and –Hemoglobin and Colour Measurement
The spectrophotometric method used in this study had been originally developed by Beutler & West for measurement of the carboxyhemoglobin content of blood. The method is based on the production of reduced myoglobin and hemoglobin by dithionite, which does not react with CO-hemoproteins. By measuring the absorbance of the pigments at 2 wavelengths (420 and 432 nm) the fraction of CO-Hp can be determined.

Preliminary experiments with myoglobin and hemoglobin from horse revealed that both pigments gave comparable results when treated with CO and analysed spectrophotometrically.

As it was found that frozen storage of tuna meat reduced the amount of CO-Hp considerably in a number of samples (Table 1, series 2), analysis of commercial products should be performed directly after sampling. However, other products expressed a greater stability of CO-Hp (Table 1, series 1). As we have no explanation for this phenomenon, interpretation of CO determination must be made with caution. Absence of measurable CO-Hp does not indicate that the product has not been treated with tasteless or liquid smoke.

On the other hand it can be concluded that such a treatment must have been performed, if the content of % CO-Hp is greater than 30 %. In any case, the method is giving only qualitative results.

The instrumentally measured redness does not really reflect differences in hemoprotein contents, especially when looking at measurements taken on samples of series 2. A better agreement can be found for series 1. The lower heme protein contents of the first 5 samples are mirrored by lower a* values compared to last 5 samples for which higher contents on heme protein obviously cause also higher a* values. To show how important the use of the instrument for colour measurement is, values taken by a second instrument (CR 300) are included in Table 1 for series 1. The much higher a* values taken by this instrument result from different standards used for calibrating the instruments. However, even this higher a* values do not correspond to the a* value of 16.2, which has been established recently by USDC as criterion to be “near normal in flesh colour” of tuna. Values greater this standard could be seen as treated with CO containing gas.

GC measurements in comparison with quantified CO-Hemoprotein
In the recent weeks we have checked a total number of 32 samples of tuna. The applied GC-method has shown it’s excellency for this special approach in the field of food control. But it is an important fact, that the reduced stability of the analyte can cause false low results.

A limited number of 11 samples has been checked for both parameters. These results are summarized in the attached Table 2. It became evident, that every freezing and thawing step, which had necessarily to be accepted, causes a significant decrease of red colour as well as detectable CO concentrations. Furthermore the weak fixation of CO to the tuna globins causes a continuous decrease of parameters even at constant storage temperatures between -20 and -30 °C. So it becomes evident, that we found only a limited correlation of R = 0.64 between GC-CO and CO-heme protein. It has to be pointed out, that GC-CO measurement has been the first step, which was followed by CO-heme protein determinations a few days or even some weeks later.

So we are able to state as follows:

1. The recommended limit of 200 µg CO per kg fish flesh can be very helpful to decide whether or not a sample has definitely been CO treated.
2. In our opinion this way of CO application can not be tolerated and has to be judged as an illegal practice.
3. Besides numerous incriminated samples tuna products are nevertheless commercially available with pretty red colour, acceptable quality and without extended CO concentrations.
Table 1: Fraction of CO-Hemoprotein and redness values of tuna muscle

<table>
<thead>
<tr>
<th>Code</th>
<th>% CO-Hemoprotein</th>
<th>a* spectro-pen</th>
<th>a* CR 300</th>
</tr>
</thead>
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<tr>
<td><strong>Series 1:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11979/02</td>
<td>56</td>
<td>2.03</td>
<td>9.93</td>
</tr>
<tr>
<td>16845/02</td>
<td>43</td>
<td>2.50</td>
<td>10.25</td>
</tr>
<tr>
<td>17881/02</td>
<td>41</td>
<td>2.06</td>
<td>10.92</td>
</tr>
<tr>
<td>18070/02</td>
<td>32</td>
<td>2.17</td>
<td>10.80</td>
</tr>
<tr>
<td>00853/03</td>
<td>38</td>
<td>1.50</td>
<td>10.08</td>
</tr>
<tr>
<td>02990/03</td>
<td>55</td>
<td>4.29</td>
<td>14.50</td>
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<td>11.13</td>
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<td>54</td>
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</tr>
<tr>
<td>01267/04</td>
<td>71</td>
<td>4.47</td>
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<td>3.4</td>
<td>n.d.</td>
</tr>
<tr>
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<td>n.d.</td>
<td>3.4</td>
<td>n.d.</td>
</tr>
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</tr>
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<td>n.d.</td>
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<td>n.d.</td>
</tr>
<tr>
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<td>14</td>
<td>4.3</td>
<td>n.d.</td>
</tr>
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<td>6</td>
<td>4.0</td>
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</tr>
<tr>
<td>0816/04</td>
<td>17</td>
<td>5.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>0854/04</td>
<td>8</td>
<td>3.2</td>
<td>n.d.</td>
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n.d. – not determined

Table 2: Comparison of CO-Hemoprotein and GC-CO

<table>
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<tr>
<th>Code</th>
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<th>GC-CO [µg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
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<td>n.d.</td>
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</tr>
<tr>
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<td>79</td>
</tr>
<tr>
<td>0440/04</td>
<td>12</td>
<td>153</td>
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<tr>
<td>0530/04</td>
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<td>257</td>
</tr>
<tr>
<td>0578/04</td>
<td>18</td>
<td>105</td>
</tr>
<tr>
<td>0632/04/1</td>
<td>15</td>
<td>950</td>
</tr>
<tr>
<td>0632/04/2</td>
<td>22</td>
<td>1610</td>
</tr>
<tr>
<td>0813/04</td>
<td>14</td>
<td>873</td>
</tr>
<tr>
<td>0814/04</td>
<td>6</td>
<td>~9</td>
</tr>
<tr>
<td>0816/04</td>
<td>17</td>
<td>433</td>
</tr>
<tr>
<td>0854/04</td>
<td>8</td>
<td>59</td>
</tr>
</tbody>
</table>

References

Jonker KM et al. (2001) Determination of Carbon Monoxide in Tuna, Keuringsdienst von Waaren Oost SANCO-2003-02727-00-00TRA-00(NL)

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4.5 CONFIRMATION OF THE ORIGIN OF SALMON - FAT ANALYSES REFLECT THE FISH DIET


The aim of the EU-financed project Confirmation of the origin of farmed and wild salmon, is to test the suitability of some analytical techniques to reveal the geographic origin of salmon and to build up a reference database with data from fish of known origin and diet. We report here the results of the gas chromatography analysis of muscle lipids.

Materials and methods

Fish.- Fish samples consisted in 161 wild and farmed *Salmo salar* sampled at different locations in Norway, Scotland, Ireland, Tasmania, Canada, Iceland and the Faroe Islands and 5 fish purchased at a local store labelled as *Onchorhynchus gorbuscha* from Alaska.

Lipid extraction and gas chromatography.- Lipids were extracted from the muscle of Atlantic salmon according to the method of Bligh and Dyer [1959], the fatty acid methyl esters were prepared according to Metcalfe et al. [1966] and gas chromatography was performed according to Aursand and Grasdalen [1992] and Aursand et al [2000]. Based on the latter work [Aursand et al, 2000], only 12 FA (14:0; 16:0; 16:1n-7; 18:0; 18:1n-9; 18:1n-7; 18:2n-6; 20:1n-9; 20:5n-3; 22:1n-9; 22:5n-3; 22:6n-3) were selected for the principal component analyses. The input data was the % area for each of these 12 peaks.

Principal component analysis (PCA) on the % area contributed by 12 FA was performed using the program RAPC ver.09 (David E. Axelson, MRI Consulting, Kingston, Ontario, Canada).

Results and discussion

Figure 1 shows the scores plot and Figure 2 the loadings plot of the PCA. All wild fish from the North Atlantic clustered together regardless of where they came from. The Norwegian farmed fish clusters apart from cultivated Icelandic, Irish, Scottish and Faroe. The figures show that: (1) all wild fish is placed opposite to fish with high content of vegetable oils, (2) most of the Norwegian, and in particular the Canadian farmed salmon had high levels of vegetable oil. The Norwegian salmons had been collected from three different companies during a one-year period. It was noticed an increasing content of vegetable fat for each sampling (the area labelled "Norway" in the first figure comprises the first collected samples, and the areas A, B, and C correspond to the last ones). (3) Fish purchased in Norway labelled as “Wild salmon; *Onchorhynchus gorbuscha*”, showed a very small amount of vegetable oil and a high content of sardine and salmon oil. This agrees with the diet of a wild salmonid feeding in the Pacific. (4) Irish and Scottish cultivated fish had a fatty acid composition similar to that of North Atlantic wild salmon, with a higher content of vegetable oil in the feed than that observed in wild salmon from the Pacific. (5) It seems that the feed used by farmers in Iceland and the Faroe resembles most what is considered the normal diet of a wild salmon from the North Atlantic, with a high content of oils characteristic for herring and capelin.

Acknowledgments

We are grateful to José Rainuzzo for his valuable contributions to the setting up of the GC analyses and the interpretation of the results. The project was funded by the EU (COFAWS, project GRD2-2000-31813) and the Norwegian Research Council (project 146932/130). Additional participants in COFAWS are: LAIEM (Nantes France), Eurofins (Nantes, France), JRC (Ispra, Italy) and the FSA (London, UK) as an observer.
Figure 1.- Scores plot of the 166 fish samples. Data are the % area of 12 selected FA. PC 1 explained 75% of the total variance and PC2 15%.

Figure 2.- Loading plot of the 12 selected FA on PC 1 and PC2. PC 1 explained 75% of the total variance and PC2 15%.

References

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4.6 FISHTRACE: A DNA DATABASE FOR EUROPEAN MARINE FISH - GENETIC CATALOGUE, BIOLOGICAL REFERENCE COLLECTIONS AND ONLINE DATABASE OF EUROPEAN MARINE FISHES (EC PROJECT QLRI-CT-2002-02755)

Véronique Verrez-Bagnis

The main aim of the FishTrace project is to pool of material and data corresponding to the genetic identification and characterisation of around 180 European marine fish species to guarantee the source and authenticity of fish and derived products. These standardized data will be compiled in a database which will be accessible to researchers and control laboratories through world wide web site (http://www.fishtrace.com/HOME.htm) at the end of 2005.

The general objectives of the project are:

- to draw up a genetic catalogue of a large, representative number of marine fish species regularly commercialised in the European markets. The catalogue will include gene characterization as a molecular marker related to morphological data as indisputable evidence for the origin of the fish and fish products;
- to pool reference biological materials (including DNA and tissue samples, otolithes and preserved fish) and to promote their use for standardisation and cross-referencing with respect to fish traceability through European markets. The long-term preservation of biological materials will be achieved by the Museums participating in this project;
- to establish a public accessible database compiling the new standardised data generated in the network (taxonomy, molecular genetics and reference collections) with existing data from other sources;
- to validate the information compiled in the database to ascertain its applicability for end-users, including biological research laboratories, control laboratories, consumers and regulatory bodies by the de novo designing and developing of cost-effective methodologies for the analysis, characterisation and commercial diagnosis of marine fish species with regard to fisheries and fish products.

All these objectives will be achieved by interaction of 10 partners belonging to different fields of knowledge, i.e. field taxonomists, natural history museums, molecular biology laboratories and software and database managing experts. The project coordinated by Dr José M. Bautista from the Universidad Complutense of Madrid is divided into the following tasks:
Sampling

A total of 143 species from 8 European sea areas will be analysed: 39 extra-European marine teleost species of food interest in the EC markets are also sampled.

Taxonomy

Species identification is made by using specialist literature (no subspecies) and species characterisation will be carried out by:

- check/verify against literature;
- basic measurements: SL head length, snout length, body depth, eye diameter, interorbital width;
- basic counts: dorsal, anal, pectoral fin rays, scales in lateral line, gill rakers, abdominal and caudal;
- biology;
- fisheries;
- socio-economic aspects.

Genetics

The constitution of the genetic catalogue is based on the sequence of two genes. This includes:

- determination of the nucleotide sequence of a complete mitochondrial gene (cytochrome b: 1141bp) and part of a nuclear gene (rhodopsin: 514bp);
- comparison of the sequences obtained;
- detection the degree of sequence variation in the genes analysed;
- extraction of specific sequences of those apparent populations;
- extraction of genotypic marks of the species;
- phylogenetic analysis and population structure analysis to confirm the biogeographically distribution of the haplotypes within species.

Collections

The coordination of the storage and long term preservation of the biological material as new collections will be achieved by the Museums. The reference biological material will be:

- two voucher specimens used to obtain molecular data preserved in 70% ethanol;
- replicate DNA samples to be frozen at -20°C;
- muscular tissue samples to be kept refrigerated in 70% ethanol;
- sagittal otoliths will be adequately stored as desiccated specimens;
- the inventories will be exchanged among the participating museums.
Database

The database will contain:
- all information about the species, its genus and bibliography;
- all information about the specimens analysed, their biogeography with GIS representation, their DNA analysis;
- all information about the extracted tissues and their actual location;
- all information about haplotyping;
- all information about the methodologies used to extract the DNA.

The database will be dynamically linked with FishBase, PescaBase and linked to GenBank.

The participants of the project are:

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4.7 IDENTIFICATION OF COMMERCIAL GADOID SPECIES BY PCR-RFLP

Miguel Angel Pardo

Introduction

Cod (Gadus morhua) is a valuable commercial bony fish belonging to the Gadidae family that is widely distributed in the markets of the EU. Although, this species has been majority consumed fresh, dried or salted, smoked and frozen, nowadays it is increasing the commercialization of cod in different ready to eat presentations as filleted, precooked with sauce, fish cakes, surimi based and so on. In those cases, it is impossible to identify the origin of the raw material due to the removal of external characteristics during the processing. European Union regulations (EC No 104/2000) indicate the necessity of labelling the seafood products with the scientific name to assure the traceability system through the whole chain.

To date, the utilisation of genetic markers has been extensively used as a tool to identify food products. According to seafood products, most of the genetic identification studies have been located on tuna species (Pardo and Pérez-Villareal, 2004). On the contrary, few studies have been carried out in order to identify gadoid species. These studies have been developed different DNA techniques to analyse an amplified marker by single strand conformation polymorphism (SSCP) (Weder et al., 2001), restriction fragment length polymorphism (RFLP) (Wolf et al., 2000 and Calo-Mata et al., 2003) and forensically informative DNA sequencing (FINS) (Bartlett and Davidson, 1992 and Calo-Mata et al., 2003). DNA sequencing is the most feasible technique because of the large amount of information that produces, but it is technically demanding. On the other hand, RFLP technique is faster and cheaper than sequencing and for that reason more convenient for routine analyses. Some authors have devised RFLP methods to identify gadoid species in fresh, frozen fish or salted fish (Wolf et al., 2000 and Calo-Mata et al., 2003), but none have identified gadoid species in precooked commercial samples.

Our work describes a simple and easy PCR-RFLP method to differentiate the most valuable gadoid species (G. morhua) from others that could be fraudulently labelled as cod. This method was tested with salted, smoked, frozen and precooked commercial samples labelled as cod.

Methodology

Specimens of nine gadoid species were obtained from a local market and then morphologically identified attending to external characters. In this way, eight cod (Gadus morhua, COD), nine Alaska pollack (Theragra chalcogramma, ALK), four haddock (Melanogrammus aeglefinus, HAD), four whiting (Merlangus merlangius, WHG), eight pollock (Pollachius pollachius, POL), eight saithe (Pollachius virens, POK), four blue ling (Molva dypterygia, BL1), eight ling (Molva molva, LIN) and five tusk (Brosme brosme, USK) were characterized.

Commercial seafood products labelled as cod were purchased at the local market. These samples had been subjected to different treatments such as, drying, salting, smoking, canning with vegetable oil, pickling, cooking in sauce or spicing.

DNA extraction method used is reported by Pardo and Pérez-Villareal, (2004).

Mitochondrial cytochrome b gene fragment GAD350 was amplified with the primers H307 (5’-CTC AGT ATG ATG AAA CTT TGG C-3’) and L307 (5’-CCT CAG AAT GAT ATT TGG CCT C-3’). Reactions were carried out as follows: 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.2 mM dNTPs, 3.5 mM MgCl₂, 1 M of primer, and 0.1-1 g of template DNA. Amplification reactions were developed in a Mastercycler Personal from Eppendorf. The reaction was developed in 40 cycles (92 ºC for 30 s, 60 ºC for 30 s and 72 ºC for 30 s).

The DNA sequencing was carried out directly on the purified fragments with a 3700 DNA Analyzer ABI PRISM, using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.0 (Applied Biosystems, Foster City, USA).

The GAD350 fragment were digested with the restriction enzymes Tsp509I (New England Biolabs, Beverly, USA), Hae II and Rsa I (Roche Applied Science, Basel, Switzerland). The reactions were carried out in a volume of 10-15 l, at each enzyme optimum temperature.

The DNA fragments obtained were separated by electrophoresis in 1-3 % (w/v) agarose and stained with ethidium bromide as described by Sambrook et al. (1989).
Sequence analysis. The multiple alignment was carried out using the Clustal X program (Thompson et al., 1997). The restriction sites analysis of the sequences was carried out using the Webcutter 2.0 program.

**Results**

Mitochondrial cytochrome \( b \) GAD350 fragments from individuals belonging to nine gadoid species (see above) were sequenced and analysed in order to find out diagnosis sites specific of species. Table 1 shows multiple alignments of compiled sequences. As a result of this alignment, six polymorphisms were found out. In some cases a polymorphism was a specific diagnosis site for a species (position 144 to \( G. \) morhua and 132 to \( B. \) brosme) but most of them were not. Nevertheless, the combination of several diagnostic polymorphic sites provided information about each species (Table 2).

Table 1. Multiple Alignment of Six Polymorphic Sites of 150 Sequences Belonging to GAD350 Fragment from Frozen Gadoid Species*.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
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<th>163-166</th>
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</table>

* N number of individuals analyzed. Each polymorphic site is delimited by its position (bp) in the sequence \( (123-126, 132-135, 142-145, 162-166, 177-180 \) and 200-204) which corresponds to the recognition site of \( Tsp509I \) enzyme except 142-145 and 200-204 which correspond to \( RsaI \) and \( HaeII \) enzymes, respectively. a Sequences obtained from the GenBank.

When it works with mitochondrial DNA the intraspecific variability between individuals belonging to the same species, plays a critical role because it exhibits a certain degree of variation (Unseld et al., 1995). Table 1 shows the degree of intraspecific variability of fragment GAD350 that was very low.
Table 2. Diagnostic Polymorphic Sites in GAD350 Fragment.

<table>
<thead>
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<th>Species</th>
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<tr>
<td></td>
<td>126 132 133 144 165 180 201</td>
</tr>
<tr>
<td><em>s morhua</em></td>
<td>A  C  C  C  T  T</td>
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<tr>
<td><em>Theragra chalcogramma</em></td>
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<td><em>Molva molva</em></td>
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<td><em>Brosme brosme</em></td>
<td>T  G  A  A  T  C</td>
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According to these results, DNA sequencing could be used to identify gadoid species by comparing an unknown sequence with reference sequences. This molecular method is technically demanding, expensive and consequently rather less suitable for routine analysis. For that reason, we developed the RFLP technique to differentiate *G. morhua* from other gadoids.

After analyzing the GAD350 sequences, a restriction enzyme (Tsp509I) what could distinguish between most of the species involved in this study was selected. The restriction patterns obtained after digesting the fragment GAD350 with Tsp509I, permitted to discriminate *G. morhua* from *M. aeglefinus*, *P. pollachius*, *B. brosme*, *M. molva*, *M. dypterygia*, *P. virens*, *M. merlangius* and *T. chalcogramma* (Figure 1A). Differentiation between *P. virens*, *M. merlangius* and *T. chalcogramma* was also possible when the fragment was digested with the combination of enzymes RsaI and HaeII (Figure 1B). Wolf described a RFLP method with three different enzymes that identified three gadoid species (Wolf et al., 2000). However this study did not take into account the intraspecific variability due to the scarce mitochondrial sequences analysed (one specimen, per species). Calo-Mata described a similar technique based on RFLP that could distinguish 16 species, but the analysis method only was demonstrated for six species. This study considered the intraspecific variability between individuals belonging to the same species, in most of the cases only was scrutinized one specimen per species (Calo-Mata et al., 2003). By contrast, we have checked the six diagnosis sites from 150 individuals without finding out intraspecific variability between individuals belong to the same species. In all cases, at least there was studied the sequences of five individuals per species were studied, and in the case of cod the number of analyzed sequences ranged nearly 90 individuals.

On the other hand, with the exception of some commercial salted cod samples (Bartlett and Davidson, 1992; Wolf et al., 2000 and Calo-Mata et al., 2003), no author has identified gadoid species in precooked commercial samples, so far. Nevertheless, we tested the methodology developed with commercial food samples labelled as cod; salted, smoked, frozen and precooked. The GAD350 fragment amplified from some of these samples were analysed by RFLP and FINS. Preliminary studies confirmed the results obtained by PCR-RFLP.
Figure 1. RFLP patterns of nine gadoid species on a 3 % (w/v) agarose gel stained with ethidium bromide. The GAD350 obtained from species were digested with Tsp509I (A) and the combination RsaI & HaeII (B) as described in the Methodology; Lanes 2-10, *Melanogrammus aeglefinus*, *Gadus morhua*, *Pollachius pollachius*, *Brosme brosme*, *Molva dypterygia*, *Molva molva*, *Pollachius virens*, *Merlangius merlangius* and *Theragra chalcogramma*, respectively; Lanes 14-16, *Merlangius merlangius*, *Theragra chalcogramma* and *Pollachius virens*, respectively; Lane 11, non-digested fragment; Lanes 1, 12 and 13, Molecular Ruler.

In conclusion, this work describes a simple and easy PCR-RFLP method to differentiate the most valuable gadoid species (*G. morhua*) from others that could be fraudulently labelled as cod in seafood products.

References


Author

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Corresponding author: mpardo@suk.azti.es
4.8 THE FISH-TRACENET PROJECT:  
A STRATEGIC RESOURCE OF INFORMATION ABOUT  
TRACEABILITY OF FISH PRODUCTS.

Maria Perez

The objective of this presentation is to introduce the main contents and characteristics addressed by the project entitled “Identification, classification and dissemination of organisational, technical and legal information resources dealing with traceability of fish products – FISH-TRACENET” which has been approved and funded by the DG Fisheries of European Commission, within the framework of Innovative Actions for the Fisheries Sector, agreement number 2003/C 115/08-34.

Partners

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Background

Traceability is at the present one of the major challenges in the food industry and administrations in Europe. All the achievements in this sense will contribute to ensuring a better quality and safety of food products and for that a better quality of life for consumers. Moreover, ensuring a wide traceability of fish products will contribute to a better and more responsible exploitation of the fishing resources.

At the present there is a great amount of disperse information about this topic on the World Wide Web. Partners at this project have found that it would be a worthy challenge to compile and organise a good part of these information and resources on a common site and to develop some complementary services that add value to the contents by pursuing the interaction and contact between the site developers and visitors.

What is the project intended to?

The principal objective of the project is to develop an internet website which compiles, classifies and facilitates the access to information and resources dealing with the traceability of fish products.
This website is being developed as an instrument for the networking, interaction and contribution of different types of stakeholders and having in mind the strategic value of such a result to contribute to a responsible exploitation of the fishing resources.

The work carried out on this project will be of interest for all those R&D institutions, companies, administrations and consumers that are concerned with the traceability of Fish Products. This site will be of help not only to protect the environment and promote a more responsible exploitation of fish resources, but also will contribute to the protection of consumers’ interests, and to competitiveness of companies in this sector (that have a strategic information tool on this site).

Contents Structure

In order to provide an easier utilization of the resources, the information is divided into three blocks, each one’s responsibility held by one partner:

1. **LEGAL AND NORMATIVE ISSUES**: Contains references of European regulations dealing with traceability as well as the regulations of at least each country collaborating in the project.
   Partner responsible: IFREMER.
   Contact people: Frédérick Bousquié (frederick.bousquie@ifremer.fr), Monique Etienne (Monique.Etienne@ifremer.fr).

2. **Scientific and Technical**: Contains references of articles and publications, projects, patents, websites…
   Partner responsible: IIM - CSIC.
   Contact people: Dr. Ricardo I. Pérez Martín (ricardo@iim.csic.es), Dr. Carmen G. Sotelo (carmen@iim.csic.es).

3. **Organisations and Products**: Contains the contact details and basic information about any kind of organisation (enterprise, administration, research centres, associations…) able to offer solutions, products or services dealing with the target topic.
   Partner responsible: La Tene Maps Ltd.
   Contact people: John Coleman (johncoleman@latene.com).

Throughout these headings it has been designed and programmed a complex database structure that will allow visitors to retrieve complete information introducing different search criteria for each information block and also to get information which is related and linked among the different blocks. This means, as an example, that someone searching for an R&D project in fish products traceability, will have the possibility not only to retrieve the main information about this project he/she is searching for, but also about the institution that has been co-ordinating such project.

Besides these contents, the website contains other relevant information about fish-products traceability and about the project itself. Some tools aiming to boost the interaction among visitors themselves and with the project partners (discussion forum, news…) are also available.

Planned Schedule

**END OF JULY 2004** – Website release at: [http://www.fishtracenet.org](http://www.fishtracenet.org)

**SEPTEMBER 2004** – Presentation of the web at the WEFTA Annual Meeting. Lübeck (Germany).

**FROM SEPTEMBER 2004 ON** – Database feeding. Organisation of dissemination activities and validation by end-users.

**JUNE 2005** – End of the project

**FISH-TRACENET is an open project**

Any organisation or person **willing to contribute with information** about itself or about other organisations and/or people considered to be relevant with regard to this topic, may just contact us and we will help to make the process as easy as possible.

We would also be grateful if you would let us know your **considerations and opinions about the website structure and contents**.
Forms and means to contact partners for this or whatever other purposes will be available at www.fishtracenet.org in the section called “Call for Cooperation”
4.9 DIFFERENTIATION OF WILD SALMON, CONVENTIONALLY AND ORGANICALLY FARmed SALMON

U. Ostermeyer

Since 1985 European salmon production has really increased which resulted in a decrease in farmed salmon prices. Limited quantities of certified organic salmon have been marketed in Germany. Organic salmon is currently produced in Ireland, Scotland and Norway. Organically farmed and wild-caught salmon are much more expensive than conventionally farmed salmon. To protect the consumer against misleading and deception a method is necessary which can distinguish between wild salmon, conventionally and organically farmed salmon and their products.

The characteristic red to pink colour of salmon flesh is due to the presence of carotenoids. Astaxanthin is the main carotenoid found in the flesh of wild Atlantic and Pacific salmon. The consumer normally expects the flesh colour of farmed salmon to be similar to that of free-living salmon. Synthetic astaxanthin and canthaxanthin are commonly used in conventional fish farms. The standards for organic aquaculture (e.g. Naturland, Soil Association) lay down that synthetic additives like dyestuffs are not permitted in the fish feed. Colourings shall have a natural origin. Shrimp shells and the yeast Xanthophyllomyces dendrorhous (formerly Phaffia rhodozyma) for example are permitted feed additives as dyestuff for organic salmon farming.

The astaxanthin molecule has two chiral centers and occurs as a mixture of three configurational isomers: two enantiomers (3R, 3’R) and (3S, 3’S) and a meso form (3R, 3’S). Synthetic astaxanthin contains the (3R, 3’R), (3R, 3’S) and (3S, 3’S) isomers in the ratio 1:2:1. The yeast Xanthophyllomyces dendrorhous is able to biosynthesize astaxanthin with the configuration (3R, 3’R). The (3S, 3’S) isomer is the main form found in wild Pacific and Atlantic salmon species.

For the differentiation between wild salmon, conventionally and organically farmed salmon, it is necessary to analyse the ratio of the configurational isomers of astaxanthin in the salmon flesh. In the present study the HPLC method based on the separation of the configurational isomers of underivatized astaxanthin on a chiral stationary phase. In addition we analysed the astaxanthin and canthaxanthin contents of the salmon samples with reversed-phase HPLC. The method was applied to fresh, smoked, graved and deep frozen salmon products.

In this study all conventionally farmed salmons were fed with synthetic astaxanthin and frequently with canthaxanthin. The diet for the organic salmon farmed in Ireland in accordance with the standards of the Naturland Verband contained the yeast Xanthophyllomyces dendrorhous. These fishes could be easily distinguished from conventionally farmed salmon and from wild salmon. However organic salmon from Scotland farmed according to the standards of the Soil Association were fed with shrimp shells. With shrimp shells one achieve the same astaxanthin profile as with synthetic astaxanthin.

Author

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5.1 HEADSPACE ANALYSIS OF VOLATILE COMPOUNDS IN CANNED WILD ALASKA PINK SALMON HAVING VARIOUS DEGREES OF WATERMARKING

Alexandra C.M. Oliveira, Charles Crapo, Brian Himelbloom, Jennifer Hoffert and Carey Vorholt.

Introduction

Wild salmon fisheries contribute significantly to the economy in the state of Alaska. In 2003, commercial salmon harvest was estimated at about 350,000 metric tons (MT) of fish (ADFG, 2004). Five species of Pacific salmon are harvested in Alaska waters, chinook (Oncorhynchus tshawytscha), sockeye (O. nerka), coho (O. kisutch), pink (O. gorbuscha) and chum (O. keta). Among these, three species are of commercial importance, pink, sockeye and chum salmon. During the past three years, pink salmon catches have averaged 130,000 to 200,000 MT of fish / year, while sockeye salmon catches ranged from 60,000 to 90,000 MT of fish / year (ADFG, 2004). Chum salmon catches have fluctuated from 110,000 MT of fish in 2000 to about 50,000 MT of fish in 2003. Annual commercial harvest of coho salmon is modest at 14,000 MT. Chinook, also known as king salmon, yearly catches have been below 4,500 MT for the past five years (ADFG, 2004).

Wild Pacific salmon are anadromous fish that undergo drastic physiological and biochemical changes during spawning migration (Reid et al., 1993). Fish do not feed during the spawning migration and metabolic degeneration takes place rapidly as fishes approach full sexual maturity (Ando et al., 1985). Chum salmon muscle undergoes a significant decrease in lipid content even before it starts upstream migration (Ando et al., 1985). Changes such as migration of lipid soluble pigments from the muscle into the skin and gonads (Kitahara, 1983; Reid et al., 1993), increase in flesh pH (Huynh and Mackey, 1990), decrease in protein content (Ando et al., 1985; Huynh and Mackey, 1990) and decrease in blood cholesterol levels (Idler and Tsuyuki, 1958) have been observed for different species of migrating Pacific salmon.

Muscle quality is greatly impacted by these biochemical changes and late-run salmon often present undesirable texture and flavor characteristics. Flesh softness, loss of mouthfeel, poor taste and the development of a distinct ‘late’ odor substantially lowers the commercial value of the product (Huynh and Mackey, 1990). Therefore, seafood processors in Alaska grade salmon according to its degree of skin watermarking. It is known by seafood processors in Alaska that moderate to heavy degree of watermarking in salmon produces an off-odor in the canned product, often defined as stale or musty. However, no information was found in the scientific literature describing the chemical compounds responsible for these undesirable sensory characteristics. The purpose of this study was to characterize the volatiles in canned pink salmon produced from different degrees of watermarked fish and determine product quality at 2 and 9 months of storage.

Materials and Methods

Collection and Canning of Salmon

Fresh Alaska pink salmon (O. gorbuscha) were collected from seafood processing plants on Kodiak Island during summer 2002. All fish used in this study were sampled during the same day and from the same fishing vessel. Fish were graded according to the Alaska Seafood Marketing Institute (ASMI, 2004) criteria for fish quality. The seven grades (A, B, C, D, E, F and G) were combined into four grades (A, BC, DE and FG) to provide distinctions. Fish were immediately eviscerated and canned during the same day of collection. Steaks of about 215 g were cut and placed in a 307 x 200.25 mm two-piece can and no salt was added. The cans were vacuum sealed, retorted and cooled in our pilot plant according to guidelines from the National Food Processors Association (NFPA, 1982).

Static headspace gas chromatography mass spectrometry (SHGCMS)

The headspaces of the liquors of eighteen cans were analyzed for each of the four grades of watermarking chosen and at 2 and 9 months of storage after canning. In addition, one commercially canned batch was
Also investigated using the same number of cans and time periods. Eighteen commercial cans (430 g net content) were provided by a local seafood processor, however information about the degree of watermarking of the raw pink salmon was not available. The liquid phase from the canned salmon was drained into a clean beaker. Two 10g portions of the aqueous phase were immediately weighted into 20 ml hypovials, which were then crimp-sealed with a Teflon/silicon septum (replicates). The SHGCMS methodology was adapted from methods described by McLachlan et al. (1999) and Girard and Nakai (1991). An HP 7694 (Agilent Technologies, Wilmington, DE) static headspace autosampler with a 44-sample capacity was used with the following conditions: oven temperature 75°C; loop temperature 85°C; transition line temperature 105°C; GC cycle time 20 min; loop capacity 3 ml; loop fill time 0.3 min; pressurization time 0.3 min; vial equilibration time 15 min; injection time 1 min; vial pressure 10 psi; shaker mode fast; GC cycle at 30 min. Volatile separation and identification was accomplished using a gas chromatograph model GC6890 interfaced with a mass spectrometer MS5973 (Agilent Technologies) and fitted with a dB-WAX capillary column of 30 m x 0.25 mm x 0.25 mm film thickness (J& W Scientific, Folsom, CA). Helium was used as carrier gas at 1 ml/min at an average velocity of 37 cm/sec in constant flow mode. Injector temperature was 100°C. Oven programming was as follows: initial temperature 58°C; hold for 5 min; increase temperature at 20°C/min to 110°C to give a total run time of 7.6 min. The MS was operated in electron impact mode under the following conditions: temperature of interface 240°C; source temperature 230°C; quadrupole temperature 150°C; solvent delay 1.35 min; 3.99 scans/sec. Headspace peaks were confirmed by comparison with pure chemical standards (Sigma-Aldrich, St. Louis, MO) and the NIST’98 mass spectral data library (Agilent Technologies). Results were subjected to factorial analysis of variance run on Statistica version 6.0 (StatSoft Inc., Tulsa, OK). For tests of statistical significance between classes and storage time, data were subjected to unequal N Tukey’s HSD test for significant differences (p<0.05).

Results and Discussion

Figure 1 depicts three of seven grades of watermarking in pink salmon used by quality control personnel from the seafood industry. Some morphological changes are readily noticeable such as increase in snout length and development of a hump in the dorsal area. It is not possible to notice the dramatic change in skin color in this scanned black and white image. However, grade A fish showed glossy silver scales, fish grade D showed an overall darkening of skin and early development of hump and snout, and fish grade G showed pronounced enlargement of hump and snout and severe darkening of skin and belly cavity. Figure 2 shows a representative chromatogram of a grade A canned pink salmon at 2 months of storage. Fourteen peaks were observed in the 7.5 min run. Table 1 shows the results in peak area percentages for the compounds quantified for the 2 and 9 months cans. A variety of sulfur-containing compounds such as methanethiol, dimethyl sulfide and carbon disulfide were identified and are in agreement with previous findings (Girard and Nakai, 1991; Milo and Grosch, 1996). Sulfur containing compounds comprised 30 to 50% of the total volatiles registered by the MS and tended to decrease with increasing degrees of watermarking. Moreover, after the cans aged for 9 month there was a significant increase in the S containing compounds for grade BC and in the commercial batch. Several furans, alcohols, aldehydes and ketones were also identified and most have already been reported as headspace constituents of fish products (Prost et al., 2004; Girard and Nakai 1991; Zhang and Lee, 1997). Propanal showed a significant increase and doubled in concentration for all groups after ageing (Table 1). Acetaldehyde showed a marked increase with more severe degrees of watermarking from 4% to a maximum of 8% of the total volatiles. Methyl-isobutyl-ketone (MIK) ranged from 1 to 3.5% of the total volatiles and was only detected in grade A canned pink salmon and in the commercially canned samples. This compound may be used as a unique marker for grade A pink salmon. Aging did not affect levels of MIK in the samples. Acetone ranged from 6 to 9% of the total volatiles and showed an increase to about 12-13% during aging for pink salmon samples grade DE and FG. Furans were present at small concentrations and the compounds accounted for less than 5% of total volatiles regardless of salmon grade or can aging. Trimethylamine and a co-eluting unknown compound ranged from 10 to 20 % of the total volatiles and showed a significant increase with increasing degree of watermarked salmon. These latter two compounds also showed a significant decrease of about 20% with aged cans for all watermarked classes. The chromatographic signal identified as unknown 2 was below detection limits for all cans studied at 2 months of storage, however this peak shows at about 1.82 min just after peak 5 (Figure 2; Table1). Based on volatile compound identification and relative quantities the commercially canned salmon quality was most similar to grade A.
Headspace analysis revealed differences in the quality and proportion of the volatiles in salmon having distinct degrees of watermarking. In addition, significant differences were also found between 2 and 9-month canned products for all watermarking levels. Future studies will build on this initial database for predicting chemical quality attributes in commercially canned salmon and expected shelf life.

References


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Figure 2. Total ion count chromatogram of grade A canned pink salmon at 2 months of storage (*not present at 2 months of storage).
<table>
<thead>
<tr>
<th>Can Age</th>
<th>2 Months Old</th>
<th>9 Months Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of Watermarking</td>
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<td>BC</td>
</tr>
<tr>
<td>sulfur containing cmpds</td>
<td>46.32 A</td>
<td>43.40 B</td>
</tr>
<tr>
<td>aldehydes (A)</td>
<td>4.96 A</td>
<td>7.04 B</td>
</tr>
<tr>
<td>ketones (K)</td>
<td>9.35 A</td>
<td>10.58 A</td>
</tr>
<tr>
<td>unknown 1 + TMA*</td>
<td>31.67 A</td>
<td>34.44 A</td>
</tr>
<tr>
<td>methanethiol**</td>
<td>1.99 A</td>
<td>2.88 A</td>
</tr>
<tr>
<td>acetaldehyde*</td>
<td>4.06 AC</td>
<td>5.95 ABC</td>
</tr>
<tr>
<td>carbon disulfide*</td>
<td>3.12 AC</td>
<td>2.64 A</td>
</tr>
<tr>
<td>dimethyl sulfide*</td>
<td>41.21 AB</td>
<td>37.88 A</td>
</tr>
<tr>
<td>unknown 2</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>propanal*</td>
<td>0.91 AD</td>
<td>1.10 AD</td>
</tr>
<tr>
<td>2-methyl-furan*</td>
<td>0.96 AC</td>
<td>1.38 B</td>
</tr>
<tr>
<td>2-butanone*</td>
<td>2.4 ABC</td>
<td>2.19 AB</td>
</tr>
<tr>
<td>2-ethyl-furan*</td>
<td>1.94 AC</td>
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<td>methyl-isobutyl-ketone*</td>
<td>1.74 A</td>
<td>BDL</td>
</tr>
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<td>1-butanol*</td>
<td>1.96 A</td>
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</tr>
<tr>
<td>unknown 3</td>
<td>2.87 ACD</td>
<td>3.53 AB</td>
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</table>

Table 1. Chemical constituents (peak area %) of canned salmon produced from watermarked pink salmon after 2 and 9 months of storage. BDL below detection limit; TMA trimethylamine; (SD) Standard deviation of the mean; Different superscript letters in a row indicate significant differences (p<0.05) between samples; Com commercial canned pink salmon; *match retention time of pure chemical compound and also NIST’98 mass spectral data library; **compound identified by NIST’98 mass spectral library at a match quality above 95%.

34th WEFTA meeting, 12-15 September 2004, Lübeck-Germany
5.2 DEVELOPMENT OF A COLORIMETRIC SENSOR FOR FISH SPOILAGE MONITORING BASED ON TOTAL VOLATILE BASIC NITROGEN (TVB-N) MEASUREMENT

Alexis Pacquit, King Tong Lau, June Frisby, Danny Diamond and Dermot Diamond

Introduction

In the fisheries industry, there is a large interest in developing accurate methods to evaluate real-time quality of fish and seafood products, particularly one that reflects and accounts for the products history and their storage conditions from harvest to home. In 2005, complete food traceability will be a requirement within the EU1. Nowadays, fish and seafood freshness judgment relies on trained assessors in auction halls, but fast, convenient and inexpensive analyses, at any point on the cold chain, requiring little investment in training is where the emphasis resides.

One concept is that of a “chemical bar-code”, in the form of on-package sensor spots that monitors spoilage in fish and seafood products. The sensor contains a pH sensitive dye that responds to basic volatile spoilage compounds, such as trimethylamine (TMA), ammonia (NH₃) and dimethylamine (DMA) collectively known as Total Volatile Basic Nitrogen (TVB-N).

When a pH indicator dye is placed in an environment that is basic enough so that deprotonation occurs, a shift in the wavelength maximum ($\lambda_{\text{max}}$) of the dye absorption spectrum takes place. For example, a shift from 438nm to 615nm is observed for bromocresol green (BCG) (Figure 1). As the fish or seafood product start to spoil, basic spoilage volatiles are gradually produced in the package headspace resulting in a pH increase and the sensor colour changing from yellow to blue, easily visible to the naked eye (Figure 4). The response is monitored with a simple, inexpensive reflectance colorimeter that we have developed based on LEDs and a photodiode.

Fig. 1: Uv/vis absorption spectra of the acidic and basic forms of bromocresol green in solution are shown. Removal of the proton causes a shift in the wavelength maximum of the acidic form of the dye, at 438nm, to the basic form of the dye, at 615nm.

Material and Methods

Sensor fabrication

Sensor spots were prepared by entrapping BCG into a plasticised cellulose acetate matrix which was then coated onto optically clear PET discs. A hydrophobic gas permeable membrane was added to protect the sensor coated surface from excess humidity while allowing gaseous compounds to go through. The optically clear PET allowed reflectance measurements from the sensor rear with minimum reflectance loss.
Sensor characterisation

100ppm synthetic ammonia gas in nitrogen was used to characterise the sensor. Further dilution of the ammonia gas with nitrogen was achieved through the use of mass flow controllers. The sensor was placed into a flow cell fitted with the optical scanner, which monitored in real time the sensor responses to changing ammonia concentration. The data was logged by a PC connected to the optical sensor.

Experimental setup

Three fish species, cod, cardinal (also known as Bulls-eye) and roundnose grenadier, were selected for investigation. The experimental design is shown in Figure 2 where ca.1gm of fish filet sample was placed in a polypropylene cap and fitted inside a well of an inverted standard 24-well plate incorporated with the sensor. The edges of each well cap were sealed with fast cure epoxy to create a permanent gas-tight seal and prevent leakage of amines. The sensor response was monitored every two hours with the optical scanner described above.

![Experimental design for fish spoilage monitoring. (Source Byrne et al.²)](image)

**Fig. 2:** Experimental design for fish spoilage monitoring. (Source Byrne et al.²)

**Results and Discussion**

Sensor characterisation

Figure 3 shows typical sensor responses to ammonia gas. The data indicates that the sensor is sensitive to relatively low concentrations of ammonia gas and a linear range between ca. 0 - 3ppm of ammonia was observed. As a mixture of amines and ammonia are produced by spoiling fish therefore the concentration of TVB-N in the headspace is sufficiently high to be detected by this proposed sensor.
Fig. 3: Sensor responses to increasing ammonia concentration monitored by the optical scanner.

Fish spoilage trial

Typical sensor responses to a spoiling fish sample over a period of 70 hours are shown in Figure 4. A clear colour change from yellow to blue was observed when the fish sample spoiled.

![Sensor responses to a spoiling fish sample over time at 20°C.](image)

**Fig 4:** Typical bromocresol green sensor response to a spoiling cod sample over time at 20°C.

Figure 5 shows the change in TVB-N level monitored by the colour sensor in spoiling cod at room temperature. For the first 18–20 hr, no colour change was detected by the reflectance colorimeter but at approximately 20 hr, a definite increase in reflectance was recorded. The sensor gradually changed colour from yellow to green then to blue in approximately 38 hr where no further colour change was observed. During this entire period, the sensors fitted in the reference wells which did not contain any fish sample remained in their yellow form.
When the experiment was repeated with cardinal and roundnose grenadier, a similar sensor response was recorded but the onset of spoilage was detected later than with the cod, at approximately 28hr. No further colour change was observed after approximately 44hr. A sigmoid model was fitted to the data and is shown in Figure 6 for cardinal.

**Conclusion and Future Development**

The results indicate that a fast and sensitive detection of spoilage compounds in fish can be achieved by colorimetric method. Before application to real fish packaging, further chemical migration and toxicity studies will be carried out to ensure food safety. This technique shows the potential of using colourimetric sensors to develop “Smart Packaging” where an immobilised on-package sensor would indicate, by a visible colour change, the freshness status of the packaged fish product.

**Acknowledgements**

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5.3 EXPLORATIVE ANALYSES OF 16S RDNA MICROBIAL COMMUNITY IN FARMED SALMON FILLETS PACKED IN MODIFIED ATMOSPHERE (MAP) WITH A CO2-EMITTER

Hansen, A. Å., Eie, T., Tamarit, M.P.C. and Rudi, K.

Introduction

Packaging salmon fillets in a modified atmosphere (MAP) in combination with low storage temperature extend the shelf life by limiting microbial growth. We want to investigate the microbial community of farmed salmon fillets in such packages. Identification and characterisation of the bacteria able to grow under these limiting conditions are important, both for determining shelf life and determination of potential health hazards. For this reason it requires a better description of the bacteria able to grow on these products. Very little is known about the total microbial flora (Cambon-Bonavita et al., 2001; Suau et al., 1999). The main focus until now, has been on specific bacteria, in particular spoilage bacteria (Gram & Huss, 1996). The most common spoilage bacteria reported in fish and fish products are *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Brochothrix thermosphacta* and lactic acid bacteria (Dalgaard, 2000). The benefits of using 16S rDNA analyses compared to traditional techniques are both that an unbiased classification of bacteria can be achieved, and that the bacteria can be analysed directly from the food matrix. 16S rDNA analyses gives a more comprehensive picture of the total microbial biodiversity in fish than previously possible. Analyses of 16S rDNA will be vital in describing microbial communities and how shelf life will be affected when using a CO2-emitter compared with traditional MA packed fish. The focus on this research was to analyse both microbial group composition and the total amounts of bacteria of the salmon samples packed in different MAP packages and vacuum after 28 days of storage. Further analyses and investigation will be conducted to reveal microbial community during the whole storage period and how this will affect the shelf life of the fish packed in MAP with a CO2-emitter.

Materials and Methods

Storage and packaging experiment of salmon

Filets of pin-boned and skinned salmon (produced at the west coast of Norway) were packed in industry packs (5400 cm³), small retail packs (127/283 cm³) and vacuum, all trays made of HDPE and with humidity absorbers. The top web was made of 52 µm PET/AlOx/PE. CO2-emitters and headspace of 100 % N2 and G/P-ratio (Gas/Product-ratio) 1:1 were used in both sizes of the trays to compare with ordinary MAP within same sizes, and vacuum. Ordinary MAP, packed with 60% CO2 and 40% N2 and a G/P-ratio of 3:1, and vacuum-packages, 6 x 6 cm fillet pieces, were used as controls. The packages were stored at 2 °C and samples were taken after 7 days, 14 days, 21 days and 28 days, 12 samples each time.

CO2-emitters were made of a dry mixture of NaHCO3 and citric acid, by placing the chemicals inside the humidity absorber, and adding 2 ml of sterile water to each absorber immediately before sealing the package. The ratio between the two chemicals in the mixture (16.8 g NaHCO3/kg salmon + 0.78 g citric acid/g NaHCO3), was chosen to give a pH-value of the dissolved chemicals close to the pH of the salmon, i.e. pH 6.2.

Direct DNA extraction from the fish matrix

The DNA extraction protocol used in this work was as described in Rudi et al. (2004). For microbial analysis fish surface area of 18 cm² in a layer of 0.5 cm was removed, placed in 100 ml peptone water (8.5 g NaCl, 1.0 g peptone, 1000 ml⁻¹) and treated for 1 minute in a stomacher. 50 ml of fish-suspension from the stomacher solution was frozen, and later on the samples were thawed overnight in the refrigerator for DNA isolation. The fish-suspension was diluted with peptone water to 100 ml and centrifuged at 700 rpm for 1 min. The supernatant was removed until 10 ml was left, 90 ml peptone water was added and the centrifugation repeated. The supernatant was added to the first tube and centrifuged at 13 000 rpm for 15 min. The pellet was diluted in 10 ml tris EDTA (TE-buffer, pH 8) and centrifuged once more at 9 000 rpm in 10 min and diluted in 5 ml TE.

The samples were treated in a Percoll gradient (Fermér & Lindqvist, 2002) before lysis and extraction of DNA. 4 x 0.9 ml samples of the final fish suspension was layered on top of 0.6 ml Percoll (Amersham) in eppendorf
tubes. The tubes were centrifuged at 13 000 rpm (Biofuge Fresco, Kendro Laboratory Products) for 2 min. The supernatant was removed until only 0.1 ml was left. 1.0 ml TE was added, mixed with the pellet and centrifuged once more at 10 000 rpm (Biofuge Fresco) for 5 min. This time the supernatant was removed until 0.3 ml was left. The contents of the 4 tubes from the same sample were pooled.

0.25 g glass beads (106 µm; Sigma, Steinheim, Germany) were added to 0.5 ml portions of suspension of the Percoll treated salmon. The bacterial cells were lysed in a Fast-Prep bead beater (Bio 101; Stratagene, La Jolla, California). The treatment was performed at maximum speed for 40 s. The DNA in the supernatant was purified with the Dneasy tissue kit (Qiagen, Hilden, Germany) following the manufacturer instructions.

Real-time quantitative PCR

The total amount of bacteria was determined by 5´nuclease PCR (Polymerase Chain Reaction) using universally conserved regions in the 16S rDNA gene as target with the primers Mangala 1F (5´-TCC TAC GGG AGG CAG CAG T-3´), Malaga 1R (5´-GGA CTA CCA GGG TAT CTA ATC GTG TT-3´) and Mangala Probe FAM (5´-6CGT ATT ACC GCG GCT GCT GGC-3´) (Sigma Genosys, USA). The amplification profile used was as follows: 95 °C for 10 min, (95 °C for 30 s and 60 °C for 1 min) x 40. The reactions were performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems). We used carboxyfluorescein (FAM) as a reporter dye, and 6-carboxy-N,N,N′,N′-tetramethylrhodamine (TAMRA) as a quencher. A threshold signal was chosen where the signal could be detected. This gave the threshold cycle (C_T), which defines the first cycle for which a signal could be detected.

16S rDNA sequence analyses of DNA directly retrieved from the fish matrix

PCR was performed on a portion of the prepared DNA samples. The primers used were the same as for real-time quantitative PCR. The amplification profile used was 95 °C for 10 min, (95 °C for 30 s and 60 °C for 30 s and 72 °C for 45 s) x 35, 72 °C for 4 min. The PCR-products were subsequently verified on a 1,5 % agarosegel, and fragments detected using a Typhoon scanner (Amersham).

The products were then cloned using the TOPO TA Cloning® kit (Invitrogen, Carlsbad, California, USA). TOP 10 One Shot® chemically competent cells were used. Transformation of the cells was performed as described in TOPO TA Cloning manual. Plasmids from the positive colonies were isolated by resuspending a colony in 50 µl water, heating to 99 °C for 10 min, removing the cell debris by centrifugation at 13 000 rpm (Biofuge Fresco) for 3 min, and transferring 30 µl to a new tube. The insert was amplified with the 5´-CGC CAG GGT TTT CCC AGT CAC GAC G-3´ (HU) and the 5´-GCT TCC GGC TCG TAT GTT GTG T GG-3´ (HR) primers, which are specific for the Bluescript vector. The following amplification reaction was used: 95 °C for 10 min, (95 °C for 30 s and 60 °C for 30 s and 72 °C for 45 s) x 35, 72 °C for 4 min. Finally, the cloned fragments were sequenced.

Sequencing

The PCR products were verified by agarose gel electrophoresis before sequencing (1,5 % agarose gel). The fragments were then sequenced using a Typhoon scanner (Amersham). The cloned fragments were presequenced including reaction treating 8 µl of the PCR product with 1 µl exonuclease I (Amersham) and 1 µl shrimp alkaline phosphatase (Amersham) at 37 °C for 15 min. The enzymes were inactivated by heating to 80 °C for 15 min. Sequencing was done using the Big Dye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA) on a 377 DNA Sequencer. Preparation of the sequencing mixture was done as recommended by the manufacturer. One of the sequencing primers used for PCR and real-time quantitative PCR (Mangala 1F, Sigma Genosys) was used.

Database searches

Searches were done in the Genebank nucleotide sequence database (June 2004) with the BLAST program (www.ncbi.nlm.nih.gov)

Results and discussion

It seems to be incidental what kind of spoilage bacteria that will be able to dominate the different fish samples after storage, and there is an indication of dominance of one bacteria in the ordinary MAP packages compared to
the other packaging methods. After 28 days of storage there was a dominance of *Photobacterium phosphoreum* in three of the packages (two packages of ordinary MAP and one package with CO$_2$-emitter). In one package Rahnella sp. dominated (vacuum), in a second Leuconostoc sp. was dominating (ordinary MAP) and in a third Carnobacterium sp. was dominating (ordinary MAP). In the 6 other fish samples (two ordinary MAP, three with CO$_2$-emitter and one vacuum) there were more than one bacteria dominating. In samples with CO$_2$-emitter Photobacterium sp. and Pseudomonas sp. were dominating, and Pseudomonas sp. and Propionibacterium sp. were found in ordinary MA packages. This is in spite of that Pseudomonas might be inhibited by CO$_2$ (Gram & Huss, 1996). In the vacuum package Enterobacteriaceae sp. were one of the dominating bacteria group. It is well established that *P. phosphoreum* is associated with colonisation of MAP fish (Dalgaard, 1995; Dalgaard et al., 1993; Debevere & Boskou, 1996; Emborg et al., 2002; Rudi et al., 2004). Our partial Photobacterium 16S rDNA sequences showed 99 % identity to this species. The other three dominating bacteria had 97 % identity to Rahnella sp., 99 % to Carnobacterium and 97 % to Leuconostoc. Rudi et al (2004) found that Brochothrix and Carnobacterium was dominating samples of salmon after 18 days of storage and that Photobacterium dominated for coalfish. Rahnella sp. (Enterobacteriaceae) have previously been isolated from environmental and human sources (Brenner et al., 1998), Leuconostoc, a lactic acid bacteria group, has been found on vegetable food (Kim et al., 2003), and Carnobacterium are found in fish intestinal. The fish samples with more than one dominating bacteria had up to 100 % identity to the Pseudomonas sp., 99 % identity to Photobacterium, 97 % identity to Propionibacterium and 96 % identity to Enterobacteriaceae. Those with lower identity might be because of poorer sequences.

The 5’ nuclease PCR quantification showed a divergence in the total amount of bacteria with C$_T$ values ranging from 26 to 33. There were no differences between the packaging methods according to the C$_T$ values, except for the vacuum packages which showed an indication of lower C$_T$ values, i.e. a higher amount of bacteria, than the other packages. According to standard curves made by Rudi et al (2004) for *Brochothrix thermosphacta* MF154, Shewanella sp. MF186 and *Carnobacterium divergens* MF151 a C$_T$ value of 26 correspond to 6 CFU log$_{10}$/cm$^2$ and a C$_T$ value of 30 correspond to 4 CFU log$_{10}$/cm$^2$.

**Figure 1.** DNA-sequence analysed from salmon packed in MAP with CO$_2$-emitter was dominated by *Photobacterium phosphoreum*.

**Conclusions**

Compared with other results it seems to be incidental what kind of bacteria that is able to dominate or weather there is one or more bacteria dominating fish samples after storage. Our results show an indication of Photobacterium sp. in MA-packages but not in vacuum packages. Vacuum packages seem to contain Enterobacteriaceae sp., and Pseudomonas sp. were found in ordinary MAP and MAP with CO$_2$-emitter. Further investigations and analyses of microbial group composition is necessary to verify these results, how the bacteria growth will affect shelf life of modified atmosphere packages of salmon and to verify the effect of CO$_2$-emitter in MAP.

**References**


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5.4 OXIDATION OF PROTEINS IN RAINBOW TROUT MUSCLE

Inger V. H. Kjærsgård & Flemming Jessen

Lipid oxidation is a well-known problem to quality of fish and substantial research has been conducted on oxidation mechanisms and products in relation to rancidity. However, aspects of protein oxidation on quality have not been given much attention. Studies have indicated that protein and lipid oxidation begin simultaneously but it is unclear whether lipid oxidation actually induces protein oxidation or vice versa. It is neither evident whether proteins and lipids interact at all at this initial stage or later during oxidation (e.g. protein oxidation could have an influence on lipid oxidation rate).

Proteins can be oxidised by reaction of reactive oxygen species (ROS) such as O₂⁻, HO• and H₂O₂ with different amino acid residues. The present study has focused on a specific protein oxidation product, namely protein carbonyls formed through reaction of ROS with proline, arginine, lysine or threonine. The aim of the investigation was to elucidate the pattern of protein oxidation (carbonylation) in muscle of rainbow trout. Are certain proteins in the muscle more susceptible to carbonylation than others during storage?

Oxidation of individual protein species was evaluated by labelling protein carbonyls with 2,4-dinitrophenyl hydrazine (DNPH) followed by immuno-blotting of proteins separated by two-dimensional gel electrophoresis (2D-GE).

High-salt and low-salt protein fractions were accessed in rainbow trout muscle after 0 and 48 hours storage at room temperature. The major amount regarding total number of carbonylated proteins and intensity of carbonylation was found among the high-salt soluble proteins as compared to the low-salt soluble proteins. The biggest increase in carbonylation during storage was found in the high-salt soluble protein fraction.

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5.5 EUROPEAN COMMUNITY RESEARCH PROJECT „SEQUID“: A NEW METHOD FOR MEASUREMENT OF THE QUALITY OF SEAFOOD – THE PART OF THE FEDERAL RESEARCH CENTRE FOR NUTRITION AND FOOD, DEPARTMENT FOR FISH QUALITY

S. Mierke-Klemeyer, J. Oehlenschläger, R. Schubring, M. von Klinkowström

Introduction

Important regulations in the food safety area shall apply from the beginning of next year in the EU. In the EC-Regulation 178/2002 the trace ability of food products is introduced and the responsibilities for food business operators are enlarged. Therefore in the field of fish processing and trading it becomes more important to enforce the development of a new method for a reliable rapid quality determination, which can be performed during fish processing. The European Community research project „SEQUID“ (QLRT-2000-01643) deals with the development of such a new method for measurement of the quality of seafood. This new method is based on very small differences in dielectric properties measured by Time-Domain-Reflectometry (TDR). These differences, which result from different storage conditions affecting quality, have to be analysed by sophisticated data processing (PCA, PLSR) (Kent et al. 2004). In order to classify this new method, frozen and chilled storage experiments with different fish species have been performed at European fishery research institutes accompanied by the determination of conventional quality parameters as well as TDR-measurements with a Prototype instrument.

Material and Methods

Ice-storage experiment

At the Federal Research Centre for Nutrition and Food and the Federal Research Centre for Fisheries the fishery research vessel “Walther Herwig III” is available. This guarantees that the experiments can start with very fresh fish, which is then kept under controlled ice storage conditions from catch to quality determination. As part of the SEQUID Project an ice storage experiments with cod was performed on the cruise to the Barents Sea in autumn 2003. During the chilled storage experiment the gutted cods were stored in melting ice and the temperature was monitored by a temperature–logger. Every second day 5 individuals were evaluated by QIM, Fischtester-readings and image processing.

QIM (Quality Index Method)

Typical QIM schemes comprise 10 quality parameters (e.g. appearance of skin, stiffness of the fish, form of the eyes, state of the cornea, colour of the pupils, gill colour, smell and mucus and colour of the blood) to be assessed preferably by a panel of experts. Between 0 and 3 well-defined demerit points are attributed to the scores depending on the parameter. The Quality Index for cod is ranging between 0-23 (Luten and Martinsdottir, 1997).

Intellectron Fischtester VI

As a comparison of existing methods for measuring freshness of fish, readings were taken on each fish using the Intellectron Fischtester VI. This well-known instrument measures the electrical conductivity through the body of the fish at two different frequencies in the low frequency range. In this way electrode polarisation can be eliminated and the true conductivity measured (Oehlenschläger, 2003). It was found earlier that such measurements relate to freshness of the fish.

Frozen storage experiments

For the frozen storage experiments the fillets were prepared properly and frozen immediately. After the end of the cruise the cod-fillets were stored –10°C, -20°C, -30°C and double-frozen at –20°C. At certain time intervals sensory evaluation, determination of drip- and cooking loss, instrumental texture- and colour measurements, image processing of the fillets were performed. These experiments will only be finished in October. But there are some results of frozen storage experiments performed as “initial trials “ with Baltic cod. The following investigations were performed:
Sensory evaluation

The fish fillet, which was treated ten minutes in 90 °C-hot water in a cooking pouch, was served to the taste panel. Each panellist had to assess the texture-attributes (tough, dry, fibrous), odour- and taste-attributes (stale, fishy) of the samples. The panellists had to set a mark on a centesimal scale in order to describe the intensity of the attribute. Five to eight panellists got 5 samples of fillets stored at the same conditions at one examination day.

Determination of drip loss

For determination of drip loss, the pouches with the fish were weighted correct to one decimal place and a corner of the pouch was cut so that the water could drip out of the pouch. Then the fish was taken out of the pouches, carefully dabbed and weighted again. The drip loss was calculated as the percentage of drip water referring to the initial weight of fish fillets.

Determination of cooking water loss

About 200 g of cod-fillets weighted correctly were put in a cooking pouch for 10 minutes into 90 °C-hot water. The cooking loss was determined by cutting of a corner of the pouch and weighting the dripped fish in the cooking pouch. The cooking loss was calculated as the percentage of cooking water referring to the weight of the fish put into the cooking pouch.

Instrumental texture, water holding, colour and DSC measurements

Methods used for instrumental measurements are recently described in detail (Schubring 2004).

Results

Ice-storage experiment with fresh cod from the Barents Sea

QIM

Figure 1 shows a linear correlation of the development of QIM-scores with days in ice.

Fig 1: QIM assessment of ice-stored cod: Quality Index as a function of days in ice
Median, 25% and 75%-percentils, min, max

The end of shelf life was reached after 17 days of storage.
Intellectron Fischtaster VI

![Graph showing Intellectron Fischtaster VI readings as a function of days in ice.](image)

Fig 2: Ice-stored cod: Intellectron Fischtaster VI readings as a function of days in ice

Figure 2 shows the development of the readings of the Intellectron Fischtaster VI with storage time. The initial readings were about 90 and dropped to values of about 10 at the end of shelf life.

Frozen-storage experiments with Baltic cod

Sensory

All sensory attributes show the same tendency for the different frozen storage conditions. In Figure 3 the development of the intensity of stale (as a taste attribute) and dry (as a texture attribute) with storage time and temperature of storage are shown.

At the beginning of the experiments the intensity of “stale” for the fillets stored at –20 °C and –30 °C was evaluated to be at a level of about ~15-20 %. During course of the experiments the intensity increased up to 50-60 %. In comparison to these evaluations the scores for double frozen fillets and fillets stored at –10°C of this attribute at the beginning at ~50 %. The arithmetic mean reached at the end of the experiments ~80-90 % intensity.

The initial scores for dry for the cod fillets stored at –10 °C and double frozen –20 °C are bigger than 60 % intensity. The initial scores for –20 °C and –30 °C were evaluated to be of nearly 40 % intensity. So the changes for this attribute observed during the experiments are not very clear.

Drip loss and cooking loss

The development of drip loss and cooking loss is shown in Table 1. Drip loss and cooking loss are remarkably higher for the cod fillets stored at –10°C and the double frozen fillets stored at –20°C than for cod stored at –20 °C and –30°C. This can be explained by the effect of frozen storage temperature ( –10°C samples) and by poor freezing conditions (double freezing). It can also be stated that the slope of the graph of –10°C storage is significant higher than the others (Oehlenschläger and Mierke-Klemeyer, 2003).
Table 1: Drip loss and cooking loss as a function of time and temperature of storage, arithmetic mean (mean), standard deviation (SD)

<table>
<thead>
<tr>
<th>Temperature of storage</th>
<th>Months of storage</th>
<th>Drip loss [%] mean</th>
<th>SD</th>
<th>Cooking loss [%] mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10°C</td>
<td>1</td>
<td>10,3</td>
<td>0,9</td>
<td>14,7</td>
<td>2,0</td>
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<td>2</td>
<td>11,1</td>
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</tr>
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<tr>
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<td>1,8</td>
<td>7,0</td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>5,9</td>
<td>0,6</td>
<td>7,3</td>
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<tr>
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</tr>
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<td>2,0</td>
<td>11,4</td>
<td>2,3</td>
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<tr>
<td>-20°C double frozen</td>
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<td>11,3</td>
<td>1,4</td>
<td>16,1</td>
<td>2,5</td>
</tr>
</tbody>
</table>

Figure 3: Sensory aspects "stale" and "dry" as a function of time and temperature of storage, arithmetic mean, standard-deviation, n=24-35
DG: double frozen
Instrumental measurements

Colour, texture and water holding capacity during frozen storage were a function of temperature and time. Changes at –10 °C were most pronounced, while those at –30 °C were negligible. Changes at –20 °C were in between both. DSC curves were widely not affected by the time of storage. However, the storage temperature affected mainly the second transition peak that is ascribed to sarcoplasmic and connective tissue proteins. The frequency of significant linear correlations between instrumental data for colour, texture and water holding capacity and the respective storage time proved to be temperature-dependent and was highest at –10 °C.

Conclusion

The results shown were used to calibrate the prototype, so that the TDR-output can be directly correlated with conventional quality parameter and frozen storage conditions with respect to cod fillets. Every fish species needs its own calibration. First results show that this new method allows efficient quality estimation of the different fish species investigated (Kent 2003).

References


Authors

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5.6 STRUCTURAL CHARACTERIZATION OF FISH MUSCLE TISSUE
BY IMAGE PROCESSING

Michael Kroeger

Introduction

„Foods have a microstructure imparted either by nature or through processing“ (Aguilera, 2000). On different spatial scales fish muscle tissue has similar inherent structures (thin filaments, thick filaments, myofibrils, muscle fibres, myotomes, figure 1). Common characteristics of all the structure elements are the linear and parallel arrangements within a neighbourhood (Kroeger, 2003). The arrangement is a basis for identification of aspects of quality, the way of farming or fish species. An area of about 10 x 10 mm² on the sample is sufficient for the analysis. However, all the image data have to involve spectroscopic and scale dependent informations gained by suitable illumination and filter techniques.

Material and Methods

Investigations in identification image were carried out on cod (gadus morhua) from Barentsea and on conventional farmed salmon (salmo salar) from Ireland and Norway, ecological farmed salmon from Ireland and wild salmon from Ireland. Surface patterns from fillets were recorded by a telecentric lens (corrected from 380 [nm] to 1000 [nm]) and a 2/3” CCD-Kamera (736x 568 Pixel). All the images were radiometric calibrated and an area of interest (AOI) of 512 x 512 Pixel was used for the postprocessing steps. The image processing system has to resolve muscle structures less than 100 [nm]. On the image (AOI) a filtermask of the size 16x16 Pixel is defined as a virtual structure element shifted pixelwise over the total AOI. By neighborhood operations the local orientation of all the filtermasks were generated and assignend with the central pixel (Bigün, 1987). By help of the structure tensor (Jähne, 2002) the original image was transformed into a coherency image. The pixelvalues of the coherency image are rotation-invariant and a quantify the linear arrangement of patterns. Local coherencies depends on the wavelength $\lambda$ of the incident light. So spectroscopic informations are essential for a successful pattern analysis. By illumination of samples with monochromatic light of narrow intervals from ultraviolet to infrared spectroscopic informations are obtained. Within the image processing steps sequential controlled LEDs (light emitted diodes) were used to generate images with spectroscopic signature. Of great significance are coherency images corresponding to different scale parameters considering hierarchical organized patterns. For a simple and rapid extraction of features histograms of all the local coherency data were used. A subset of about 10 equidistant data from the histograms was used as a feature vector for a classifier (PCA, network, Fuzzy system). Additional informations were gained from scale dependent spectral coherency images by computing local wavenumbers, local energies and local phases by application of quadrature filters.
Results

The transformation of a radiometric calibrated greylevel image (figure 2) to a coherency image in different scales for conventional famed salmon is demonstrated in figures 2 b-d for a wavelength 525 [nm].

Samples of cod were stored on ice and samples of salmon were stored at a constant temperature (-10°C). Results in identification of quality aspects for cod and of way of farming for salmon depend on the combination suitable wavelengths and scales. Figure 3 demonstrates the prediction of storage time from coherency images as a function of 3 wavelengths for cod. As a classifier the Partial Least Square (PLS) method was used (Wold, 1983). PLS had insignificant better results than the principle component analysis (PCA) or a network. Table 1 demonstrates the effect of a combination of different wavelengths for the differentiation between conventional and ecological farmed salmon and wild salmon. A combination of 4 wavelengths is sufficient for salmon.
use of images for only one scale resulted in more than 80 percent correct assignment of images to the storage time for cod and the way of farming for salmon. The coupling of another scale using the same wavelengths combination and considering local energies increases the correct assignment of images to about 89 percent. However the time for evaluation increases to about 500 percent.

Figure 3 Prediction of storage time for cod

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>SEC</th>
<th>SEP</th>
<th>R2</th>
<th>RMSEC</th>
<th>RMSEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>0.82551</td>
<td>0.85954</td>
<td>0.02647</td>
<td>0.73836</td>
<td>0.81543</td>
</tr>
<tr>
<td>400+470</td>
<td>0.67670</td>
<td>0.67937</td>
<td>0.77104</td>
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<tr>
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<td>0.48733</td>
<td>0.88926</td>
<td>0.29010</td>
<td>0.46232</td>
</tr>
<tr>
<td>400+470+525+595+660</td>
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<td>0.87494</td>
<td>0.27620</td>
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</tr>
<tr>
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<td>0.78378</td>
<td>0.41591</td>
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</tr>
<tr>
<td>470+525+595+660+940</td>
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<td>0.46797</td>
<td>0.85083</td>
<td>0.30898</td>
<td>0.44396</td>
</tr>
<tr>
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<td>0.29433</td>
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</tr>
<tr>
<td>595+660+940</td>
<td>0.30458</td>
<td>0.61208</td>
<td>0.90838</td>
<td>0.27242</td>
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</tr>
<tr>
<td>660+940</td>
<td>0.51529</td>
<td>0.57314</td>
<td>0.69022</td>
<td>0.43113</td>
<td>0.54373</td>
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<tr>
<td>940</td>
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<td>0.86407</td>
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<td>0.81973</td>
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<tr>
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<td>0.83108</td>
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<tr>
<td>400+470+525+595</td>
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<td>0.48733</td>
<td>0.88926</td>
<td>0.29010</td>
<td>0.46232</td>
</tr>
</tbody>
</table>

Table 1 Effect of combination of wavelengths to the assignment of images to the way of farming

| SEC       | standard error of calculation | RMSEC | root mean squared error calibration |
| SEP       | standard error of prediction  | RMSEV | root mean squared error validation |
| R2        | coefficient of determination |
Conclusion

Image analysis is a useful tool for the analysis of fish muscle tissue. Surface patterns of fish fillets gives information about quality, way of farming and species. The correct assignment of images from samples from cod and salmon depends on the geometry of the optical system and the size of the filtermask. Spectroscopic and scale informations are essential for the quality of results. A coupling of additional local features increases the correct assignment of images to the samples. Most important is the process of wavelengths combination. However a natural law for combination is yet unknown. Shorter wavelengths are more important for an extraction of structure features than long wavelengths (Dufour, 2002).

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References


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5.7 EVOLUTION OF K VALUE IN FARMED GILTHEAD SEABREAM, SEABASS AND SENEGALESE SOLE

Tejada, Margarita; Huidobro, Almudena and Mohamed, Gamal

Introduction

K value is defined as a percentage of the ratio of inosine (Ino) and hypoxantine (Hypo) to adenosine-5-triphosphate (ATP) and its breakdown products (adenosine-5-diphosphate (ADP), adenosine-5-monophosphate (AMP), inosine-5-monophosphate (IMP), Ino and Hypo) (Saito et al, 1959). K value has been used for decades as a freshness indicator for chilled fish and as a reference for the shelf life of fresh fish. Although the breakdown of nucleotides is not necessarily the cause of loss of freshness, however, it does coincide with it. It is considered that IMP contributes to flavour enhancement in fish products (Kuninaka et al, 1964). Traditionally the degradation of ATP until IMP has been attributed to muscle enzymes meanwhile the degradation of IMP to Ino and Hypo has been also connected with the growth of bacteria. Nevertheless the last assessment has not been proved in fish muscle stored aseptically (Uchiyama and Ehira, 1974). For some species to be consumed raw, where the freshness of the fish muscle is very important, maximum K value has been set at 20% (Saito et al, 1959). The rate of breakdown of ATP and related products depends on handling before and after slaughter, seasonal variations, and inter- and intra- species differences (Huidobro et al, 2001). It has been demonstrated that K values remain low for gilthead seabream even when the fish is close to the point of rejection by a sensory panel (Huidobro et al, 2001)

Materials and methods

Farmed gilthead seabream (Sparus aurata) (GSB), seabass (Dicentrarchus labrax) (SB) and Senegalese sole (Solea senegalensis) (SS) were used in the study, each lot consisting of ≥40 kg of fish. The fish was fasted for 48h before slaughtering (November 2003) and killed by immersion in ice-water slurry. GSB and SB were farmed and slaughtered at CULMAREX, Águilas, Murcia, Spain. After death the fish were packed in expanded polystyrene boxes with perforated bottoms containing approximately 6 kg fish each, the fish covered by a perforated plastic film with ice flakes on top and freighted to the laboratory at the Instituto del Frío (IF) by regular transport in refrigerated trucks. SS were farmed and slaughtered at PISCIFACTORIA MURTERAR, Mallorca, Spain and sent by airmail to de IF in expanded polystyrene sealed boxes containing approximately 10 kg fish each. In this case the ice flakes were packed in sealed plastic bags to avoid leaks. Once at the IF, the bottom of the boxes containing SS was perforated to allow draining of the melted ice. All the fish were kept in perforated boxes with ice in cold stores at 2 ± 1 ºC, and ice was added to the boxes as required. The fish was stored until rejected by a sensory panel (15, 23 and 28 days for GSB, SB and SS respectively). The mean weight of the fish was 504.6±26.8 g; 620.0±68.3 g; 250.7±40.5 g and the mean length 23.5±0.67 cm; 32.3±198 cm and 22.1±1.33 cm for GSB, SB and SS respectively.

ATP and their breakdown products were extracted with 0.6 M perchloric acid according to Ryder (1985) from the dorsal muscle of three individuals (post rigor mortis) and stored at –80 °C until analysed (≤30 days). Immediately before analysis, the extracts were thawed and passed through 0.45 μm Nylon filters [Micro Filtration Systems (MSF) Inc., Pleasanton, CA], and 10 or 20 µL was injected. Chromatography was carried out on a Waters HPLC system (distributed by Waters Cromatografía, S.A., Madrid, Spain) equipped with a binary HPLC pump (model 1525), a dual λ Absorbance Detector (model 2487) (set at 254 nm ), an autosampler (model 717plus) and equipped with a Waters Breeze™ software. Determinations of ATP, ADP, AMP, IMP, Ino and Hypo were done on a Waters Symmetry® C-18 5.0 µm. 4.6 mm * 150 mm column (Millipore Corporation, Milford, MA) using 0.04 M KH₂PO₄-0.06 M K₂HPO₄ buffer pumped at 1 mL*min⁻¹. Run time was 20 min. External calibration was used with standards obtained from Sigma (Sigma Chemical Company, St. Louis, MO, USA). The means of six measurements were calculated. Amounts of ATP and breakdown products were expressed as μmol*g⁻¹ sample. K value (as a percentage of the ratio between Ino + Hx to all ATP related products) was calculated according to Saito et al. (1959).
Results and discussion

In all three species, the evolution of the K-value with storage time was linear (GSB: $y = 1.8169x + 3.0173$, $R^2 = 0.9893$; SB: $y = 3.0671x + 15.7111$, $R^2 = 0.9559$ and SS: $y = 1.1042x + 7.7102$, $R^2 = 0.927$) (Fig. 1). The increase was sharper in seabass than in the other two species. At the end of the storage period, K values were around 80% in SB, and lower than 40% and 30% for SS and GSB respectively. The low K values obtained for GSB have been previously reported (Huidobro et al., 2001). These differences are due to the slower rate of degradation of IMP for gilthead seabream and Senegalese sole, since the degradation of ATP, ADP and AMP was very fast in all three species. For these two species the decrease of IMP was less than half of its initial value by the end of the storage period. SB presented the sharpest increase of Ino, while SS presented the highest rate of Hypo formation and lowest Ino values (Fig. 2).

Differences among species were also observed in the total amount of ATP and breakdown products obtained. Significantly lowest values were observed in SS and a decrease of total ATP and derivatives during ice storage were observed in SB. Apparent disappearance of Hx as a result of bacterial action has been reported by Surete at
al. (1988). Nevertheless, in the present case, bacterial count (aerobic plate count at 15°C (APC at 15°)) luminous bacteria, H2S producing bacteria and Enterobacteriaceae) for GSB and SB did not show significant differences during storage (results not shown).

Two unknown peaks were observed in the HPLC chromatograms, with retention time AMP<X<Hypo<Y<Ino (Fig. 3). Comparison of the areas of the peaks as they relate to IMP, Ino and Hypo shows that the area of peak Y decreased during storage, meanwhile the area of peak X remain quite stable in GSB and SB and does not appear in SS. This could indicate that some intermediate peaks occur in the early stages of storage of the fish in ice, or else that there is some degradation during storage of the extracts at -80°C.

Conclusions

The increase of K value in fish muscle during the ice storage of fish, used for several species as an early index for freshness of fish has to be determined in each species and related with changes in sensory parameters used to determine fish quality.

The index has a good linear correlation with storage time on ice although the slope of the curves depends on the species. For this reason, in species where K value is low (lower than 40%) at the end of the storage period, individual differences among fish may give a wide prediction error in storage time. Nevertheless, this index increases from the beginning of the storage in ice of fish and have good correlation with sensory measurements as the quality index (QIM) (results not shown). At present K value is compared with the dielectric response of the fish (skin) and minced muscle in theses species in order to develop simple tools to measure fish quality.

References


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**5.8 EVALUATION OF SEAFOOD PROTEOLYSIS BY IMMUNOLOGICAL TECHNIQUES: α-ACTININ AS A BIOMARKER OF SHELF-LIFE IN CHILLED PRODUCTS**

Mónica Carrera, Vanesa Losada, Carmen Piñeiro, Lorena Barros, José Manuel Gallardo, Jorge Barros-Velázquez and Santiago P. Aubourg

**Introduction**

Post-mortem tenderisation of fish muscle is one of the major problems related to freshness loss in chilled seafood products. One of the causes of post-mortem tenderisation in fish muscle is the breakdown of Z-line structure of myofibrils (Ando et al., 1991). The principal protein of Z-line, α-actinin, plays a key role in post-mortem changes of the Z-line structure (Papa et al., 1996; Seki and Tsuchiya, 1991; Astier et al., 1991). This protein (100 kDa, pI 5.6), (Papa et al., 1995), represents in the fish muscle the 2% of myofibril protein total weight (Takahashi and Hatori, 1992). The release of α-actinin from the myofibrilar protein fraction depends on different proteolytic mechanisms such as the activity of proteases as calpains and cathepsins (Delbarre et al., 2004a; Delbarre et al., 2004b; Ladrat et al., 2003, Verrez-Bagnis et al., 2002, Lamare et al., 2002, Aoki et al., 2000).

Actually, the methods for monitoring the changes associated with freshness can be classified as sensory, physical, physico-chemical, chemical and microbiological (Alomirah et al., 1998). For this reason, the study of release of α-actinin to sarcoplasmic fraction can be established as a method to monitor the proteolysis degree in fish and to define it as biomarker of quality and freshness in chilled fish (Tsuchiya, et al., 1992; Papa et al., 1996; Verrez-Bagnis et al., 1999; Delbarre-Ladrat et al., 2004b). Thus, we have evaluated the α-actinin content by means of a specific enzyme-linked immunosorbent assay (ELISA) in the sarcoplasmic muscle protein fraction of three different chilled fish species (European hake, farmed turbot and horse mackerel). The results obtained by immunological procedures were correlated with the changes observed in electrophoretic profiles and other biochemical parameters.

**Materials and methods**

**Fish material, processing and sampling.**

European hake (*Merluccius merluccius*) and horse mackerel (*Trachurus trachurus*) specimens were caught from the Galician Atlantic coast and kept in flake ice till they arrived at laboratory (six hours after). Farmed turbots (*Psetta maxima*) specimens were obtained from Stolt Sea Farm, SA (Carnota, Galicia, Spain) and slaughtered in ice for 6 hours until they arrived at laboratory. All the fish specimens were directly immersed in flake ice without being headed or gutted in an isothermal room at 2°C.

**Sensory analysis**

Sensory analysis was conducted by a sensory panel consisting of five experienced judges, according to guidelines concerning fresh and refrigerated fish (DOCE, 1989; Rodriguez et al., 2003). Sensory assessment included the following parameters: skin, external odour, gills, consistency and flesh odour. All analyses were performed in triplicate.

**Preparation of sarcoplasmic proteins**

Sarcoplasmic protein extracts were prepared in a low-ionic-strength buffer as previously described (Piñeiro et al., 1998). All extracts were maintained at –80°C until analysis. Protein concentrations in the extracts were determined by means of the protein microassay method (Bio-Rad Laboratories Inc. Hercules, CA). A standard curve constructed for bovine serum albumin was used as reference.

**Nucleotide degradation analyses.**

Analysis of the nucleotide autolytic degradation was carried out by the method of Ryder (Ryder, 1985). This procedure is based in the separation of nucleotides by means of high performance liquid chromatography (HPLC).
The K value was calculated according to the following concentration ratio: 

\[ K \text{ value} = 100 \times \frac{\text{hypoxanthine + inosine}}{\text{adenosine triphosphate + adenosine diphosphate + adenosine monophosphate + inosine monophosphate + inosine + hypoxanthine}}. \]

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoretic analyses were carried out by means in commercial horizontal SDS-PAGE gels (245x110x1mm Excel-Gel SDS Homogeneous 15%, Amersham Biosciences) and silver stained according to described procedure (Piñeiro et al., 1998).

Enzyme-linked immunosorbent assay (ELISA)

Detection of \(\alpha\)-actinin in the protein samples was assessed by a conventional indirect ELISA. Briefly, 96-well high binding plates (Costar Corning Incorporated, NY, USA) were coated with aliquots from sarcoplasmic protein fraction and incubated at 4 °C overnight. Then, wells were blocked and washed. Afterward, wells were incubated with mouse monoclonal anti-\(\alpha\)-actinin Sarcomeric (Mab) (Sigma, St Louis, MO). This was followed by washing. Bound antibodies were detected using horseradish peroxidase (HRP)-labelled goat anti-mouse Igs (Sigma). After washing, the colorimetric reaction was developed with the substrate ortho-phenylene-diamine (OPD, Sigma). Absorbance at 492 nm was measured in a ELISA Microplate Reader. In all ELISAs, we used a control negative and three replicate wells for sample.

Results

Sensory analyses

European hake specimens stored in ice maintained good quality only until the second day of storage (Table 1). After this time, sensory quality decreased and on day 8 this batch was no longer acceptable (Losada et al., 2004). For farmed turbot, the specimens maintained good quality up to day 14, afterwards the batch exhibited unacceptable quality on day 19. Finally, the horse mackerel specimens maintained good quality up to day 2 and were rejected at day 8.

Table 1. Summary of sensory analysis during chilled storage of the different species \(^a\).

<table>
<thead>
<tr>
<th>Freshness categories</th>
<th>European hake</th>
<th>Turbot</th>
<th>Horse mackerel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Storage time (days)</td>
<td>2(^b)</td>
<td>8</td>
<td>14</td>
</tr>
</tbody>
</table>

\(^a\)Freshness categories: A (good) and C (unacceptable). \(^b\) Limit day for freshness category indicated

Electrophoretic profiles of sarcoplasmic proteins

Previous reports by other authors have proposed certain soluble polypeptides as spoilage or freshness biomarkers (Morzel, et al., 2000; Papa et al., 1996, Verrez-Bagnis et al., 1999). These observations agree with the results obtained in this work. This can be observed in the profiles obtained by electrophoretic techniques of sarcoplasmic proteins in the Figure 1. In the profiles obtained for hake (Figure 1.i), we observed a marked increase of two protein bands (about 23 and 24.5 kDa) at day 5 of storage, which were subsequently degraded at day 15. In farmed turbot (Figure 1.ii), the profiles obtained for specimens stored in ice show the increase of one polypeptide (around 22 kDa) from day 29 to the end to storage. Finally, horse mackerel specimens show differences among different times of storage (Figure 1.iii). In this way, a correlation between the spoilage degree and the appearance of two polypeptides (25 and 14 kDa) could be observed. The amount of such polypeptides increased notably at day 19 of storage.
Figure 1: Comparative electrophoretic profiles obtained in 15% ExcelGel homogenous SDS-PAGE from sarcoplasmic proteins of hake (i), turbot (ii) and horse mackerel (iii), during storage in ice. Different lines include a low molecular weight standard (st: 14-94 kDa) and the different storage times (days). Black arrows indicate the positions of the proteins undergoing changes during the storage.

Correlation between K value and enzyme-linked immunosorbent assay (ELISA) for α-actinin.

Figure 2 (lines) shows the results obtained by ELISA analysis of each fish species analyzed, with a α-actinin MAb for sarcoplasmic protein samples, throughout the storage time. The absorbance values for negative samples were substracted from the positive values.

An indirect ELISA assay was chosen for all species using a commercial α-actinin mouse MAbs as capture antibodies. The assays were then developed through a series of optimization steps

We observed (Figure 2) a correlation between ELISA (line) and K value (bars), during storage. This correlation is species-dependent. In the case of the European hake (Figure 2.i), along the storage time, we showed in sarcoplasmic fraction (black line), a little increase of absorbance until day 12, however the greater increase of absorbance was obtained from day 12 until the end for values of α-actinin. This result agrees with the increase revealed in K value for that day (Figure 2.i). For turbot, the optic density for α-actinin in the protein sarcoplasmic fraction, shows a gradual increase until 40 days. This datum agrees with a gradual increase of K value. Finally, in the case of horse mackerel specimens, a remarkable tendency in the ELISA results does not exist until day 15. After 15 days of storage, specimens show a notable increase of absorbance. This fact is in correlation to the maximum levels of K value.
Discussion and conclusions

We evaluated muscle degradation in the post mortem state of three different chilled fish species (European hake, turbot and horse mackerel). For this purpose, we studied the correlation between the $\alpha$-actinin removal from the Z-line to sarcoplasmic fraction and other biochemical parameters and sensorial, along the storage time. We observed differences in the degree of proteolysis (Whittle, et al., 1990; Love, 1997). Specimens of European hake show high values of proteolysis between days 5 and 8 of storage. This conclusion is correlated with an increase of K value, the appearance of two peptides (23 and 24.5 kDa) at day 5, and an unacceptable sensory quality. In addition, the release of $\alpha$-actinin to sarcoplasmic fraction studied by ELISA, reveals an increase at the same time. In the case of farmed turbot the results show the presence of high values of absorbance in ELISA after 14 days of chilled storage in concordance with the maximum values of K index and an unacceptable sensory quality. To conclude, specimens of horse mackerel present bad quality from day 5, this fact is remarked with an increase of K value and the appearance of two proteolysis products (25 and 14 kDa), in sarcoplasmic protein profiles. However, the optic density for $\alpha$-actinin presents a small increase until day 15th. Based on these results, we expect that this protein could be used as a biochemical maker of fish muscle degradation. Besides, the ELISA procedure is a simple method and short time consumer, and it can be carried out into a field test kit for use onsite by fish processor and inspectors.

References


Authors

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5.9 TWO-DIMENSIONAL GEL ELECTROPHORESIS ANALYSIS OF FISH MEAL PRODUCTION

Morten Ruud, Harald B. Jensen and Eyolf Langmyhr

Two-dimensional gel electrophoresis (2-DE) followed by spot identification with mass spectrometry is a powerful and widely used method for the analysis of complex protein mixtures. 2-DE separates proteins according to charge (pI) by isoelectric focusing in the first dimension and according to size (Mr) by SDS-PAGE in the second dimension, capable of resolving 2000-3000 proteins in a single gel. We have used this approach to study the changes in protein composition during production of fish meal using Blue Whiting (Micromesistius poutassou) as raw material, and to identify the major proteins in fish meal. First, a study to find the optimal sample preparation conditions for 2-DE of fish meal was carried out. 13 different solubilisation/rehydration buffers were tested and the buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, and 20 mM DTT was found to be best. To study the fish processing, samples were collected from a commercial fish meal factory at different steps in the fish meal production. Fish meal was also produced in laboratory-scale with varying temperatures during the cooking, evaporation and drying process to investigate the effect of the thermal processing. The 2-DE was performed under reducing and non-reducing conditions in order to follow formation of disulphide bonds. 2-DE maps of these experiments were visibly different and the preliminary results are presented here.

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5.10 DEVELOPMENT OF AN ENZYME IMMUNOASSAY FOR TYPE-I BREVETOXIN DETECTION IN SHELLFISH

Argarate, N., Pérez Villarreal, B. and Alfaro Redondo B.

The massive proliferation of the Gymnodinium breve dinoflagellate produce a potent group of neurotoxins known as brevetoxins that cause a syndrome in humans named neurotoxic shellfish poisoning (NSP). The most frequently produced toxin is brevetoxin-2 (PbTx-2), being 80 µg per 100 g of tissue the maximum permitted level of brevetoxin-2 by Health Authorities. The immunological methods developed in this project aim respond to the need for new, faster and more sensible methods for the routine analysis of marine biotoxins.

The main objective of this research project is the development of an enzyme linked immunosorbent assay (ELISA) based on the availability of specific monoclonal antibodies (mAb) to brevetoxin type-1 detection in shellfish samples to assure the safety of seafood and fish products. The assay utilizes mice anti-brevetoxin monoclonal antibodies obtained after immunization with bovine serum albumin-brevetoxin conjugate. The purified antibodies produced will be labelled with biotin and two alternative ELISA formats will be evaluated. Additionally, we will compare different sample extraction procedures for minimization of matrix effects in the detection of brevetoxins by immunodiagnostic techniques.

The implementation of immunodiagnostic tools will allow to determine the presence of brevetoxins in shellfish samples. An enzyme immunoassay could be an appropriate method to closely monitor brevetoxin as a screening method. The ELISA procedure proposed in this project can be a simple, rapid and low-cost method with a high sensitivity and specificity toward brevetoxins, and can potentially be adapted to formats suitable for field applications.

Introduction

The bloom-forming Gymnodinium breve dinoflagellate produces a potent group of neurotoxins known as Brevetoxins (PbTx). Blooms of this toxic dinoflagellate have caused massive fish kills, marine mammals and sea bird deaths, and mollusc contamination, which, if consumed, result in human neurotoxic shellfish poisoning (NSP).

The brevetoxins are lipid-soluble polyether toxins that activate the voltage-sensitive sodium channels of nerve and muscle tissue, which leads to cell death (Naar et al., 2002). Brevetoxins consist of at least nine congeners and are divided into two groups based on their polyether backbone structure (type A or 1 and Type B or 2). The principal toxins in G. breve are the A type brevetoxin PbTx-1, and B-types PbTx-2 and –3. Of these, PbTx-2 is the most abundant being 80 µg per 100 g of tissue the maximum permitted level of brevetoxin-2 by Health Authorities.

The growing threat of seafood intoxication has become evident in recent times. In Europe, the economy of many coastal cities is linked to seafood production; hence toxin detecting is of extreme importance. However, the actual monitoring of shellfish by mouse bioassay is slow, with a low throughput, causing delays in the reopening of shellfish beds. Development of rapid alternative methods for brevetoxin detection in seafood is important for those involved in seafood regulation and the shell fishing industry as well as for those concerned with public health. Enzyme immunoassays are sensitive methods for quantifying many biologically active small molecules. During the last 15 years, intensive efforts have developed both polyclonal and monoclonal antibodies raised against brevetoxins (Trainer et al., 1991; Levine et al., 1992; Poli et al., 1995).

In this work, we will develop a quick, sensitive, and accurate ELISA method to quantify brevetoxins in seafood. The main objectives will be to develop an ELISA immunoassay based on the availability of specific monoclonal antibodies (mAb) to brevetoxin PbTx-2 detection in shellfish samples.

Materials and Methods

Brevetoxin PbTx-2 was purchased from Latoxan (Valence, France). The brevetoxin was conjugated with bovine serum albumin (BSA) using the aldehyde function of the hapten. Monitoring of positive fraction was performed
using UV detection at 280 nm and collected. Then, the mice were immunised by intraperitoneal (I.P) injection of the immunogen brevetoxin-BSA.

Splenocytes from the immunised mice were mixed with murine myeloma cells. The fused cells were cultured in hypoxanthine aminopterin thymidine medium (HAT medium), and the culture medium screened for anti-brevetoxin antibody by ELISA. Hybridoma cell populations secreting anti brevetoxin antibody were cloned. The cloned hybridoma cell line was injected into Freund-primed mice. The anti brevetoxin mAb was purified from the ascites supernatant by affinity chromatography on a protein A-agarose gel (Pharmacia Biotech., Uppsala, Sweden).

The purified antibodies produced will be labelled with biotin and two alternative ELISA formats will be evaluated. Finally, once the ELISA will be characterised we will compare different simple extraction procedures for mussel sample analysis. Acetone (Hannah et al. 1995) and ethanol (Garthwaite et al. 2001) extraction will be tested for minimization of the matrix effects.

Results

The brevetoxin PbTx-2 is (C_{50}H_{70}O_{11}) is the target antigen with a molecular weight of 895.09. Like most other marine toxins, the brevetoxin, a hapten, has no immunogenicity and must be conjugated to a protein carrier to add immunogenicity. Therefore, in the present study, we conjugated the brevetoxin PbTx-2 to the protein carrier bovine sera albumin (BSA).

Three fusions were done and three hybridoma secreting IG-M antibodies against brevetoxins PbTx-2 were selected. Moreover, recent work is focused on purified antibodies production and on the antibodies characterisation.

In future work, we will investigate the application of this ELISA in liquid samples and seafood samples by spiking the toxin and analysing the matrix effect. The recoveries of shellfish homogenate, acetone extract, ethanol extract and buffer will be tested. The detection limit that is expected will be about 2.5-5 µg/100 g shellfish meat.

The development of ELISA is still being done and we will critically evaluate and select the most appropriate assay methods and formats for use within food processing environments. Selection criteria will include robustness, antibody availability, cost of assay manufacture, and ease of manufacture.
Conclusion

For international health authorities and the fishing industry, guaranteeing the safety of seafood like bivalve mollusks and fish is a high priority. In order to achieve this aim, methods capable of detecting marine toxins in amounts lower than the maximum permitted levels (MPLs) for these substances are essential.

The official methods currently in use are time-consuming (24 hours), expensive, and non-specific. For that reason, alternative validated methods are needed to replace or to complement existing methods. This ELISA in progress is expected to be a sensitive method for detecting brevetoxins in complex matrices. These methods must be fast, easy to use, sensitive, specific, reproducible, inexpensive, and susceptible to automation (Rodriguez et al., 1990). These methods must be considered as complementary to conventional techniques, rather than as substitutes for them.

References


Authors

Argarate, N., Pérez Villarreal, B. and Alfaro Redondo B.

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5.11 EVALUATION OF THE EUROPEAN FOUR-PLATE TEST FOR SCREENING DIFFERENT ANTIBIOTIC RESIDUES IN TROUTS

Berna Kilinc, Sukran Cakli, Carsten Meyer

Introduction

The detection of antibacterial residues in food requires screening methods sensitive at antibiotic concentrations close to the maximum Residue Limit (MRL). Four Plate Test method is commonly used for screening. The method proposed is a four plate agar diffusion test in which two different microorganisms (Bacillus subtilis) and (Micrococcus luteus) ATCC 9341 are used as indicator organisms, besides three different pH-es of the media (Bogaerts & Brussels, 1980). The fish samples are applied to four plates of agar media inoculated with Bacillus subtilis spores (at Ph 6, 7.2, 8.0) and Micrococcus luteus (at pH 8.0). Diffusion of an antibacterial substance is shown by the formation of zones of inhibition of one or both microorganisms (Okerman, Wasch & Hoof, 1998). There were not found so much studies about determination of antibiotic residues by using ECC-Four plate test. The antibiotic residues were determined in meat samples (Okerman, & Hoof, 1997), in pork (Chang, Thai, & Li, 2000), chloramphenicol residues in the tissues (Lynas, Currie, Elliot, McEvoy, & Hewitt, 1998), oxytetracycline treatment on the immune response of turbot (Tafalla, Novoa, Alvarez, & Figueras, 1999). The aim of present study was to investigate first measuring the inhibition zones for penicillin, sulfadimidine and streptomycin which were used in the four plate test and then preparing paper discs with different concentrations of antibiotics for determining the detection limits. The next step was the detection of antibiotics in fish samples from fish which were fed with antibiotics by using ECC- Four Pate Test.

Material and Methods

Fish samples and diet preparation

In the study trouts were obtained from the fish farm of BAĞCI in Turkey. 225 Fish (five fish/ day) samples were used in this study. 5 of these fish were the negative control for all antibiotics group, 50 trouts were fed with oxytetracycline (100 mg powder per kg of body weight of fish per day), 50 trouts were fed with Tribrissen (80 mg powder per kg of body weight of fish per day), 50 trouts were fed with (75 mg ciprofloxacin and 50 mg enrofloxacin per kg of body weight of fish per day) were used. The treatment continued for a period of 10 days for each antibiotic. From day 11 on the trouts were feeded with normal pellets (Trouvit) without antibiotics. During the first 10 days 5 trouts were taken daily, after break off the diet every fifth day 5 trouts were taken. All trouts were slaughtered and frozen for the experiments.

Sample preparation

Frozen fish samples were thawed at 4°C overnight. The fish fillets were taken and homogenized by using Ultra Turrax T25. Almost 10 g of fish homogenate were taken for centrifugation. Fish homogenate centrifugated. 10µl of supernatant for each fish were put directly on paper discs (Mast Diagnostics, BD0638W) and then dried at 40°C for 10 minutes. These dried paper discs were put on the inoculated agar plates which were prepared before. The fish supernatants were applied to four plates of agar media.

Analysis

This is a microbiological agar diffusion test with two different microorganisms, it consists in four different assay plates.

Plates I : Melted agar medium Test Agar pH 6.0 (Merck, 10663) is inoculated with Bacillus subtilis, at a final concentration of 10^4 spore/ml then 13 ml of the inoculated medium is transferred in a petri dish 90 mm diameter. Plates are incubated 18 hours at 30°C.

Plates II : Melted agar medium Test Agar pH 7.2 (Merck, 15787) with trimethoprim (Riedel- de Haen, 46984) added to a final concentration of 50µg/l medium is inoculated with Bacillus subtilis, at a final concentration of 10^4 spore/ml then 13 ml of the inoculated medium is transferred in a petri dish 90 mm diameter. Plates are incubated 18 hours at 30°C.
Results and Discussions

The effects of the fish supernatant on detection limits of antibiotics with microbiological inhibition tests were measured. Fish supernatants were laid directly on top of paper disks impregnated with aqueous antibiotic solutions. Inhibition zones were compared with those obtained by the same standard solution without fish supernatant. Penicillin, sulfadimidine, streptomycin, sulfadiazine/trimethoprim groups of antibiotics were determined by using ECC-Four Plate Test method. Comparison of inhibition zones observed with paper disks impregnated with aqueous antibiotic solution and their detection limits are shown in Table 1.

Table 1 Comparison of inhibition zones observed with paper disks impregnated with aqueous antibiotic solution

<table>
<thead>
<tr>
<th>Medium</th>
<th>Antibiotic (and detection limit)</th>
<th>paper disk tested /ng antibiotic</th>
<th>Diameter of zones without tissue (range of six observations)/ mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>PenicillinG (0.4 ng)</td>
<td>6.2</td>
<td>10-8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.1</td>
<td>8-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6</td>
<td>6-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8</td>
<td>5-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4*</td>
<td>2-0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Sulfadimidine (31.2 ng)</td>
<td>500</td>
<td>8-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>125</td>
<td>5-4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62.5</td>
<td>4-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31.2*</td>
<td>1-0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Sulfadiazine/ (1.95 ng)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Trimethoprim</td>
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<td></td>
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<td></td>
<td></td>
<td>500</td>
<td>15-14</td>
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<td></td>
<td></td>
<td>250</td>
<td>12</td>
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<td></td>
<td></td>
<td>125</td>
<td>11-10</td>
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<tr>
<td></td>
<td></td>
<td>62.5</td>
<td>10-9</td>
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<tr>
<td></td>
<td></td>
<td>31.2</td>
<td>7</td>
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<td></td>
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<td>15.6</td>
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<td>7.8</td>
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<td></td>
<td>3.9</td>
<td>3-2</td>
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<td></td>
<td></td>
<td>1.95*</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.98</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>Streptomycin (62.5 ng)</td>
<td>500</td>
<td>7-6</td>
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<tr>
<td></td>
<td></td>
<td>250</td>
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<td>31.5</td>
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<td>0</td>
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<tr>
<td>IV</td>
<td>Streptomycin (125 ng)</td>
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<td>31.2</td>
<td>0</td>
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<td></td>
<td></td>
<td>15.6</td>
<td>0</td>
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</table>

*: Detection limit
Six observations were obtained with each concentration of antibiotic without fish. Inhibition zones were measured and correlated to antibiotic concentrations. Detection limits were as follows: on medium I penicillin G (sodium salt), 0.4 ng; on medium II sulfadimidin, 31.2 ng; on medium III streptomycin, 62.5 ng; on medium IV streptomycin, 125 ng. Fish fed with pellets containing antibiotics the inhibition zones increased according to time of feeding. In the control group, no inhibition zones were detected.

References


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5.12 LOSS OF REDNESS (a*) AS A METHOD TO FOLLOW HEMOGLOBIN-MEDIATED LIPID OXIDATION IN FISH MINCE

Daniel Wetterskog and Ingrid Undeland

Introduction

Heme-proteins, especially the oxidized met-forms, have been identified as highly critical for the onset of lipid oxidation in muscle, particularly in fish from cold waters (Richards & Hultin, 2002; Undeland et al., 2004). In studies of lipid oxidation in minced herring (Undeland et al., 1998), minced/sliced tuna (Lee et al., 2002) and washed cod mince model systems containing fish hemoglobin (Hb’s) (Richards et al., 2002ab; Richards & Hultin, 2003; Undeland et al., 2004), it was measured instrumentally and visually that there was a dramatic loss of red colour (a*) in parallel with hydroperoxide and TBARS development during cold storage. Lee et al. (2002) concluded that the decrease in a*-value of tuna was due to conversion of the bright red oxy-myoglobin (Mb) to the brownish met-Mb form.

In this study, we wished to evaluate whether the brownish colour of the highly pro-oxidative met-Hb could be taken advantage of as a basis for detecting the oxidative changes in the fish muscle that it gives rise to. More specifically, our aim was to evaluate whether loss of redness (a*) in a Hb-containing fish muscle model system could be used as an indirect tool to follow Hb-mediated lipid oxidation during ice storage. Lipid oxidation was followed in terms of TBARS and painty odor, and the model systems were designed to develop lipid oxidation at different rates. The latter was achieved by adding an antioxidative cod muscle press juice and by varying the pH of the model system. To confirm the involvement of met-Hb in the colour changes, spectrophotometric analyses of a buffer-based model system at different pH-values was done in parallel with colorimetric analyses of redness (a*).

Materials and methods

The preparation of washed cod mince (at 70% or 81% moisture), trout blood hemolysate and cod muscle press juice was done according to Undeland et al. (2003; 2004). Samples were prepared by adjusting the washed cod mince made at 81% moisture to pH 6.1, 6.5 or 6.9. In another set of samples, the washed cod made at 70% moisture was brought to 81% moisture with either 50 mM phosphate buffer or antioxidative cod muscle press juice (hereby diluted 1.8 times). Streptomycin and hemolysate were then added to each sample to reach final levels in the moisture fraction of 200 ppm and 15 µM hemoglobin, respectively. Each sample (final weight ~20 g) were then flattened out with an L-shaped stainless steel spatula in the bottom of a 250 mL screw-capped glass Erlenmeyer flasks resulting in a sample thickness of ∼5-6 mm. Samples were stored on ice in darkness for up to 14 days.

During storage of the Erlenmeyer flasks, changes in redness (a*), lightness (L*) and yellowness (b*) of the cod mince samples were measured using a colorimeter (Minolta Chroma Meter CR-300 Minolta Corp., Ramsey, NJ) using the CIE Lab color scale. The head space of the sample flasks were also regularly smelled for “painty odour” (Undeland et al., 2003). One gram sample “plugs” were also taken for TBARS analyses (Lemon et al., 1975).

An aqueous model system made to mimic the muscle system was used to spectrally follow conformational changes in the hemoglobin molecule during ice storage. Sixteen ml of phosphate buffer (50 mM, pH 6.4) was mixed with 200 ppm streptomycin sulfate and 15 µM trout Hb. A blank was constructed by excluding the addition of trout Hb. Twenty mL of the samples were stored on ice in 250 ml Erlenmeyer flasks for 24 days. At regular intervals, 1 ml samples were scanned against blanks between 650 and 450 nm. The peak values around 576 nm and 630 nm as well as the valley value at 560 nm was recorded for all the samples. Calculations of the oxy- deoxy- and met-Hb were done according to the equations described by Benesch et al. (1973).

Results

In all fish mince samples developing lipid oxidation, a*-values correlated well with TBARS and painty odor; r = -0.95 and r = -0.77, respectively. Press juice containing samples did not develop any lipid oxidation products, but
still lost some redness, why the correlation between a* and TBARS was lower (r=0.55) (Figure 1). Based on a*-values, one could distinguish between “oxidizing” and stable samples already within 1 day, which was before any lipid oxidation products had developed. The kinetics of the a*-value drop in a sample subjected to lipid oxidation could be distinguished into three different phases that were called “initial phase”, “differentiation phase” and “stationary phase” (Figure 1). In the “differentiation phase” both the lipid oxidation data and a*-value responses changed rapidly. In the other tow phases, changes were less pronounced. Both the method of preparing the washed cod model system, and the pH of the system affected absolute initial a*-readings given by the added 15 µM trout Hb. The span of variation was from ~3-4.5.

An aqueous model system was setup to confirm that the formation of met-Hb paralleled a*-value loss. The redness dropped significantly slower in this aqueous system than in the mince system. However, significant alterations in the relative levels of oxy-, deoxy- and met-Hb were seen already within 0.8 days. The increase in met-Hb formation in this period was from 0-40%. After 10 days, the formation levelled off at ~85%. Deoxy-Hb and oxy-Hb levels decreased from 60% and 50% down to 28 % and 32 %, respectively, during the first 0.8 days. Thereafter, the decrease continued but more slowly. After 10 days, the changes levelled off at just below 10% for both deoxy-Hb and oxy-Hb.

Discussion

Our study showed that the factors controlling TBARS and painty odor development also controlled redness (a*-value)-loss in a similar way. However, in samples where lipid oxidation was not detected at all during storage, there were still a slight reduction in a*-values; possibly due to some drying of the surface, and/or slight met-Hb formation. The a*-value data were best used as a lipid oxidation index by calculating the rate of a*-decrease (k) in the “initial phase” of the storage (before accumulation of lipid oxidation products) or in the “differentiation phase” (during the exponential raise in TBARS/painty odor) (Figure 1). Here, there were clear differences between stable and “oxidizing” samples. Based on that the initial a*-values of the various samples differed somewhat depending on the preparation technique, it is suggested that a*-value measurements must be calibrated against lipid oxidation products for each specific sample type. In a solution consisting of phosphate buffer and trout hemolysate, spectrophotometric analyses confirmed that the loss of a*-value corresponded to a buildup of brownish met-Hb at the expense of reduced oxy- and deoxy-Hb. The faster loss of redness in the muscle based

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**Figure 1:** Storage induced development of TBARS and loss of redness (a*-value) in washed cod mince with and without added cod muscle press juice at a 1.8-fold dilution. Both samples contained 15 µM Hb, 81% moisture and adjusted to pH 6.5.
sample than buffer based sample could suggest that the development of lipid oxidation products also triggered met-Hb formation; thus, not only the opposite.

**Conclusion**

In conclusion, instrumental redness-analysis of fish mince could become a very early and sensitive tool to follow lipid oxidation. However, the relation between a*-values and build-up of oxidation products must be carefully controlled for each specific system.

**References**


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5.13 QUANTITATIVE DETERMINATION OF POLYPHOSPHATES ADDED TO FRESH AND DEEP FROZEN FISH BY MEANS OF THERMO DIFFERENTIAL PHOTOMETRY

Reinhard Kruse

Introduction
Polyphosphates are widely used as proper agents to enhance water retaining power of different foods. In the special field of official quality control of fish analytical techniques are needed under two corresponding aspects: First of all qualitative information is required whether polyphosphates have been added illegally to fresh fish and frozen fish respectively. In order to check the presence or absence of these compounds, thin layer chromatography (TLC) has ever been the method of choice. In addition to the merely qualitative procedure above reliable knowledge is necessary about definite concentration of both di- and triphosphate-ions in fish. Only quantitative determinations can proof an agreement between official legal limits and added amounts.

Our method
Realizing specific weak points in actually applied quantitative methods like densitometry of thin layers, enzymatic determination or photometry of total or soluble P, we felt motivated to create a new procedure. Due to its main technique it may be called “Thermo Differential Photometry”. It depends on the different reaction speeds, under which – on the one hand – the monophosphate-anion and – on the other hand – the polyphosphates (di- and tri-ions) are converted into yellow phosphovanadic molybdic acid, which is the well known and established step in many applications of photometric phosphate-analysis. The differences in kinetic behaviour offer a simple and reliable way to quantify polyphosphates in addition to the usual monophosphate determination procedure. Significant influence of temperatures is given not only on reaction speed but also on the intensity of light absorbance. Thus the derivatisation procedures and even more the photometric measurements at higher temperature levels have to be performed under precise temperature control. For this reason the usual laboratory equipment for photometry has to be completed by a commercially available thermo regulated cuvette holder.

Experimental Procedure
Sample handling and measurement:
1. Add 46 ccm TCA (w = 10 %) to 5 g homogenate
2. Apply Ultra Turrax homogenisation
3. Prepare a filtrate or perform a centrifugation
4. Transfer 5 ml of filtrate or supernatant into a 50 ml flask
5. Add 15 ml of MoVa reagent and complete volume with water
6. Position the flask into 60 °C water bath
7. after 15 min.: Transfer a first small aliquote to a heated cuvette (60°C) and measure extinction at 430 nm
8. after 90 min.: Repeat measurement in the same way using another transferred volume

Polyphosphate concentration is negligible if extinction has increased less than 0.01 between 15 and 90 min. In this case no further efforts are necessary, sample checking may be stopped as far as monophosphate concentration is out of interest. If extinction difference is clearly more than 0.01, polyphosphates are obviously present. Additional quantification steps become necessary as follows:
Calibration

1. Prepare separated aqueous stock solutions of both mono- and triphosphates each containing 5 mg P$_2$O$_5$/ml, e.g. 958.6 mg KH$_2$PO$_4$ resp. 861.4 mg Na$_5$P$_3$O$_10$ in 100 ml water.
2. Transfer 0, 100, 200, 300, 400, 500 and 600 µl of each stock solution into 50 ml flasks.
3. Add 15 ml of MoVa reagent and complete volume with water.
4. Treat solutions the same way as sample extracts above.

Calculation

1. Draw the calibration curve for polyphosphate by plotting Δ Ext. (Ext. 90 – Ext. 15) versus concentration (mg P$_2$O$_5$ / 50 ml)
2. Insert lower and upper extinction value of the sample into the curve, find out the lower and upper corresponding concentration values C$_1$ and C$_2$.
3. The difference C$_2$ – C$_1$ stands for the P$_2$O$_5$ concentration in the sample solution or in 1/10 of the initial weight of the sample.

Results and Discussion

This method is suitable for the determination of soluble polyphosphates as well as for monophosphate. It may be used solitarily or in context with other ones like TLC. In recent weeks we could identify several products like frozen and fresh fish fillets and deep frozen fish fingers containing amounts of mainly triphosphate up to 20 mg / g. The method’s practical limit of quantification is calculated between 0,1 and 0,2 mg / g.

We are convinced our method is a better tool to find out illegal use of polyphosphates and thus to prevent illegal addition of water to fresh or frozen fish.

References

Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG, Methode 06.00 – 10: Bestimmung des Säure löslichen Phosphorgehaltes in Fleisch und Fleischerzeugnissen

Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG, Methode 06.00 – 15: Nachweis von kondensierten Phosphaten in Fleisch und Fleischerzeugnissen

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5.14 QUALITY CHANGES IN FISH BY-PRODUCTS EVALUATED BY HIGH RESOLUTION NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

E. Falch, T. Størseth and M. Aursand

Introduction

Marine raw material contains health beneficial marine lipids [Dyerberg et al., 1978; Vanschoonbeek et al., 2003] with applications in food, healthcare and pharmaceutical products. However, marine lipids are highly susceptible to lipid oxidation and lipolysis caused by enzymes. The lipid composition in fish is a complex mixture of fatty acids esterified in neutral and polar lipids, sterols, vitamin etc. Although lipids in fish muscle are relatively well characterized, more effort is needed in characterizing the chemical composition of the potential by-products (heads, cut-offs and visceral fractions) to increase the value of the total catch of fish [Rustad and Falch, 2003]. Recently, the lipid components in selected by-products from different cod species are characterized [Falch et al., 2004a], and data on effect of fishing ground will also soon be released [unpublished data]. Lipids in different compartments of the fish head is characterised by Stoknes et al. [2004].

The lipid composition in this material becomes even more complex when the degradation reactions are elapsing. It is therefore necessary to know and have control of these reactions. Reaction products from lipid oxidation and the products from lipolysis (free fatty acids) are known to affect the sensory acceptance of the products, but concern should also be taken to the possible heath affect of reaction products generated during lipid oxidation. Some of the compounds are toxic at high concentrations and studies have shown that hydroperoxides and aldehydes might cause damage of DNA [Yang and Schaich, 1996].

Reliable methods to follow the changes in marine lipids should be developed. To date most analytical techniques are supplying information on changes in one of the lipid substances, but do not provide information of what other consequences or parallel chemical activities that proceeds. NMR spectroscopy to study mixtures of compounds gives information on a broad range of chemical compounds that would demand the use of numerous conventional analysis, such as GC, HPLC and TLC methods, to obtain. Analysing extracts from samples using NMR would provide information on all compounds that are observable by NMR and soluble in the solvent used for extraction in one analysis. While NMR on extracts has the limitations of the solubility of compounds in different solvents, the high resolution magic angle spinning NMR (HR-MAS NMR) technique may be used directly on solids and semi solids [Cheng et al., 1997], such as cells and tissues, and give information on all NMR observable compounds in the sample regardless of the solubility characteristics of the compounds.

Using HR-MAS NMR would then provide information on both polar and non-polar compounds observable by NMR in one analysis. This gives the unique opportunity to study chemical variations in fats during different processes while at the same time obtaining information on the changes in polar compounds in the sample, giving a more complete understanding of the processes. Studying solids and semi-solids HR-MAS NMR furthermore has the advantage of the ease of sample preparation; A typical sample procedure would be to insert the sample into the MAS test tube, the rotor, and add deuterated solvent for field frequency locking and insert the sample in the magnet. The typical MAS rotor has a volume of 12 or 50 µl. Methods could be developed around the HR MAS technique as alternatives to conventional methods this would mean benefits both in work hours and the amount of chemicals used.

1H-MR has been successful in studying the changes in the lipid composition in marine lipids due to lipid oxidation [Falch et al., 2004b] and the groups of reaction products from lipid oxidation are assigned. The amount of docosahexaenoic acid (DHA) and n-3 fatty acids are quantified in fish oils [Igarashi et al., 2000] and assessments of a broad range of lipid compounds in the 1H-MR spectra of fish is reported by Aursand et al. [1994]. 13C-MR is less sensitive compared to 1H-MR but has shown potential in analysis of fatty acid composition, positional distribution of fatty acids in acylglycerols [Maninna et al., 1999; Aursand et al., 1994] and acyl stereospecific analysis of tuna phospholipids [Medina et al., 1998].

Materials and methods

These experiments were performed on raw visceral fractions of cod (Gadus morhua). Lipids were extracted according to the method of Bligh and Dyer [1959]. The fatty acid methyl esters were prepared according to Metcalfe et al. [1966] and gas chromatography was performed as reported in Aursand et al., [1992]. Lipid classes were separated by thin layer chromatography (Iatroscan) according to the method reported in Aursand et
al., [1992]. Lipid oxidation was analysed by measuring peroxide value [Undeland et al., 1998] and thiobarbiture acid reactive substances (TBARS) [Ke and Woyewoda, 1979].

The NMR analysis were all performed on a 600 MHz magnet (Bruker, Germany), The lipid extracts were analysed by $^{13}$C-MR [modified method of Aursand et al., 1993] and $^1$H-MR as reported in Falch et al. [2004b]. A set of two dimensional NMR analysis was also recorded. The HR-MAS analysis was performed on samples during a temperature controlled storage at 4°C to be able to study chemical changes during storage. The samples were kept in the magnet during recording of $^1$H, $^{13}$C, COSY NMR analysis with storage time up to three days.

**Results and discussion**

Within short time storage at 4°C significant changes in both lipid oxidation and in lipid classes (formation of free fatty acids) were found by use of the traditional analytical methods. The MAS analysis provides spectra with a high amount of signals with relatively good resolution in certain regions. The signals are generally broader than what is obtained for in the lipid extracts. Spectra of cod roe is used to illustrate the differences between the two techniques and the spectra of lipid extract are used to separate the lipid signals from the other signals obtained in the MAS spectra (Figure 1).

![Figure 1. Extended region (0-3 ppm) of $^1$H- MR spectra of stored cod roe. The upper spectrum is from the MAS experiment, while the lipid extract is below. The spectra of lipid extracts helps the assignments of peaks in the spectra from direct measurement (MAS) since these spectra will also have signals form other components than lipids. Example of information from this region is: all fatty acids except n-3 (-CH$_3$) at 0.85-0.98 ppm, n-3 fatty acids (-CH$_3$) at 0.95-0.98 ppm and C22:6/DHA at 2.38 ppm. Aursand et al., [1994] provides further peak assignmen](image-url)
Figure 2. HR- MAS (1H MR) spectra of cod roe during storage at 4°C in the magnet. Changes in specific parts of the spectra specially due to hydrolysis of lipids and proteins are observed. The two upper spectra are recorded on roe before any storage (control) while the two spectra below are from the same roe that has been stored in the magnet.

The storage experiment performed in the magnet did succeed in detecting changes in the chemical composition, and specially differences due to proteolytic- (increasing levels of free amino acids) and lipolytic activity (release of free fatty acids from phospholipids) was found. (Figure 2).

Conclusions

HR-MAS NMR is a promising method for obtaining information on a broad range of chemical compounds in a sample, which would need multiple conventional analysis methods. Our results show that this method may be used independently to study the processes related to hydrolysis (lipolysis and proteolysis) providing information on lipophilic and hydrophilic compounds in one analysis. HR-MAS NMR may also be used to obtain an overview of the compounds that vary the most, determining which conventional methods should be used to understand the processes in the best possible way.

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5.15 SPECTRAL CHARACTERISATION OF COD MUSCLE AND NEMATODES

Heidi Nilsen, Karsten Heia, Agnar H. Sivertsen, Svein K. Stormo and Edel Elvevoll

Abstract

The objective of this work was to characterize the spectral signatures of nematodes (Anisakis simplex and Pseudoterranova decipiens) and compare these with characteristic spectra from the muscle of Atlantic cod (Gadus morhua). This is important information from a spectroscopy point of view when methodology for nematode detection is developed.

Spectra from solvents with the two types of nematodes have been recorded to obtain spectral responses of Anisakis simplex and Pseudoterranova decipiens, respectively. Correspondingly, spectral responses of intact cod muscle with and without nematodes present has been recorded.

The transmission spectra of homogenised nematode solutions show interesting absorbance peaks that are not present in transmission spectra from homogenised fish muscle solutions. Working on intact fish muscle with or without embedded nematodes provides more complex spectra. Structure and thickness of the fish muscle influence the recorded spectra, and nematode spectra are thus not pure nematode spectra. Since the nematodes are embedded in the fish muscle the recorded nematode spectra is a combination of a fish muscle spectrum and a nematode spectrum. The deeper the nematode is located, the less influence the nematode spectrum has on the combined spectrum. Therefore it is reasonable to assume that nematodes closer to the surface is easier to detect than nematodes deeply embedded into the fish muscle.

By combining a nematode spectrum with a fish spectrum from the same sample (equal sample thickness) the identified absorbance peaks from the homogenised solutions are also identified in intact samples. This shows that a methodology based on spectroscopy can be used for nematode detection.

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5.16 NEMATODE DETECTION IN COD FILLETS BASED ON IMAGING SPECTROSCOPY

Karsten Heia, Heidi Nilsen, Agnar H. Sivertsen and Jens Petter Wold

Abstract

The objective of this work was to develop methodology for detection of nematodes (Anisakis simplex and Pseudoterranova decipiens) embedded in Atlantic cod (Gadus morhua) fillets. The measurement technique applied was Imaging Spectroscopy. From this instrumentation both spatial and spectral representation of the sample is obtained. Each point in the two-dimensional spatial representation of the sample contains full spectral information in the range from 400 nm to 800 nm. In other words the instrumentation creates a high number of images of the sample where each image is a recording of light at different wavelengths.

To separate nematodes from fish muscle a two step procedure was carried out. First a discriminate partial least square (DPLS) algorithm was applied on all spectra followed by a threshold to distinguish nematodes from other types of tissue like fish muscle, blood, black lining and skin remnants. This step reduces the data set to a single binary image, embedding the information; nematode or not nematode. Depending on the threshold value this binary image can be rather noisy. To reduce the noise level a median filter was applied.

Results so far show that surface nematodes are easily detected whereas nematodes deeply embedded in the fillet are more difficult to detect. The “world record” within this work is to detect a nematode located 9 mm into the fillet. Compared to possible manual detection in a fish processing plant (6 mm) this is an improvement of 50 %.

Based on these laboratory results a full scale experiment is prepared to test the performance under industrial conditions.

The results obtained in this work indicate that within two years instrumental on-line nematode detection can be achieved in the fish processing industry.

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5.17 ALGAL TOXIN TESTING IN MUSSELS BY USING CHEMICAL AND BIOLOGICAL METHODS: TWO EQUIVALENT APPROACHES?

Stefan Effkemann, Ernst Jütting, Ingo Nausch, Reinhard Tiebach, Frerk Feldhusen

Introduction

The occurrence of harmful algal blooms, followed by accumulation of algal toxins in mussels poses a threat to human consumers. In addition the presence of these compounds in mussels can lead to significant economic consequences for the mussel fishery. According to decision 2002/225/EG chemical analytical techniques (e.g. HPLC, LC-MS) can be used as well as biological methods (e.g. mouse bioassay), while they guarantee a comparable consumer protection.

Experimental part

Despite preventive algal toxin testing was performed in different EU countries in 2002, many cases of diarrhetic shellfish poisoning (DSP) occurred. In Germany for instance diarrhea, nausea were observed in case of 30 people who joined a reception and consumed mussels originating from the Isefjord, Denmark. Although 3576 µg okadaic acid per kg hepatopancreas were found by LC-MS, no mice died in the mouse bioassay, which had been initially performed by the responsible authority, in order to release the mussels for human consumption.

In another investigation, different mussel batches coming from the Flensborg inlet (Denmark, harvested between November 3\textsuperscript{rd}-6\textsuperscript{th} 2003) were released due to a negative mouse bioassay result. Due to a request of a mussel processing company further LC-MS investigations were carried out by a German authority. Although the mouse bioassay led to a negative result, the okadaic acid concentration partly exceeded 200 µg/kg mussel (whole body). In contrast to the results of the mouse bioassay the corresponding findings based on a LC-MS method were unequivocally positive. The detailed results are presented in Figure 1. The big differences of the okadaic acid concentration within mussel samples harvested in the same area at the same time are notable.

![Figure 1: Distribution of okadaic acid in mussels (whole body), harvested between November 3\textsuperscript{rd}-6\textsuperscript{th}, 2003 in the Flensborg Inlet, Denmark.](image)

In contrast to findings described above, mice died in other studies, although the okadaic acid concentration did not exceed 160 µg/kg (whole body) \[1\]. False positive results are the consequence. Other toxins were not detectable in these studies. Since chemical methods are usually based on HPLC with fluorescence or mass detection (limit of detection, okadaic acid (LOD): 5 µg/kg) they enable a very sensitive and reliable...
determination of marine biotoxins. These powerful techniques can be used in order to establish a “biotoxin early warning system”. Since the course of occurrence of DSP is characterized by a parabolic curve an estimation of the further development of the concentration of these toxins in mussels is enabled. A typical diagram showing the occurrence of okadaic acid in the hepatopancreas in blue mussels (Mellum, Germany, 1995) is illustrated in Figure 2:

Figure 2: Occurrence of DSP (okadaic acid) in blue mussels.

The authors of this presentation suggest the use of an DSP action level. If DSP values exceed a concentration of provisionally 20 µg/kg control activities should be intensified for preventive consumer protection. Due to the known inhomogeneity of DSP values within one harvesting area (see Figure 1) much more samples coming from the respective area have to be analyzed. In addition the analyzing interval has to be shortened in order to get more reliable data.

In contrast to the mouse bioassay analytical methods preferably based on LC-MS/MS are suitable for monitoring purposes. Despite grave deficiencies of the mouse bioassay with respect to the analytical problems stated above, it can be used in very exceptional cases for solution of toxicological questions, particularly in order to clarify a potential toxicological risk for the health of consumers by unknown compounds. This was shown in 2003: Vietnamese apple snails were analyzed for PSP toxins according to LAWRENCE et al. This method is based on a derivatization reaction with subsequent HPLC/fluorescence detection. The corresponding chromatogram is presented in Figure 3 a). In addition a chromatogram of a sample containing various known PSP standards is shown in Figure 3 b). Subsequently, spike experiments were carried out. It was shown, that the retention time of the sample peak did not exactly correspond to any known PSP standard.
In order to assess the toxicity of the unknown compound a mouse bioassay was performed. In these experiments it was shown, that a few mice died at approximately 24 hours after injection of the sample extract. In addition saxitoxin standard solution was injected. In case of the standard solution injection death time was just a few seconds. 
It was shown by performance of these experiments, that a health risk for human consumers by the unknown compound can almost be excluded.

**Conclusions**

- Mouse bioassay is not suitable for monitoring of threshold value levels. Wrong results can be expected.
- Due to its low sensitivity no prediction of an upcoming DSP problem (exceeding of the threshold value) possible.
- Mouse bioassay cannot be validated.
- Mouse bioassay just provides semi-quantitative results.
- Mouse bioassay does not provide information concerning the type of toxin. Every positive result has to be confirmed by using chemical methods, e.g. LC-MS/MS.

If no alternative is available, the mouse bioassay can be used in very exceptional cases for solution of toxicological questions, particularly in order to clarify a potential toxicological risk for the health of consumers by unknown compounds.

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Session 5

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34th WEFTA meeting, 12-15 September 2004, Lübeck-Germany
6.1 CHARACTERIZATION OF LIVERS LIPIDS FROM FISH SPECIES HARVESTED IN ALASKA

Alexandra C.M. Oliveira and Peter J. Bechtel

In Alaska there is over one million metric tons of fish processing byproducts produced annually. One of the major byproducts is viscera, which contain substantial quantities of liver. In Alaska most fish liver is made into fishmeal and oil or it is discarded. The purpose of this study was to characterize and compare lipid contents and fatty acid profiles of fish livers from walleye pollock (WP), pink salmon (PS), big mouth sculpin (BS), pacific halibut (PH), arrow tooth flounder (AF), flat head sole (FS) and spiny head rock fish (RF). Results show remarkable differences in lipid content for these species which ranged from 50.3% (wet wt.) in WP livers to as low as 3.3% (wet wt.) in PS livers. Interestingly, PS shows the highest content of ω-3 fatty acids at about 336 mg/g of oil as well as the highest P/S ratio at about 3.3. The contribution of docosahexaenoic acid (DHA) to the total content of ω-3 in PS livers is very high at about 179 mg/g of oil. AF and FS show high content of monounsaturated fatty acids at about 475 mg/g of oil, mainly due to the large content of oleic acid. Livers studied had variable quantities of lipid and distinct fatty acid profiles and can be used for the development of unique ingredients for the manufacturing of specialized aquaculture feeds.

Introduction

There is growing interest in feed ingredients with distinct nutritional characteristics that can better suit the dietary requirements of a variety of aquaculture fish species. This is mainly attributed to the rapid growth and diversification of the aquaculture industry worldwide. A significant amount of information addressing the potential uses of fish byproducts for the manufacturing of feed ingredients is available in the current literature (Aidos et al., 2002; Gunasekera et al., 2002; Oliveira and Bechtel, 2004; Bechtel, 2003). Fish byproduct utilization in the state of Alaska is increasing in importance due to environmental regulations which restrict and penalize discarding of processing waste (Smiley et al., 2003).

Commercial wild stock fisheries generate considerable revenue in Alaska with walleye pollock annual catches averaging more than one million MT. This fishery alone yields over 700,000 MT of byproducts of which about 240,000 MT is viscera (Crapo and Bechtel, 2003). Pink salmon catches average approximately 300,000 MT annually with about 30,000 MT of viscera byproducts, which is used in the manufacturing of fishmeal and fish oil or discarded (Crapo and Bechtel, 2003). Harvests of other commercially important fish species such as Pacific halibut and rockfish are approximately 33,000 MT and 10,000 MT, respectively. Total harvest of flatfish in Alaska during 2000 was about 140,000 MT (Crapo and Bechtel, 2003). In addition, there are large biomasses available in Alaskan waters of a number of underutilized species such as arrow tooth flounder and big mouth sculpin.

Liver, in certain fish species, is a significant component of the viscera. The estimated ranges of weight ratios for pollock and salmon viscera in relation to fish weight are about 9% to 32% and 6% to 16%, respectively (Babbitt 1990). These ranges fluctuate due to seasonality and fish size (Kizevetter, 1971). During mechanized fish processing the livers can be easily separated from remaining viscera tissue and used in the manufacturing of specific co products with unique chemical and physical characteristics The objective of this study was to characterize and compare the lipid content and fatty acid profile of fish livers from several commercially important species harvested in Alaska and also some underutilized Alaska fish species such as arrow tooth flounder. This research is part of an ongoing effort to enhance utilization of Alaska fish byproducts by developing novel food and feed ingredients.

Materials and Methods

Sampling

A total of six livers per species were obtained from arrow tooth founder (AF), walleye pollock (WP), pacific halibut (PH) and flathead sole (FS) in November 2002 as part of a University of Alaska survey trawl. Three livers of big mouth sculpin were also collected during the survey. Fish were frozen on board and livers removed in the pilot plant within a few days after catch. Five pink salmon and spiny head rock fish livers were collected during July from 1 commercial fish processing plants. Livers were immediately frozen at -70°C until analysis. All analyses were carried out in a timely manner to avoid lipid degradation.
Proximate Analysis

Protein (AOAC, 1990; method 968.06), moisture (AOAC, 1990; method 952.08), ash (AOAC, 1990; method 938.08) and lipid (AOAC, 1990; method 948.16 using petroleum ether) content were determined in triplicate for each liver sample. After lipid extraction solvent was removed at 49°C on a rotary evaporator (Büchi Rotavapor R-205, Westbury, NY) and lipids transferred into a pre-weighed 10ml amber screw top vial. The remaining solvent was removed under a N₂ gas stream until constant weight and percent lipids determined. Oils were stored in chloroform containing 0.01% BHT at -70°C until analysis.

Fatty Acid Profile and GC Analysis

Fatty acid methyl esters (FAME) were prepared using Methanol/BF₃ method and C23:0 as internal standard (AOAC method 969.33). A gas chromatograph model 6850 (Agilent Technologies, Wilmington, Delaware) equipped with an autosampler, flame ionization detector, and a DB-225 capillary column (DB-225 50% Cyanopropyl, J&W Scientific, Folsom, CA) was used to quantify the fatty acid methyl esters. Helium was used as carrier gas at constant flow of 1.0ml/min. Injector and detector temperature were held at 250°C and the split ratio was 25:1. The oven was held at 140°C for 5 minutes then increased at a rate of 3°C to 220°C. A hold step at 220°C for 15 minutes ensured column clean up for a total run time of about 46 minutes. The ChemStation enhanced integrator program was used to integrate the chromatogram peaks. Five-point calibration tables were determined using Supelco 37 FAME (Supelco®, Bellefonte, PA). Mixtures, BAME, PUFA-1 and PUFA-3 (Supelco®) were used to identify additional fatty acids. All samples were run in duplicates.

Statistical Analysis

The weighted means are derived from an analysis of variance run on Statistica version 6.0 (StatSoft Inc., Tulsa, OK). For tests of statistical significance between livers from species data was subjected to unequal N Tukey’s HSD test for significant differences (p<0.05).

Results and Discussion

WP livers had the highest lipid content at 50.3% followed by RF and FS at 33.1% and 24.9%, respectively (Table 1). The lipid contents of AF and PH were not significantly different from one another at 19.4 and 12.0%, and AF had a much higher standard deviation between replicates than PH livers. The lowest fat content was found in livers from BS at 8.7% and PS at 3.3%, which were not significantly different form one another. Moisture contents were inversely proportional to lipids, as expected. Ash content showed small but significant differences with WP livers presenting the lowest value at about 0.9%, and AF livers the highest value at about 1.5%. Protein content was significantly higher in BS and PS livers at 18.4 to 18.6%, while PH, AF and RF livers presented intermediate levels of protein ranging from 11.9 to 13.7%. Lower levels of protein were determined for FS and WP livers at 8.8 and 7.8, respectively.

Table 1. Proximate composition of Alaska fish livers (weight percent)

<table>
<thead>
<tr>
<th>Species</th>
<th>Protein</th>
<th>Moisture</th>
<th>Ash</th>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP</td>
<td>7.77(0.92)</td>
<td>41.04 (5.77)</td>
<td>0.89 (0.07)</td>
<td>50.30 (5.13)</td>
</tr>
<tr>
<td>BS</td>
<td>18.35 (0.79)</td>
<td>71.40 (1.05)</td>
<td>1.53 (0.22)</td>
<td>8.72 (1.36)</td>
</tr>
<tr>
<td>PH</td>
<td>13.36 (0.78)</td>
<td>73.31 (2.75)</td>
<td>1.30 (0.24)</td>
<td>12.04 (3.46)</td>
</tr>
<tr>
<td>AF</td>
<td>13.70 (1.74)</td>
<td>65.32 (4.23)</td>
<td>1.54 (0.43)</td>
<td>19.44 (6.34)</td>
</tr>
<tr>
<td>FS</td>
<td>8.77 (0.37)</td>
<td>65.30 (2.11)</td>
<td>1.05 (0.08)</td>
<td>24.88 (2.43)</td>
</tr>
<tr>
<td>PS</td>
<td>18.61 (1.29)</td>
<td>76.60 (1.86)</td>
<td>1.50 (0.05)</td>
<td>3.30 (0.94)</td>
</tr>
<tr>
<td>RF</td>
<td>11.85 (1.06)</td>
<td>54.13 (3.74)</td>
<td>0.93 (0.13)</td>
<td>33.09 (4.84)</td>
</tr>
</tbody>
</table>

WP walleye pollock; BS big mouth sculpin; PH pacific halibut; AF arrow tooth flounder; FS flat head sole; PS pink salmon; RF spiny head rock fish; (SD) Standard deviation of the mean; Different superscript letters indicate significant differences between species (p<0.05) by column.
Quantities of twenty eight fatty acids were determined in the liver samples. PS livers presented the highest levels of polyunsaturated fatty acids (PUFA) and ω-3 fatty acids at 376.5 and 336.4 mg/g of oil, respectively (Table 2).

Table 2. Summary of results from fatty acids methyl esters in Alaska fish livers (mg/g of oil)

<table>
<thead>
<tr>
<th>FA Profile</th>
<th>BS</th>
<th>FS</th>
<th>AF</th>
<th>RF</th>
<th>PS</th>
<th>WP</th>
<th>PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total FA</td>
<td>760.94</td>
<td>805.78</td>
<td>738.15</td>
<td>673.58</td>
<td>759.63</td>
<td>754.77</td>
<td>694.29</td>
</tr>
<tr>
<td></td>
<td>(4.23)</td>
<td>(33.37)</td>
<td>(16.21)</td>
<td>(11.05)</td>
<td>(28.15)</td>
<td>(32.13)</td>
<td>(51.55)</td>
</tr>
<tr>
<td>SFA (S)</td>
<td>1.434.3</td>
<td>180.92</td>
<td>143.02</td>
<td>112.81</td>
<td>113.98</td>
<td>207.46</td>
<td>143.88</td>
</tr>
<tr>
<td></td>
<td>(9.98)</td>
<td>(12.76)</td>
<td>(7.62)</td>
<td>(7.23)</td>
<td>(7.23)</td>
<td>(18.74)</td>
<td>(12.36)</td>
</tr>
<tr>
<td>MUSA</td>
<td>342.05</td>
<td>477.76</td>
<td>474.70</td>
<td>401.24</td>
<td>269.20</td>
<td>343.25</td>
<td>334.45</td>
</tr>
<tr>
<td></td>
<td>(11.09)</td>
<td>(22.63)</td>
<td>(23.80)</td>
<td>(8.67)</td>
<td>(11.05)</td>
<td>(22.27)</td>
<td>(48.48)</td>
</tr>
<tr>
<td>PUFA (P)</td>
<td>275.47</td>
<td>147.11</td>
<td>120.43</td>
<td>159.53</td>
<td>376.45</td>
<td>204.05</td>
<td>215.96</td>
</tr>
<tr>
<td></td>
<td>(3.59)</td>
<td>(11.76)</td>
<td>(13.42)</td>
<td>(4.04)</td>
<td>(17.80)</td>
<td>(13.70)</td>
<td>(17.02)</td>
</tr>
<tr>
<td>ω-3</td>
<td>239.34</td>
<td>130.04</td>
<td>109.85</td>
<td>141.80</td>
<td>336.42</td>
<td>182.83</td>
<td>193.15</td>
</tr>
<tr>
<td></td>
<td>(5.31)</td>
<td>(11.51)</td>
<td>(13.27)</td>
<td>(17.76)</td>
<td>(12.03)</td>
<td>(14.79)</td>
<td></td>
</tr>
<tr>
<td>ω-6</td>
<td>52.29</td>
<td>12.54</td>
<td>9.17</td>
<td>8.57</td>
<td>32.42</td>
<td>12.35</td>
<td>16.91</td>
</tr>
<tr>
<td></td>
<td>(3.24)</td>
<td>(2.61)</td>
<td>(0.54)</td>
<td>(0.57)</td>
<td>(1.88)</td>
<td>(1.39)</td>
<td>(3.73)</td>
</tr>
<tr>
<td></td>
<td>(1.13)</td>
<td>(2.46)</td>
<td>(1.41)</td>
<td>(0.81)</td>
<td>(0.93)</td>
<td>(1.31)</td>
<td>(2.24)</td>
</tr>
<tr>
<td>P/S</td>
<td>1.93</td>
<td>0.82</td>
<td>0.84</td>
<td>1.42</td>
<td>3.31</td>
<td>0.99</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>(0.13)</td>
<td>(0.09)</td>
<td>(0.09)</td>
<td>(0.13)</td>
<td>(0.22)</td>
<td>(0.10)</td>
<td>(0.24)</td>
</tr>
</tbody>
</table>

WP walleye pollock; BS big mouth sculpin; PH Pacific halibut; AF arrow tooth flounder; FS flathead sole; PS pink salmon; RF spiny head rock fish; (SD) Standard deviation of the mean, Different superscript letters indicate significant differences between species (p<0.05) by row.

The contribution of docosahexaenoic acid (22:6 ω 3) (DHA) to the total content of ω-3 in PS livers was very high at about 179 mg/g of oil (Table 3). PUFA levels were lowest in AF livers at 120.4 mg/g of oil (p<0.05). The ω-3/ω-6 ratios for all livers were high with RF having the highest ratio at 16.6 (p<0.05). Gunasekera et al. (2002) reported ω-3/ω-6 ratio in farmed trout offal, carp offal and frames from marine fish wastes of 0.76, 1.47, and 2.5, respectively. The ω-3/ω-6 ratio from trout intestine (from the fishing industry) was 4.7 (Kotzamanis et al., 2001). PS livers had a P/S ratio significantly higher than other livers (p<0.05). BS and PH had intermediate levels of DHA, which ranged from 80 to 90 mg/g of oil (Table 3). PS and AF livers presented the lowest concentration of DHA, which ranged from about 22 to 31 mg/g of oil. Aidos et al. (2002) reported levels of DHA and PUFA for headless herring products of about 95 g/kg of lipid and 235 g/kg of lipid, respectively.

Levels of monounsaturated fatty acids (MUSA) were significantly higher in FS and AF livers at approximately 475 mg/g of oil, followed by RF livers at 401.2 mg/g of MUSA per g of oil. MUSA in all livers was predominantly cis-oleic acid (18:1ω9), which was highest in FS and AF at approximately 250 mg/g of oil. Quantities of MUFA for WP, PH and BS livers were not significantly different and ranged from 324.5 to 343.3 mg/g of oil. Other abundant MUSA found in liver oils were palmitoleic (16:1ω7) and cis-vaccenic (18:1ω7). PS livers presented the lowest MUSA content at 269.2 mg/g of oil. WP livers contain the highest amount of saturated fatty acids (SFA) at 207.5 mg/g of oil, followed by FS livers at 180.9 mg/g of oil. Lowest levels of SFA were found in RF and PS livers at about 113 mg/g of oil. Palmitic acid (16:0) was the most abundant SFA in all species but varied widely from a high of 193.15 mg/g of oil to a low of 81.8 mg/g of oil.

Concentration of linoleic acid was low in all samples as expected for marine oils from wild fish stocks (Joseph and Ackman, 1992). Levels of arachidonic acid (20:4ω6) varied from 27.7 to 2.9 mg/g oil, with PS and BS livers containing significantly higher amounts (23.5 and 27.7 mg/g oil) than liver tissues from all other fish species (2.9 to 9.3 mg/g oil). Values for both gadoleic (20:1ω11) and cetoleic (22:1ω11) fatty acids varied with species and had high standard deviations. Oliveira and Bechtel (2004) reported similar high standard deviations for these two fatty acids in pollock and salmon byproducts. Eicosapentaenoic acid (EPA) content also varied widely among livers (47.0 to 120.4 mg/g oil) with WP, PS and BS having concentrations about two times higher than EPA level found in AF. Erucic acid (22:1ω11) was not detected in BS and FS but PS had levels of about 142.6 mg/g of oil. Nervonic acid (24:1ω9) was not detected in most livers but FS livers had significant concentrations (43.5 mg/g oil) of this MUSA.

In conclusion, lipids from livers of seven different Alaska fish species were characterized and found to have different and fatty acids profiles, PUFA and ω-3 fatty acids content, as well as ω-3/ω-6 ratios and P/S ratios. These unique properties can be used to formulate food and feed ingredients.
References


Authors

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### Table 3. Fatty acids methyl esters in Alaska fish livers (mg/g of oil)

<table>
<thead>
<tr>
<th>FAME</th>
<th>BS</th>
<th>FS</th>
<th>AF</th>
<th>RF</th>
<th>PS</th>
<th>WP</th>
<th>PH</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>6.80 AC (1.14)</td>
<td>22.55 B (3.14)</td>
<td>23.29 B (2.54)</td>
<td>12.53 C (1.19)</td>
<td>8.22 C (1.65)</td>
<td>33.64 D (5.72)</td>
<td>24.70 B (3.10)</td>
</tr>
<tr>
<td>C15:0</td>
<td>ND</td>
<td>2.85 A (0.43)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.41 AB (1.38)</td>
<td>4.50 AB (2.18)</td>
</tr>
<tr>
<td>C16:0</td>
<td>109.74 AD (7.07)</td>
<td>138.95 B (9.59)</td>
<td>98.08 ACD (6.30)</td>
<td>81.79 C (6.19)</td>
<td>95.12 CD (3.18)</td>
<td>139.79 B (14.17)</td>
<td>102.25 B (12.58)</td>
</tr>
<tr>
<td>C16:1ω9</td>
<td>6.51 AB (1.37)</td>
<td>6.27 A (3.37)</td>
<td>3.61 AB (0.32)</td>
<td>2.09 B (0.07)</td>
<td>ND</td>
<td>3.62 AD (0.71)</td>
<td>5.60 BC (1.33)</td>
</tr>
<tr>
<td>C16:1ω7</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.85 A (0.43)</td>
<td>2.47 AB (0.56)</td>
<td>2.07 B (0.17)</td>
</tr>
<tr>
<td>C16:2ω4</td>
<td>3.94 ACD (1.19)</td>
<td>2.03 A (0.39)</td>
<td>ND</td>
<td>6.30 B (0.04)</td>
<td>3.62 AD (0.71)</td>
<td>4.50 AB (2.18)</td>
<td>3.67 D (1.34)</td>
</tr>
<tr>
<td>C17:0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.28 A (0.42)</td>
<td>2.83 A (0.63)</td>
<td>1.34 B (0.22)</td>
</tr>
<tr>
<td>C17:1ω9</td>
<td>20.47 AB (0.90)</td>
<td>15.73 A (3.61)</td>
<td>29.27 B (8.79)</td>
<td>14.97 B (8.79)</td>
<td>21.97 B (2.66)</td>
<td>17.29 A (1.73)</td>
<td>17.29 A (1.73)</td>
</tr>
<tr>
<td>C18:0</td>
<td>26.89 AD (5.39)</td>
<td>19.42 AB (2.68)</td>
<td>21.66 AB (2.47)</td>
<td>17.45 AD (6.30)</td>
<td>4.50 C (0.88)</td>
<td>28.73 B (3.45)</td>
<td>13.52 C (4.07)</td>
</tr>
<tr>
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<td>156.00 CD (2.44)</td>
<td>245.23 B (29.90)</td>
<td>269.66 B (21.36)</td>
<td>196.48 C (7.59)</td>
<td>170.24 CD (13.25)</td>
<td>149.31 B (12.85)</td>
<td>112.39 A (19.99)</td>
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<tr>
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<td>84.98 A (10.30)</td>
<td>58.46 AB (13.31)</td>
<td>51.04 B (3.47)</td>
<td>58.59 AB (1.20)</td>
<td>21.62 C (4.47)</td>
<td>75.95 A (12.77)</td>
<td>41.11 BC (15.05)</td>
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<tr>
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<td>ND</td>
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<td>3.11 A (0.68)</td>
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<tr>
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<td>4.66 A (1.88)</td>
<td>4.26 A (0.44)</td>
<td>5.38 AB (0.24)</td>
<td>4.69 A (0.64)</td>
<td>7.30 B (1.04)</td>
<td>5.46 AB (1.29)</td>
</tr>
<tr>
<td>C18:2ω4</td>
<td>2.96 AC (0.21)</td>
<td>2.50 A (0.31)</td>
<td>1.40 B (0.69)</td>
<td>2.86 A (0.22)</td>
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<td>3.28 AC (0.83)</td>
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</tr>
<tr>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.22 A (0.30)</td>
<td>4.01 B (0.13)</td>
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<tr>
<td>C18:4ω3</td>
<td>3.93 A (1.85)</td>
<td>4.95 A (0.33)</td>
<td>5.63 A (2.39)</td>
<td>5.01 A (0.34)</td>
<td>6.48 A (0.38)</td>
<td>15.63 B (2.91)</td>
<td>5.35 A (2.71)</td>
</tr>
<tr>
<td>C20:1ω9 cis</td>
<td>19.13 AB (3.11)</td>
<td>19.13 AB (3.11)</td>
<td>19.13 AB (3.11)</td>
<td>19.13 AB (3.11)</td>
<td>19.13 AB (3.11)</td>
<td>19.13 AB (3.11)</td>
<td>19.13 AB (3.11)</td>
</tr>
<tr>
<td>C20:1ω5</td>
<td>9.19 ACD (0.39)</td>
<td>4.88 (4.88)</td>
<td>31.04 B (5.90)</td>
<td>13.87 AD (0.53)</td>
<td>3.88 C (0.90)</td>
<td>8.33 AC (4.33)</td>
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<td>7.22 AD (1.34)</td>
<td>4.21 AB (3.31)</td>
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<td>4.23 AD (0.42)</td>
<td>2.41 BC (0.29)</td>
<td>1.66 C (1.35)</td>
<td>7.72 B (2.29)</td>
</tr>
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<td>ND</td>
<td>ND</td>
<td>2.00 B (0.34)</td>
<td>ND</td>
<td>ND</td>
<td>1.20 B (0.97)</td>
<td>2.13 A (0.17)</td>
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<td>23.53 A (3.32)</td>
<td>7.88 B (1.15)</td>
<td>2.91 B (0.26)</td>
<td>3.19 B (0.44)</td>
<td>27.33 A (2.50)</td>
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</tr>
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<td>4.45 A (1.06)</td>
<td>2.79 A (0.40)</td>
<td>4 A (2.11)</td>
<td>4.34 A (0.23)</td>
<td>11.09 A (1.37)</td>
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<td>4.96 A (2.07)</td>
</tr>
<tr>
<td>C20:5ω3</td>
<td>120.35 A (10.24)</td>
<td>95.32 B (10.24)</td>
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<td>75.11 C (5.72)</td>
<td>102.25 B (2.89)</td>
<td>111.35 A (8.58)</td>
<td>79.38 B (8.43)</td>
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<td>ND</td>
<td>13.67 A (8.24)</td>
<td>21.42 AC (1.11)</td>
<td>42.56 B (2.45)</td>
<td>9.33 A (4.65)</td>
<td>26.46 C (11.45)</td>
</tr>
<tr>
<td>C22:2ω5</td>
<td>20.20 AB (4.07)</td>
<td>4.78 (1.26)</td>
<td>18.90 AB (5.95)</td>
<td>11.66 AD (0.25)</td>
<td>33.69 B (3.88)</td>
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<td>22.28 B (2.45)</td>
<td>30.66 AC (3.48)</td>
<td>42.47 CD (1.46)</td>
<td>178.90 C (17.28)</td>
<td>45.93 A (5.70)</td>
<td>79.74 A (10.07)</td>
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<tr>
<td>C24:1ω9</td>
<td>ND</td>
<td>43.53 A (17.77)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>8.55 B (1.34)</td>
</tr>
</tbody>
</table>

WP walleye pollock; BS big mouth sculpin; PH pacific halibut; AF arrow tooth flounder; FS flat head sole; PS pink salmon; RF spiny head rock fish.

(SD) Standard deviation of the mean; Superscript letters indicate significant differences between species (p<0.05) by row; ND under detection or integration limits.
6.2 UTILISATION OF BY-PRODUCTS FROM FARMED ATLANTIC SALMON (SALMO SALAR)

Hege Michelsen¹, Eva Falch¹,², Turid Rustad¹

The global production of farmed Atlantic salmon are close to 700 000 tons (year 2000) and more than ½ of this weight is regarded as by-products or waste. The largest fractions constitute the cut-offs (incl. backbone) (14%), viscera (13%) and head (10%), and these fractions might serve as source of valuable marine lipids and proteins. In this work, the viscera and fractions of backbone were hydrolysed using a commercial protease (Alcalase 2.4 L). The effect of pre-inactivation of endogenous enzymes, water addition and centrifugal conditions were evaluated regarding the yields and quality of the lipid and protein fractions. After hydrolysis and separation, 4 fractions were collected: oil, emulsion, water soluble proteins (FPH) and water insoluble components. Overall, the degree of hydrolysis in the FPH were higher in the viscera (~40%) compared to the backbone (~25%). Addition of water (0.5-1 part water : 1 part raw material) gave better separation of the 4 phases with less emulsion. Pre-inactivation of endogenous enzymes led to denaturation of protein and thereby a higher amount of emulsion and more lipids in the protein phases. While the use of commercial enzyme was necessary for hydrolysing the backbone fraction, minor effect of enzyme addition were found for the viscera. The water binding capacity and gelling properties of the fish protein hydrolysates was evaluated in minced cod, both properties were highest for the FPH from viscera independent of processing parameters. The emulsion properties were highest in the fish protein hydrolysates from backbone, and the highest stability and volume of the emulsion were produced in hydrolysates produced with added water. Enzyme addition did not influence the evaluated functional properties. The highest yield, lowest amount of free fatty acids and lightest colour of the oil were obtained when the oil was separated by centrifugation before hydrolysis.

Characteristics of the raw material, yield and quality of the bulk-product and functional properties will be presented.

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6.3 PREPARATION AND CHARACTERISTICS OF PROTEASES FROM ATLANTIC COD AND THEIR APPLICATIONS IN INDUSTRY AND MEDICINE (ENZYPRO, QLK1-CT-2002-70871)

Linda Helgadottir, Sigridur Olafsdottir and Jon Bragi Bjarnason

Introduction

Cold-adaptation of ectothermic organisms, such as fishes, involves compensations in the efficiency of enzyme catalyzed reactions, either through alterations in the catalytic efficiency of the enzymes or through increasing enzyme concentrations (Hazel & Prosser 1974; Hochachka & Somero 1985). The optimization of an enzyme towards a low temperature environment presumably involves reducing the rigidity of the enzyme molecule, which would lead to a measurable reduction in stability properties of the enzyme (Ásgeirsson et al. 1989). Thus, in cold-adapted poikilotherms, natural selection would be expected to favor enzymes with increased catalytic efficiency at low temperatures, although other factors, such as structural stability, may restrict the degree of optimization. We have been studying digestive enzymes from the Atlantic cod as a possible source of industrial and medical enzymes with unique and useful properties for a couple of decades.

The present report describes the components of a mixture of proteolytic digestive enzymes, called Cryotin, which has been prepared by neutral extraction from the pyloric caeca of Atlantic cod. This proteinase mixture has many unique characteristics. The proteinases in the mixture, studied so far, are more active at low temperatures, when compared to their mammalian counterparts. They are also thermo-labile as well as acid sensitive. Cryotin has been shown to contain trypsin, chymotrypsin, and elastase and, perhaps most importantly, collagenolytic enzymes, as well as other proteolytic and peptidolytic activities, but it is practically devoid of lipase, amylase and nuclease activities (Ásgeirsson et al. 1989; Ásgeirsson & Bjarnason, 1991; Ásgeirsson & Bjarnason, 1993; Kristjánsson et al., 1993).

Hydrolytic enzymes, especially proteases, have many uses and potential applications in industry, medicine and research. Among these are detergent production, leather processing, chemical modifications and food processing. Enzymes isolated from cold water marine organisms may prove to be especially useful for these purposes. The cold-active or psychrophilic enzymes are frequently more active at low temperatures than their mammalian or bacterial counterparts, a characteristic, which could be beneficial in many industrial processes and medical applications. Already, a mixture of proteases is being used in a patented process to produce seafood flavors, bases and stocks for the food industry. These products termed NorthTaste are natural digests of seafood such as lobster, shrimp, crab and cod, containing no additives. A preparation of a highly purified proteases termed Penzyme has been isolated and purified from cod offal by aqueous extraction and fractionation on chromatography columns. Penzyme is presently used as the active ingredient in a patented process to produce the natural skin care product PENZIM for the treatment of various skin indications, inflammation, pain, for wound healing and more.

The present report describes the preparation of a mixture of proteolytic digestive enzymes, called crude Cryotin, which is prepared by neutral aqueous extraction from the Atlantic cod offal. In this investigation a novel Cryotin A protease mixture derived from crude Cryotin, and several purified protease preparations, termed Cryotins B to H derived from Cryotin A and crude Cryotin, were developed for testing their use in cheese ripening, leather processing and skinning of squid. These Cryotin preparations contain various amounts and proportions of the proteases trypsin, chymotrypsin, elastase and serine collagenase in differing degrees of purity (see further in table 1). The purpose of this investigation was to develop stable formulations for the Cryotins for their storage, transport and use in the applications of the project. The investigation has been funded by the European Union, Craft project ENZYPRO, QLK1-CT-2002-70871.

Methods and results

Enzyme assay methods. For unit definitions synthetic chromogenic substrates are used in the enzymatic assays. On cleavage of the peptide bond the synthetic substrate releases a chromogenic compound, p-nitroaniline, which absorbs light at 410 nm with an absorption coefficient \( \varepsilon = 8800 \text{ M}^{-1}\text{cm}^{-1} \). From the change in A410 with time the
rate of peptide bond cleavage can be calculated. One enzymatic unit is defined as the amount of enzyme that cleaves one µmole of substrate per minute. A simple and reliable enzyme activity assay method had been developed to monitor enzyme recoveries and yields during process development and for use in quality control and experimental design. The chymotrypsin and elastase assay based on that chymotrypsin and elastase activity were routinely determined at 25°C in a Tris-HCl buffer solution at pH 8.0. For chymotrypsin activity Succinyl-alanine-alanine-proline-phenylanaline-p-nitroanilide (SucAAPF-pNA) was used for substrate, and for elastase activity Succinyl-alanine-alanine-alanine-p-nitroanilide (SucAAA-pNA) substrate was used. Chromatographic analysis methods have been developed to monitor enzyme composition and purity during process development and for use in quality control. Cryotin B and G is analysed for elastase by Mini-S PE 4.6/50 and Cryotin C is analysed for chymotrypsin and Cryotin H is analysed for trypsin by Mono-Q HR5/5 ion exchange column. Both columns were linked to an Äkta Purifier FPLC instrument.

Atlantic cod intestinal extract contains trypsin, chymotrypsin and elastase activities. Serine collagenases of two types have also been detected in the extract, one, which has a chymotrypsin-like specificity, and one that possesses a trypsin-like specificity. The trypsin–like collagenase is removed from the extract with other trypsins affinity chromatography. The remaining proteases in the raw material for this project, Cryotin A, are chymotrypsins, chymotrypsin-like collagenase and elastases. In order to assess the amount of each type of protease in the mixture two different enzymatic assays are used: Assay for chymotrypsin activity and assay for elastase activity. The chymotrypsin assay does not distinguish between the chymotrypsins and the chymotrypsin-like collagenase.

Cryotin A contains chymotrypsin, elastase and serine collagenase. Four different enzyme mixtures are produced from Cryotin A, that is Cryotin B and G that contain purified elastase in differing concentrations, Cryotin C contains purified chymotrypsin and Cryotin H contains purified trypsin. Cryotin D is mixture of Cryotin G and C. Cryotin E is concentrated Cryotin A, Cryotin F is mixture of Cryotin A and Crude Cryotin in ratio (9:1). In all, a total of nine enzyme preparations have been prepared at the Science Institute, see further in Table 1.

Table 1. Definition of different Cryotins

<table>
<thead>
<tr>
<th>Samples</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Cryotin</td>
<td>Trypsin, chymotrypsin, elastase and serine collagenase</td>
</tr>
<tr>
<td>Cryotin A</td>
<td>Chymotrypsin, elastase and serine collagenase</td>
</tr>
<tr>
<td>Cryotin B</td>
<td>Concentrated purified elastase</td>
</tr>
<tr>
<td>Cryotin C</td>
<td>Purified chymotrypsin</td>
</tr>
<tr>
<td>Cryotin D</td>
<td>Mixture of Cryotin G (elastase) and Cryotin C (chymotrypsin)</td>
</tr>
<tr>
<td>Cryotin E</td>
<td>Concentrated Cryotin A</td>
</tr>
<tr>
<td>Cryotin F</td>
<td>Cryotin A + Crude Cryotin ( ratio 9:1)</td>
</tr>
<tr>
<td>Cryotin G</td>
<td>Purified elastase</td>
</tr>
<tr>
<td>Cryotin H</td>
<td>Purified trypsin</td>
</tr>
</tbody>
</table>

Formulations and stability of Cryotins

Formulations for the Cryotins have been developed for the purpose of stabilizing all of the enzyme mixtures for their storage, transportation and use. Formulation of Crude Cryotin, Cryotin A and E is unbuffered by added buffer material, containing 36% glycerol. Cryotin B, C, D, G and H contain 50% glycerol, 2 mM CaCl₂ and 5 mM Tris-HCl, pH between 8 and 9.

Trypsin, chymotrypsin and elastase stability of Crude Cryotin in 36 % glycerol was measured at three different temperatures –20°C, 4°C and 25°C for 310 days (44 weeks). Results show that there is great difference in stability of the samples at 25 and –20°C. The best conditions to keep the samples were at –20°C. After 310 days at –20°C no discernable trypsin activity was lost. In comparison 44 % chymotrypsin activity and 18 % elastase activity were lost.
It is known from previous studies in our laboratory that raising the glycerol concentration improves the stability of serine proteases from cod. It was, therefore, decided to increase the glycerol concentration to 50% in Cryotin A to improve the stability of the enzymes.

Stability of Cryotin A has been measured in samples that were stored at three different temperatures, 4°C, -20°C and 25°C for 90 days, samples were both kept in 36 % and 50% glycerol. Both elastase and chymotrypsin activities were measured. Results from both elastase and chymotrypsin measurements show that storing the samples in 50 % glycerol is better for the stability. However chymotrypsin is much less stable than elastase in Cryotin A under these conditions both in 50% and 36 % glycerol. These results show that the best conditions for the samples is to keep them in 50 % glycerol and at temperatures of -20°C.

Stability of Cryotin C (chymotrypsin) in 50% glycerol formulations has been measured at three different temperatures, -20°C, 4°C and 25°C for 320 days (42 weeks). Cryotin C samples kept at 4°C and –18 °C are stable for 320 days but the chymotryptic activity in the sample kept at 25°C had lost 90% of its activity.

Stability of Cryotin G (elastase) in a 50% glycerol formulation has been measured at three different temperatures –18°C, 4°C and 25°C for 440 days. The results show that the elastase activity in Cryotin G remains stable at 4°C and –18°C for 440 days but has lost 25% activity at 25°C.

Comparative stability measurements of elastase activity in Cryotin B (170 U/ml) and Cryotin G (20 U/ml) were conducted to see the effect of the concentration of elastase on its stability. Three different temperatures were tested. Results from these measurements show that after 80 days there is little difference in residual activity of Cryotin B and G at any of the three temperatures. At 25°C about 27 % activity was lost, at 4°C about 10 % activity was lost and at –18°C 5% activity was lost. Thus, after 80 days it can be concluded that the different enzyme concentrations do not affect the activity decay of elastase to a great extent, indicating that elastase is not very susceptible to autolysis.

Stability of Cryotin H (purified trypsin) has been measured in samples that were stored at two different temperatures 4°C and 25°C for 157 days. The results show that at 4°C, only 7% of the trypsin activity was lost. But when the sample was kept at 25°C it had lost 55 % of it’s activity. These stability experiments show that storage and transportation of Cryotin solutions in 50% glycerol that takes 2-5 days at room temperatures should not compromise the activity of the three major proteases in the Cryotins. After the enzyme solutions arrive at the final destination they should be kept refrigerated or frozen until used.

These stability experiments show that storage and transportation of Cryotin solutions in 50% glycerol that takes 2-5 days should not compromise the activity of the three major proteases in the Cryotins. After the enzyme solutions arrive at the final destination they should be kept refrigerated or frozen until used.

Discussion and conclusions

A mixture of proteinases, called Cryotin, was prepared by neutral extraction from frozen and homogenized pyloric caeca from Atlantic cod. The preparation was shown to contain trypsin, chymotrypsin, elastase and collagenases. Cryotin, the mixture of proteinases from Atlantic cod, has many unique characteristics for a pancreatic enzyme mixture. It contains practically no lipase, amylase or nuclease activities, which may be due to proteolytic breakdown of these enzymes in the initial homogenate. The proteinases in Cryotin have higher catalytic activities, even at very low temperatures, than comparable mammalian enzymes, permitting the use of lower amounts of enzyme adjuncts in various processes. They are more temperature and acid sensitive than enzymes from conventional sources, allowing the use of milder conditions to destroy residual enzyme activities if needed, after processing is completed.

The cold-active proteinases, in the various Cryotin formulations, have many potential uses in industry, medicine and research, especially in food processing applications, which require hydrolysis at low temperatures, inactivation under mild conditions or native collagen digestion. It has proven promising in various fish processing applications such as skinning of fish, removal of membranes and ripening of herring. Cryotin also has potential as a digestive aid, both for humans and animals. In the present investigation, funded by the European Union, Craft project ENZYPRO, QLK1-CT-2002-70871, Cryotins were prepared in the laboratory and on a pilot plant scale for application tests for cheese ripening, leather processing and skinning of squid. Purification processes of various Cryotin derivatives were developed, as well as formulations for the Cryotins for the purpose of stabilizing the proteinase activities of trypsin, chymotrypsin and elastase in the different Cryotins.
Crude Cryotin is now being used in a patented process to prepare high quality all-natural flavorings for food processing and innovative cooking. Penzyme, a pure superactive proteinase from cod, is presently produced in Iceland as an active enzyme ingredient in a patented skin ointment called PENZIM gel and lotion for a patented use in pharmaceuticals and cosmetics. The PENZIM ointment is a soothing, moisturizing, cleansing and nourishing skin healing treatment for dry or chapped skin. PENZIM is also used for the treatment of various skin indications, inflammation, pain, for wound healing and more.

References


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6.4 PREVENTION OF HB CATALYZED OXIDATION IN WASHED COD MUSCLE BY AN AQUEOUS FRACTION OF HERRING (CLUPEA HARENGUS)

Thippeswamy Sannaveerappa, Ingrid Undeland and Ann-Sofie Sandberg

Introduction

Fish derived water soluble antioxidants have been matter of focus in the recent years. In a previous study it was reported that aqueous fractions (press juice, PJ) from white muscle fishes like cod, haddock, dab and winter flounder showed antioxidative properties against hemoglobin catalyzed oxidation of cod muscle membranes (Undeland et al., 2003). Water soluble compounds like taurine have also been suggested as cardio protective agents (Schaffer et al., 2000) in addition to omega 3 fatty acids. The aim of this study was to evaluate the antioxidative properties of herring muscle press juice. This was both to understand how the endogenous antioxidative system of herring is built up and to look into possible practical uses of herring byproducts (wash water, frames etc). The water soluble fraction of herring we investigated for its antioxidative properties in the washed cod mince. An attempt was also made to characterize the antioxidative components of herring PJ in terms of its molecular size, heat stability, sensitivity to dilution and synergetic effects.

Materials and Methods

Fish

Fresh cod (Gadhus morhua) and herring (CLUPEA harengus) were collected from the Gothenburg fish harbor (Sweden). Light muscle was manually cut out and minced using a kitchen grinder (Ultra Power, Model KSM90, Kitchen Aid, St.Joseph, Michigan USA). These minces were used either for PJ preparation (herring) or for model system preparation (cod).

Washed minced cod muscle model system

The washing procedure was adapted to minimize the water content of the mince so that maximum amount of PJ could be added to the model without increasing the final moisture content above the physiological 81%. The procedure for washing and thawing was done according to Undeland et al. (2003).

Preparation of press juice

Press juice was prepared from minced herring light muscle according to Undeland et al. (2003). However centrifugation was done at 18000g for 2h at 4°C. Heat treatment, ultra filtration and dialysis of the PJ was performed according to Undeland et al. (2003), with a slight modification in the dialysis procedure; it was carried out for 24h with three times buffer change. Some of heated herring PJ was not centrifuged, but used directly in to the model system.

Bleeding of fish, preparation and analysis of hb

Rainbow trout (Onchorhynchus mykiss) were bled according to Rowley (1990). Hemolysate was prepared as described by Fyhn et al. (1979). The method described by Brown (1961) was adapted for quantifying the heme content in hemolysate and PJ.

Preparation of oxidation system

Appropriate amount of low moisture washed cod mince was taken, to which was added untreated PJ or treated PJ. Treatments included ultra filtered <1KDa, dialysis >3.5KDa, or >50KDa, heated plus centrifuged or only heated PJs to bring the moisture from ~75% to 81%. In controls, the PJ was replaced by 50mM phosphate buffer at pH 6.2. To avoid microbial spoilage 200ppm streptomycin was added based on the moisture content. pH was adjusted to 6.2 by adding 1M NaOH/HCl. Oxidation was initiated by addition of trout hemolysate to a final concentration of 20µM Hb based on the moisture content of the model. The samples were spread evenly in the
bottom of 250ml Erlenmeyer flasks to a thickness of 4-6mm. The flasks were stored on ice in cold room at 4°C until the samples were microbially spoiled, which typically took 7-9 days.

Sensory analysis

Twice a day 2-3 trained panelists (Richards, 1998) sniffed the headspace above the samples by opening the flasks. Panelists were asked to detect the fishy, rancid and stale odor using a scale of 0-100 with 100 being the strongest. The lag phase for development of different odors is defined as the time elapsing until a threshold of intensity 10 was reached.

TBARS

Sample plugs of around 1g were taken every day to follow the lipid oxidation in terms of TBARS. TBARS was analyzed according to Lemon (1975).

Redness

The redness measurement was done according to Wetterskog and Undeland.

Results and discussion

Results of the study indicated that in controls containing 20µM Hb, oxidation developed within 1.5-3.5 days during ice storage. Added PJ when diluted 1.5-4.5 times, fully inhibited the development of this Hb catalyzed oxidation of the cod muscle membranes during the storage period (7-9 days). Low molecular weight fraction (LMW, <1kDa) of herring PJ could only prolong the oxidation lag phase with 1day compared to controls. The high molecular weight fraction (>3.5kDa, or >50kDa) prolonged the lag phase with up to 3.5 days showing somewhat higher antioxidative activity compared to LMW fraction. However, it was still not as efficient as the whole PJ indicating some synergic effects. When PJ was heated at 100°C for 10min, and added after removal of heat coagulated proteins, it did not retain the antioxidative property fully. Since the herring PJ contained 11.47µM Hb, a possible reason could be release of pro oxidative heme due to heating (Eriksson et al., 1971). It was interesting that when, added without protein removal, it inhibited oxidation fully. The heat studies indicated that the antioxidative property may not be due to antioxidative enzymes, but that the sarcoplasmic proteins in general exert some antioxidative property.

Herring PJ may be a promising agent against rancidity during preservation and processing of muscle based food products. However further investigations have to be made to see its suitability for different commercial applications.

Reference


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6.5 NEW WAYS TO A BETTER UTILIZATION OF THE RAW FISH: FILLET-LIKE RESTRUCTURATES FROM MINCED FISH

Christoph Schneider

Introduction

More and more people living on the earth need more and more fish for their nutrition. But the amount of fish being harvested from the oceans and from the fresh water remains stagnant for several years. And there is no hope for an improvement during the coming years. In contrast a dramatic further shortage threatens (Zimmermann, 2003) because of overfishing the fish stocks. Only the aquaculture will be able to deliver raising amounts of fish for the human nutrition, especially if it will be possible to develop fish feed originating from vegetable raw materials.

Another problem beside the shortage of raw fish consists in a relatively low yield of edible products made from fish. Mostly, especially in the western countries, the fishes come on the table as fillets, being baked, cooked, smoked or marinated. But the filleting process is connected with a loss of a certain amount of fish meat, e.g. remaining between the backbone. The share of that loss depends upon the size and shape of the fish body. But it is also influenced by the filleting technique being used.

Some of the fish species are fitted out with so many bones, especially pin bones, that it is impossible to produce fillets being practically free of bones. Therefore such fishes are treated as an undesired by-catch which is discarded.

The fish meat remaining after filleting is very often thrown away with the carcass, mostly it is given into the production of fish meal. In some cases the remaining meat is separated from skin and bones using a skin and bone separator. The resulting minced fish is our raw material. That means that we use raw fish meat which is not so attractive within the conventional fish processing. Mostly Hamburgers or similar products are made from minced fish and certain amounts are added to frozen blocks of frozen fish. Furthermore minced fish is usually produced in relatively large amounts as a preliminary product for Surimi. In general the potential use and the acceptance of minced fish on the market of fish products for human nutrition is limited because of

- High price and limited consumption in case of Surimi.
- The significant difference in mouthfeeling, texture and structure between fish burgers and a true fish muscle. Most people who like fish in their diet prefer the whole fish muscle or pieces of it.
- The relatively low market value of the conventional products made from minced fish.

Now a new product (so called NEWFISH) has been introduced which is made from minced fish but with an anisotropic and lamellar structure similar to an original muscle. In terms of food science NEWFISH means a restructure. It took several years in Europe for the development of NEWFISH starting at the end of the 80’s with a first patent. Now NEWFISH is presented as an industrially practicable and versatile process with moderate cost as a base for a broad range of new and innovative products.

We want to make it absolutely clear that we do not intend to substitute fillets or other traditional products. We are introducing NEWFISH to facilitate new products not only for the established fish market of today but especially also for the fast growing market segments of tomorrow like convenience, healthy food and so on.

Some characteristics and details of NEWFISH

1. What does NEWFISH mean ?

NEWFISH is a fish product being completely free of bones and nearly free of skin with an anisotropic structure and with a mouthfeeling mostly resembling a fish fillet. NEWFISH is significantly different from known products commonly made from minced fish. Structure and mouthfeeling are adjustable in a relatively wide range.

NEWFISH is available during the whole year in constant and standardizable quality.

2. What about the composition of NEWFISH ?

NEWFISH consists of fresh meat without skin and bones. You only have to add a small amount of other food components such as salt, water and in some cases fat (fish oils other edible oils). No further additives such as binding agents, phosphates etc. are needed. To realize a wide spread variety of new products, it is possible to
incorporate flavours, herbs, seasonings, natural colouring agents, vegetables, cheese and other ingredients. There is a nearly unlimited number of options to develop tailor made products to meet exactly the demands of the customer.

3. What kind of raw materials can be used for NEWFISH?

Principally every species from fresh water up to deep sea fishes is suitable for NEWFISH. Naturally some species are better suited than others but by adjusting the recipes or by mixing different species, in most cases good results are obtainable. This is one difference to the Surimi process because Surimi of good quality can be produced only from such fishes providing a high strength of their myofibrillar proteins to establish a gel network.

Among others especially all kinds of Salmon provide an excellent raw material for the new process. Considering the aspect that NEWFISH should not substitute but supplement the traditional processing, NEWFISH will be produced preferably from such fish meat being disadvantageous for traditional filleting. That includes

- Underutilized species such as fishes with an undesired anatomy (size, number and arrangement of the bones within the body, undesired properties of the meat and so on). If one proceeds in this way a significant percentage of the so called by-catch can be changed into products with a good market value.
- Fish meat remaining after traditional processing, for instance between the backbone, as undesired pieces after calibration, as sawdust from sawing frozen fillet blocks into fish fingers or into other portions.

Using the new process, it is possible to utilize nearly 100% of the fish meat from a fish body.

4. How the manufacturing process of NEWFISH can be described?

As described earlier the best suited raw material is minced fish produced with a skin and bone separator. Pieces of a fish muscle (trimmings) can be used too if it is sure that they are free of bones and skin.

The first processing step includes an intensive mincing or comminuting with the aim to destroy the myofibrillar structure of the muscle, resulting in a homogeneous mixture together with the other components of the recipe. If you wish to incorporate visible pieces such as vegetables, you can it do so under gentle conditions after mincing. The mixture is than filled into sausage coatings, in block cartons or other moulds.

The second processing step consists of building up a new structure by a very special freezing procedure. From the so called freeze structurization a semi fabricated frozen bulk product results. Finding the best suited special freeze conditions will determine to a high degree whether you can get a good structure or not.

The third step includes the fixation of the newly built structure by one of the known denaturation procedures of the proteins like boiling, frying, smoking and so on.

In practice the semi fabricated bulk product may be processed further by the same manner as a frozen block of fish fillets. The only difference may be that the restructurate must be kept frozen up to the denaturation step because the newly built up structure in some cases may be not stable enough for that.

The semi fabricated frozen restructurate itself may be a consumer product, if it is sliced in the frozen state in portions, breaded or coated and packed. In that case the fixation will be carried out by the consumer itself by heating the restructurate. Furthermore it is possible to produce boiled, fried or smoked products from the restructurate in any kind of shape and/or in combination with sauces, dressings, vegetables, noodles, rice and so on.

5. Incorporation of NEWFISH into existing fish processing facilities and outlook for a completely wastefree fish processing

As described earlier NEWFISH is not a substitute but a supplement to the traditional fish processing and expands the range of valuable fish products especially in direction of convenience and healthy food.

A combination of the traditional filleting facility with a restructuring unit followed by the processing of the waste to a marketable product and some amounts of contaminated water leads to a completely waste-free fish processing. Such a concept gives new perspectives for the fish processors.

Depending on the market situation the following products form the “waste” (skin, bones, gut) can be made:

- Fodder for pigs, minks and other animals by enzymatic treatment of waste. That way is suited for all kinds of fish waste.
- A dry fodder rich in protein and calcium for dogs and cats, suited for skin, bones and heads.
- A sauce concentrate to make sauces and soups for fish dishes, for which skin, bones and heads can be used, too.
Some combinations of the different waste processing procedures are possible to optimize the utilization of the whole fish and to maximize the profit coming from fish processing.

6. What are the main targets of the new restructuring process in the future?

Analysing the targets we will take into account the following aspects:
- Situation of the raw material
- Demands on a modern production
- Supplying the customer with fish products
- Meeting the main trends of food development in the future

6.1 Situation of the raw material

No doubt – we are not able to catch as much fish from the sea or from fresh water as we want to have. The new aquaculture can be only a limited supplement in order to harvest more and more fish. Since 1989 the yield of fish caught has remained about 89 tons per year. Additionally about 20 to 30 mio tons are immediately discarded. One third of the fish is processed into fodder for animals. The other two thirds are provided for human nutrition. This is a significant example for the extravagance concerning a valuable raw material.

From the 56 mio tons of catch between 30 to 50 % are lost during the traditional processing such as filleting. Possibly no more than 30 mio tons of fish can be found on the table.

All these facts show the huge importance of NEWFISH concerning the yield of products similar to fillets (no burger).

An example may be the filleting of Salmon. Up to 200 grams of meat of best quality per body remains after filleting between the backbone and in other parts. With the production of Salmon restructurate you may get a higher yield of marketable products without a more extended use of raw fish.

6.2 Demands concerning an optimized production

One of the main problems existing in fish processing is the difficulty to calculate exactly the costs of the processed fish products over a longer time. Price and availability of the raw fishes go up and down within a short time and it is nearly impossible to have a stable balance between the raw material, the facilities for processing and the possibilities to sell the products on the market.

NEWFISH offers some solutions to overcome such problems because the production of the semi fabricated bulk product can be adjusted to the availability of the raw material. The semi fabricated product can be stored deep frozen for some months. Fat oxidation may be prevented by addition of antioxidants. It will be possible to buy the raw fish when the price is low and free capacities within the processing plant are available. The costs for the restructurization procedure are nearly constant and so one may have very clear and constant calculation. Furthermore one is able to work constantly over the whole year. So NEWFISH may be offered over a longer time with a standardizable and constant high quality and a fixed price. This is one way to come away from the more or less hand-made fish processing.

6.3 Supplying consumers with fish products in the future

In many countries the degree of acceptance and consumption of fish and related products is limited not only by the price but mainly by three factors:
- Preparation of fish dishes is relatively complicated.
- Fish has too many bones.
- Fish smells unpleasant.

All three obstacles can be overcome by NEWFISH: We have bonefree fish products and therefore NEWFISH is suited especially for catering, for fish dishes in hospitals, old people’s homes, nursery schools and similar establishments.

With NEWFISH odour and taste of fish can be influenced in a desired manner. In general the intensity of the fish odour is a little bit lowered by the comminuting procedure. Last but not least NEWFISH is an important component to create convenience food.
6.4 NEWFISH meets main trends in food development

Estimating the general direction of food development for the next years two main trends are especially important for NEWFISH:

• Convenience
• Food providing healthy benefits by their consumption (functional food, healthy food, food for the not-so-healthy, nutraceuticals).

NEWFISH is best suited to be a main part of convenience food. A wide range of ready-to-eat or ready-to-cook dishes are realizable with it. Often it is connected with a change of the marketing philosophy: The goal is not to sell fish but to sell a delicious dish containing fish. Convenience will push the production of NEWFISH within a relatively short time.

NEWFISH is an ideal basis for food providing a special benefit for health because it is possible to incorporate all the necessary components into the product during the processing. We did some experimental work among others with fish oil being rich in ω-3-fatty acids, vitamins, antioxidants and other substances with very good results.

Conclusions

NEWFISH and its processing procedure is a great step forward in fish processing and marketing. The main features are

• Suited for nearly all fish species.
• Suited for products with constant quality and costs.
• Better utilization of the fish resources.
• Meeting the main trends of food development of the coming years.
• Process with relatively simple equipment and moderate costs.

A short time ago food consult started with NEWFISH in Europe and could sign two license agreements with fish processors in Norway and Germany.

Food consult is able to give licences for the described process and products to interested companies. Such a license includes not only the exact description of the procedures and recipes for different products but also a list of the needed equipment and the training for the personnel in the facilities of the licensee. It is also possible to make individual arrangements for companies being interested in special developments adjusted to the respective demands concerning product properties and raw materials.

References


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6.6 THE POSSIBILITY FOR INDUSTRIAL PRODUCTION OF DRIED FISH HEADS IN THE NORTHERN PART OF NORWAY

Hilde Herland, Morten Heide, Even Tidemann

Utilisation of the entire catch is given a lot of attention. In Norway, fish heads are one of the by-products considered a waste problem instead of a possible product for human consumption. Most of the heads are thrown out at sea; only a small amount is landed. The landed heads are dried outdoors in a traditional way, and most are sold to EU-countries as animal feed. Smaller amounts are sold to Nigeria, the only market for Icelandic dried fish heads. This market pays approximately 18 NOK/kg (2002) for Icelandic fish heads, while Norwegian fish heads are paid approximately 14 NOK/kg in the same market. When used for animal feed, the price is approx 2 NOK/kg. The lower Norwegian price might be due to a poorer quality, less stable delivery and different size grading. Unlike fish heads produced in Iceland, the Norwegian fish heads are without collarbones, which is less preferred by the buyers (less flesh).

In the Northern parts of Norway, such as in the western part of Finnmark County, the potential amount of fish heads for drying is 5600 tons (cod, haddock and saithe). Landings are spread quite evenly throughout the year, facilitating an industrial production where a continuous supply of raw material is advantageous. The size of the landed fish is mostly of medium size, giving medium sized heads. The Nigerian market prefers smaller heads, as they are buying per kg and selling per piece. The price per head is almost the same regardless of the size of the heads.

In the industrial production of dried fish-heads in Iceland, geothermal heat is used to provide the heat needed for drying. The heads are dried in two steps at low temperatures (15-25 °C) for 5-6 days. This process yield is app. 1 kg of dried heads out of 5 kg of raw fish heads (20 % yield). Unprocessed fish heads may be transported either fresh or frozen, making it possible to utilize heads from other regions in Norway, if the supply in one region should be to small.

The new oil refinery in Hammerfest could be a possible supplier of excess heat for drying. The amount of energy available is vast, making it possible to establish a factory in this area. Based on the previous mentioned amount of 5600 tons of unprocessed heads, one could produce some 1120 tons of dried heads annually (a value of 15-20 mill NOK). Estimates made by the authors indicate that the contribution margin per kg dried product is 7,10 NOK.

The conclusion of the work is that our estimates indicate that such a production could be economically viable, especially at places where excess heat from other industrial activities is available. There are a few obstacles though, where the major is to collect the heads and to get them on shore. Better outcome and a higher quality when de-heading the fish closer to further processing could promote such a production. This could also be a way to increase fishermen’s earnings. As the process can be used to produce dried collarbones and dried backbones, there is a production volume potential which is higher than the 1120 tons of dried heads estimated in our report.

Authors

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6.7 CHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF A FINFISH SPECIES REJECTED TO SEA: ROCK COD


Introduction
The fishing grounds of the Patagonian shelf support some of the most important fisheries in the world. Hakes (Merluccius hubbsi and Merluccius australis) and cephalopods (Illex argentinus and Loligo gahi) have been found to be the main commercial species, with important amounts of accompanying species such as Patagonotothen species which are discarded. The genus Patagonotothen has 14 species (P. Ramsayi, P. Guntheri, P. Magellanica,….) in southern South America (Falkland Islands). P. Ramsayi is the most abundant (Ekau, 1982; Norman, 1937; Hart, 1946). Little is known about the biology, ecology, chemical composition and nutritional value of these species. The Craft Project, Proposal No CRAFT-1999-71709, “Promoting higher added value to a finfish species rejected to sea” is aimed to examine various aspects of the biology of these species with a particular emphasis on chemical composition and nutritional value. The aptitude of these species to be on-board processed and frozen stored was also studied.

Materials and methods
All solvents used were analytical grade and were supported by Merck and Prolabo. All reactives used were ultra pure grade and were supported by Sigma-Aldrich (FeCl₃⋅4 H₂O, FeCl₂ 4 H₂O, tetraethoxy propane, phosphatidil choline dipalmitoil, picric acid, trimethyl amine clorhidrate, nonadecanoic acid, albumin bovine minimum 96%, phenol, hydrazine sulphate, glucose), Merck, (ammonium tiocianate, tiobarbituric acid), Larodan (cholesterol) and Supelco (Fame standard mix).

Raw material
Fish were caught at different periods of the year in the waters of the Falkland Shelf (Southern South America) longitude 59°W-60°W and latitude 51°S-52°S. They were on-board frozen and were sent to the Institute of Marine Research (Vigo). They were hold frozen at –20 ºC until analysis.

Sensorial evaluation
Sensory assessments of general appearance, firmness and raw odour and taste of each fish were carried out according to DOCE, 1989.

Biochemical and quality analysis
Total Volatile Bases (TVB-N).was measured by the Antonacopoulos (1960) method with some modifications. Ten grams fish muscle were extracted with perchloric acid (6 %) and made up to 50 mL. TVB-N content was obtained by steam distillation of the acid extracts made alkaline to pH 13 with NaOH (20 %), followed by titration of the distillate with 10 mM hydrochloric acid. Data are expressed as mg TVB-N/100g muscle.

Trimethyl amine content (TMA) was determined according to the Analytical Methods Committee (Analyst, 1979). Peroxide Value (PV) was determined according to Chapman and Makay (1949).

Aldehyde formation (i-TBA, mg malondialdehyde/kg sample) was determined according to Vyncke (1970).

Proximate composition and nutritional value
Content of Carbon, Hydrogen and Nitrogen was determined in a Perkin-Elmer 2400 CHN Elemental Analyzer. Total lipids were obtained by the Bligh & Dyer method (1954) and were quantified by gravimetric analysis (Herbes & Allen, 1984).

Fatty acid composition was determined by gas cromatography (8700 Perkin-Elmer) (Christie, 1982). Fatty acids were methylated with a solution of sulphuric acid in methanol (Medina et al., 1994).

Protein content was determined according to Lowry (1954) from a muscle homogenate in NaOH 0.5N.
**Tocopherol** was extracted using a modification of Burton et al. (1985) and analysed by HPLC according to Cabrini (1992).

**Cholesterol** content was determined using TLC according to Christie (1982).

**Carbohydrates** content was determined according to Strickland et al. (1968).

### Results

#### Characterisation of raw material

Three species of *Patagonotothen* have been studied: *P. Ramsayi*, *P. Guntheri* and *P. Magellanic*. The study is mostly focused on *P. Ramsayi*, the most abundant species, in which, the seasonal variation of the parameters related to nutritional value and quality was also determined.

#### Sensorial evaluation

Sensorial analysis of raw *P. Guntheri* and *P. Ramsayi* showed firm and elastic texture and white colour muscle. Odour was fresh. Texture of *P. Magellanic* was rapidly deteriorated at refrigerated temperatures. *P. Magellanic* muscle was darker than the others. Odour was also fresh. No seasonal variations in organoleptic characters were observed.

#### Biochemical and quality analysis

Quality values regarding to the formation of volatile bases and amines (Table 1) were low and revealed good and acceptable initial and storage conditions. Initial quality measurements regarding to lipid deterioration and rancidity (i-TBA) were low and no indicated deterioration (Table 1). There is not significant seasonal variation in the above mentioned indexes (Table 2).

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>TVB$^a$</th>
<th>TMA$^b$</th>
<th>i-TBA$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. GUNThERI</td>
<td>16.8 ± 0.41</td>
<td>0.049 ± 0.003</td>
<td>1.58 ± 0.03</td>
</tr>
<tr>
<td>P. RAMSAYI</td>
<td>21.9 ± 0.81</td>
<td>0.262 ± 0.004</td>
<td>1.20 ± 0.02</td>
</tr>
<tr>
<td>P. MAGELLANICA</td>
<td>11.93 ± 4.08</td>
<td>0.0129 ± 0.004</td>
<td>0.39 ± 0.03</td>
</tr>
</tbody>
</table>

*a* expressed as mg N/100 g wet muscle.

*b* expressed as mg N/100 g wet muscle.

*c* expressed as mmol MDA/g wet muscle.

<table>
<thead>
<tr>
<th>TVB$^a$</th>
<th>TMA$^b$</th>
<th>PV$^c$</th>
<th>i-TBA$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUSTRAL AUTUMN</td>
<td>21.89</td>
<td>0.262</td>
<td>0.39-1.20</td>
</tr>
<tr>
<td>0.81</td>
<td>0.004</td>
<td>0.03-0.02</td>
<td></td>
</tr>
<tr>
<td>AUSTRAL WINTER</td>
<td>17.32-26.42</td>
<td>0.1902-0.5971</td>
<td>1.04-2.78</td>
</tr>
<tr>
<td>(0.34-0.00)</td>
<td>0.0013-0.0002</td>
<td>0.21-0.08</td>
<td>0.02-0.01</td>
</tr>
<tr>
<td>AUSTRAL SPRING</td>
<td>16.10-22.33</td>
<td>0.3669-0.7965</td>
<td>1.29-9.04</td>
</tr>
<tr>
<td>(0.57-0.19)</td>
<td>0.0088-0.0073</td>
<td>0.02-0.3</td>
<td>0.01-0.01</td>
</tr>
<tr>
<td>AUSTRAL SUMMER</td>
<td>15.15-18.33</td>
<td>0.1053-0.2937</td>
<td>1.54-8.47</td>
</tr>
<tr>
<td>(0.11-0.11)</td>
<td>0.0027-0.0044</td>
<td>0.01-0.04</td>
<td>0.002-0.01</td>
</tr>
</tbody>
</table>

*a* expressed as mg N/100 g wet muscle.

*b* expressed as mg N/100 g wet muscle.

*c* expressed mequiv O$_2$/Kg wet muscle

*d* expressed as mmol MDA/g wet muscle.
Proximate composition and nutritional value

The content in C (~48%), N (~14%) and H (~4%) was similar in the three species. *P. Guntheri* and *P. Ramsayi* showed similar protein content, (19.76% and 18.06% respectively). *P. Magellanic* had less protein content and higher water content. *P. Ramsayi* and *P. Magellanic* have a moderate fat content (0.51-1.41% and 1.45% respectively) and *P. Guntheri* was the fattest (3.18%). Water content ranged between 76-79% in all species. Carbohydrates were low (0.1%) and minerals measured as ashes were agree with the content described in other species.

Nutritional value showed that all species had a high content in n-3 polyunsaturated fatty acids: DHA (22:6w3) and EPA (20:5w3), which achieved amounts of 30-42 and 12-18 % respectively of total lipids. In addition to this, *P. Ramsayi* showed important levels of vitamin E (167 µg/g fat) and under levels of cholesterol, about 15 mg/100 g of wet muscle.

Related to seasonal variations, (Table 3 and Fig. 1), the values showed a slight variation in fat and water content during the ripening state, autumn (ustral spring). Related to fatty acids (FA) composition, during the austral spring was observed the biggest quantity of DHA (about a 45%).

Table 3. Proximate composition. Seasonal variation (interval, stdev of each value)

<table>
<thead>
<tr>
<th></th>
<th>a % WATER</th>
<th>b % FAT</th>
<th>c % PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUSTRAL AUTUMN</td>
<td>78.43</td>
<td>1.32</td>
<td>18.06</td>
</tr>
<tr>
<td>AUSTRAL WINTER</td>
<td>79.6-80-15</td>
<td>0.63-1.08</td>
<td>16.16-18.30</td>
</tr>
<tr>
<td>(0.47-0.09)</td>
<td>(0.13-0.00)</td>
<td>(4.31-3.30)</td>
<td></td>
</tr>
<tr>
<td>AUSTRAL SPRING</td>
<td>80.06-82.99</td>
<td>0.51-1.41</td>
<td>11.91-15.65</td>
</tr>
<tr>
<td>(0.01-0.06)</td>
<td>(0.04-0.21)</td>
<td>(3.49-0.84)</td>
<td></td>
</tr>
<tr>
<td>AUSTRAL SUMMER</td>
<td>79.83-82.92</td>
<td>0.66-1.41</td>
<td>15.68-22.08</td>
</tr>
<tr>
<td>(0.33-0.019)</td>
<td>(0.17-0.30)</td>
<td>(0.31-5.07)</td>
<td></td>
</tr>
</tbody>
</table>

a, b and c expressed as % of wet muscle

Fig. 1. Seasonal variation in the major FA expressed as mg FA/mg total lipids

Aptitude to be frozen stored

It has been studied the aptitude to be frozen storage of *P. Ramsayi*. After six months of frozen storage biochemical measurements (i-TBA, TMA, TVB) didn’t show a significant increase. Only PV was significantly higher. These values were correlated with sensorial analysis and the product was found to be acceptable.
Discussion

All individuals analysed showed good initial sensorial quality. It didn’t notice significant differences between individuals of different size or sex. However, texture of *P. Magellanic* species was rapidly deteriorated at 4°C. *P. Guntheri* and *P. Ramsayi* showed good muscle properties with high water retention and firm texture. *P. Guntheri* and *P. Ramsayi* showed similar protein content. *P. Magellanic* had less protein content and higher water content. A slight seasonal variation was observed in *P. Ramsayi*.

The three species showed a high content of polyunsaturated fatty acids, with high contents of EPA and DHA (n-3 polyunsaturated fatty acids). Lipids of *P. Guntheri* were more saturated than the other. *P. Ramsayi* showed a small content in cholesterol and important content in tocopherol.

Quality values regarding to the formation of volatile bases and amines were low and revealed good and acceptable storage and initial conditions. Quality measurements regarding to lipid deterioration and rancidity were low and no indicated deterioration after six months storage.

Conclusion

The chemical and biochemical analyses performed demonstrated that *P. Guntheri* and *P. Ramsayi* could be very acceptable for consumption and posterior frozen storage. All analysis demonstrated, therefore that *P. Ramsayi* can be considered a healthful species because of the high PUFA levels (especially DHA and EPA), vitamin E levels and under cholesterol levels.

Acknowledgments

The authors acknowledge financial support for the Craft Project, Proposal, Nº CRAF-1999-71709, “Promoting higher added value to a finfish species rejected to sea” from European Community and Xunta de Galicia (Project PGIDIT04PXIC40201PM).

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Authors

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34th WEFTA meeting, 12-15 September 2004, Lübeck, Germany
6.8 GELATIN EXTRACTION FROM CAPE HAKE AND BLUE SHARK SKIN

Irineu Batista, Patricia Fradinho and Célia Silvestre

Introduction

Fish wastes from the fish processing factories represent a valuable raw material for many purposes. About one third of these wastes is skin and bone, which are a rich source of collagen. Heat denaturation of collagen produces gelatin. This proteic compound presents a wide range of applications, which includes the food, pharmaceutical and photographic industries. The quality of fish gelatin, as referred by Gómez-Guillén et al. (2002), is greatly dependent on the fish species, which present intrinsic differences in the collagen molecules. According to above authors, the fish collagen is also more susceptible to degradation than the gelatin from mammals due to the lower content in intra- and interchain non-reducible crosslinks. The properties of fish gelatins from several species have been studied but there is still little information concerning gelatin extraction from the skin of many marine species.

The availability of raw material is also an important factor to take into account. Thus, with this in mind, the skin from Cape hake (Merluccius capensis) and blue shark (Prionace glauca) were chosen in this study. High amounts of skins from these two species are produced and generally wasted. So, their utilisation for the extraction of fish gelatin could be an interesting way of upgrading those by-products. They are also quite different species, which could determine very different physicochemical characteristics of the gelatin extracted.

In this work two different gelatin extraction methods were studied, the yields achieved were calculated and some properties of the gelatins obtained were measured.

Materials and Methods

Skins from Cape hake (Merluccius capensis) and blue shark (Prionace glauca) were used for the extraction of gelatin and collagen.

Gelatin was prepared following the method described by Gudmundsson and Hafsteinsson (1997) (method A) and by hydrolysis of collagen (method B), which was essentially extracted according to the procedure described by Montero and Gómez-Guillén (2000). For the hydrolysis the collagen powder was dissolved in distilled water (1:6 fish skin weight/distilled water volume) at 45 ºC and kept at this temperature for 22 h. Gelatin yield was calculated as dry weight gelatin/wet weight fish skins x 100.

The concentration of the solutions used in the both preparation methods is shown in next Table 1.

Table 1. Concentration of the solutions and extraction temperature used in methods A and B

<table>
<thead>
<tr>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH 0.1 – 0.5 %</td>
<td>NaCl 0.8 M</td>
</tr>
<tr>
<td>H₂SO₄ 0.2 %</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>Citric acid 0.7 %</td>
<td>0.5 M</td>
</tr>
<tr>
<td>Extraction temperature 45 ºC or 70 ºC</td>
<td></td>
</tr>
</tbody>
</table>

Moisture, fat and ash were determined according to the Portuguese Standards NP 2282 (1991), NP 1972 (1992) and NP 2032 (1988), respectively. Crude protein was determined according to AOAC (1995) procedure in a Kjeltec System 1026 Distilling Unit. Crude protein content was calculated by multiplying total nitrogen by the factor 5.3 (Montero et al., 1999).

The pH of gelatin solutions was measured at 28 ºC using a WTW pH meter. Gel strength was determined on a 6.67 % (w/w) gelatin solution, formed by dissolving the powder in distilled water at 60 ºC and maintained during 16-18 h at 4 ºC. The gel strength was determined on a 6.67 % (w/w) gel. An Instron model 4301 Universal Testing Machine with a load cell of 1 kN, cross head speed 1 mm/s equipped with a 1.27 cm diameter flat-faced cylindrical plugger. All determinations were performed at 4ºC and the results were averages of 3 measurements. Viscosity measurements of 6.67 % (w/w) gelatin solutions were done at 28, 40 and 60 ºC on a Rheology Internacional model RI:2:L. Dynamic viscoelastic studies of 6.67 % (w/w) gelatin solutions were performed on a rheometer RheoStress RS-75 using a parallel-plates geometry. Cooling from 30 ºC to 5 ºC was performed at a scan rate 0.5 ºC/min, frequency 1 Hz, and oscillating applied stress of 50 Pa.
Results and discussion

Very low yields were achieved in the extraction of gelatin from Cape hake skin following the method A at 45 ºC (Table 2). A slightly higher value was obtained at 70 ºC but both results were very low when compared with those referred by Gudmundsson and Hafsteinsson (1997) for the gelatin extraction cod skin. This could be related to the occurrence of a high degree of cross-linking in the Cape hake collagen making its extraction very difficult. The method B allowed to obtaining a much higher yield but the gelatin had a high salt content. In the case of blue shark skin the gelatin extraction was easier than from Cape hake skin following method A and a considerably higher yield was attained. A slightly higher increase was also obtained by method B.

Table 2. Yields obtained in the gelatin extraction from Cape hake and blue shark skin by the methods A and B

<table>
<thead>
<tr>
<th></th>
<th>Cape hake skin (%)</th>
<th>Blue shark skin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method A</td>
<td>1.3-1.5 (a)</td>
<td>10.7 (c) (BSA1)</td>
</tr>
<tr>
<td></td>
<td>2.8 (b) (HA)</td>
<td>14.7 (d) (BSA2)</td>
</tr>
<tr>
<td>Method B</td>
<td>11.1 (e) (HB)</td>
<td>12.9 (e) (BSB)</td>
</tr>
</tbody>
</table>

(a) Mean of two experiments, extract. temp. 45 ºC; (b) Mean of five experiments, extract. temp. 70 ºC; (c) Mean of three experiments, extract temp. 45 ºC; (d) Mean of two experiments, extract temp. 70 ºC; (e) Mean of two experiments.

The most relevant feature to be stressed on the proximate chemical composition of gelatins obtained (Table 3) is the high ash content of products prepared by method B. This results from the presence of NaCl, which was not conveniently removed during preparation.

Table 3. Proximate chemical composition of fish gelatins

<table>
<thead>
<tr>
<th></th>
<th>Moisture</th>
<th>Protein</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Cape Hake skin</td>
<td>6.52 (HA)</td>
<td>8.17 (HB)</td>
<td>80.6 (HA)</td>
</tr>
<tr>
<td>Blue shark skin</td>
<td>8.04 (BSA1)</td>
<td>5.36 (BSB)</td>
<td>77.69 (BSA1)</td>
</tr>
<tr>
<td></td>
<td>6.31 (BSA2)</td>
<td>78.12 (BSA2)</td>
<td>1.00 (BSA2)</td>
</tr>
</tbody>
</table>

The pH values of fish gelatins (Table 4) prepared by the method A were generally lower than those of gelatins obtained by the method B. These results reflect the methodology followed in the preparation and the pH values of products prepared according to the method A were similar to those reported by Gudmundsson and Hafsteinsson (1997).

Table 4. pH values of fish gelatins

<table>
<thead>
<tr>
<th></th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cape hake skin</td>
<td>3.42 (HA)</td>
<td>5.29 (HB)</td>
</tr>
<tr>
<td>Blue shark skin</td>
<td>3.80 (BSA1)</td>
<td>5.18 (BSB)</td>
</tr>
<tr>
<td></td>
<td>3.5 (BSA2)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Gel strength of fish gelatins.
The results in Fig. 1 put into evidence the effect of the extraction method on the gel strength (GS) of the fish gelatin prepared. The preparation conditions of method B are milder than those of method A, which could explain the difference between GS of gelatins prepared by both methods from the same species in the case of Cape hake gelatin. However, the same trend was not observed with the gelatin from blue shark. The much higher GS of blue shark gelatin extracted at 45 °C (BSA1) also evidences the effect of the temperature on this gelatin characteristic. The solutions of all gelatin samples exhibited at 28 °C a Newtonian behaviour, with the exception of BSA1 sample, which presented typical pseudoplastic flow behaviour (Fig. 2). However, at higher temperatures (40 °C and 60 °C) the solutions of all samples were Newtonian fluids. The relatively low viscosity of samples HA, HB, BSA2, and BSB may be due to the presence of high percentage of low molecular weight molecules. They are also in accordance with the low gel strength of those gelatins. The viscosity of Cape hake gelatin solutions of both products was lower than the values reported by Gudmundsson and Hafsteinsson (1997) for cod gelatin. On the other hand, the viscosity of gelatin solution BSA1 at 40 °C and 60 °C was similar to the values reported by the above authors. The solution of gelatins HB and BSB had lower viscosity values than those reported by Montero and Gómez-Guillén (2000) for the megrim gelatin also obtained from the hydrolysis of megrim collagen extracted.

In Fig. 3 is shown the evolution of elastic modulus (G') and viscosity modulus (G'') of gelatin BSA2 during cooling. The gelling temperature was determined where G’ and G’’ intersect and the results obtained for some gelatin samples is shown in Table 5.

Table 5. Gelling temperature of gelatin samples

<table>
<thead>
<tr>
<th>Gelatin sample</th>
<th>Gelling temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>1</td>
</tr>
<tr>
<td>HB</td>
<td>5</td>
</tr>
<tr>
<td>BSA2</td>
<td>5</td>
</tr>
<tr>
<td>BSB</td>
<td>5</td>
</tr>
</tbody>
</table>

Both viscoelastic modulus of gelatins HB and BSB were lower than those reported by Montero and Gómez-Guillén (2000) for megrim gelatin prepared from collagen. These modulus of gelatins HA and BSA2 were also lower than those reported by Gudmundsson (2002) for cod gelatin extracted under similar conditions. The gelling temperature of the gelatins prepared in this work was also very low when compared with the values reported by other authors (Montero and Gómez-Guillén, 2000; Gudmundsson, 2002).
Conclusions

The extraction of gelatin from Cape hake skin by the methods described by Gudmundsson and Hafsteinsson (1997) and Montero and Gómez-Guillén (2000) was very difficult and low yields were achieved. The gelatin of blue shark skin was easily extracted using the above-mentioned methods and reasonable yields were obtained. The low gel strength, viscosity and gelling temperature of all gelatins, with the exception of gelatin BSA1, indicate the presence of high level of low molecular weight molecules in those products.

Acknowledgements

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6.9 IMPROVING PRODUCTION OF MINCED FISH PRODUCTS

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Introduction

Seafood products made of minced fish, like fish cakes, fish balls and fish pudding, are passed through a traditional double heat process with baking (emulsification and top encrustation), chilling, vacuum packaging, pasteurisation and cooling. The production method is time- and energy consuming due to two heating steps with intervening chilling. In 2002 the seafood industry in Norway initiated a project to modernise the production of minced seafood products with a specific aim to improve the sensory quality and to make a more cost-effective production. A target requirement was to obtain a shelf life of 8-12 weeks in chilled storage (0-4 °C) without increased safety hazards. Fish pudding was chosen as a general model product and the production and improvements were carried out during ordinary production at an industrial plant. The work was focused on heat treatment, new hygienic production layout and to maintain comparable shelf life and sensory quality.

Materials and methods

Fish puddings (800g) were produced at a local producer near Stavanger, Norway. Ingredients are greater argentine (Argentina silus), haddock (Melanogrammus aeglefinus) and saithe (Pollachius virens) together with milk, starch, salt and spices.

Pudding samples of 25 g were homogenised in 225 ml of peptone water (0.9 % NaCl (w/v), 0.1% peptone (w/v) and homogenised for 2 min in a Stomacher 400 Laboratory Blender (Seward, Medical, England). After suitable dilutions, 1.0 ml duplicate samples were added to melted Plate Count Agar (Merk) and incubated at 30 °C for 3 days to enumerate total aerobic plate counts (APC).

The texture analyses were performed using a Texture Analyser TA.XTplus (Stable Micro Systems Ltd, UK), equipped with a 5 kg load cell and a 5mm Ø stainless steel spherical probe (P/5S). Cylindrical samples for texture analysis (30mm high, 30 mm Ø) were cut from fish pudding preheated to 20 °C. The samples were wrapped in plastic film to avoid drying of the surface. The gel strength was defined as Force*Distance and the brittleness as Force/Distance at the breaking point.

Results

Modification of the baking line

The baking line was modified with increased temperature and time, in order to achieve a comparable pasteurisation value as in the original process with both baking and pasteurization. A heat transfer model was programmed in FemLab (FemLab 2.3/ 3.0, Comsol AB) based on measurement of conductivity in the pudding and emissivity of the surface. Convection heating was estimated by experiments. Further experiments were done to verify and optimise the model. The General Method used for calculation of inactivation is based on the following assumptions:

In general, the pasteurising value is determined from the following equation:

\[ P_{\text{ref}} = \int_0^{t} \left( \frac{T(t) - T_{\text{ref}}}{T_{\text{ref}} - T_{\text{ref}}} \right)^{10} \cdot dt \]

Test procedures for heat penetration tests are based on recommendations from Institute for Thermal Processing Specialists (IFTPS, 1995). Test procedures for temperature distribution tests are based on recommendations from Institute for Thermal Processing Specialists (IFTPS, 1992).
Table 1. Pasteurisation values in the core of the minced fish product

<table>
<thead>
<tr>
<th>Method</th>
<th>A - $P_{90}^{10}$</th>
<th>A - $P_{80}^{10}$</th>
<th>B - $P_{90}^{10}$</th>
<th>B - $P_{80}^{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked and Pasteurised (BP)</td>
<td>4.1</td>
<td>41.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Baked (B)</td>
<td>3.1</td>
<td>30.7</td>
<td>8</td>
<td>80</td>
</tr>
</tbody>
</table>

Fig. 1. Core temperature profiles in 1) baking and pasteurisation (BP) and 2) only baking (B) with extended heat processing

It is a commonly accepted concept to use calculations to gain a 6 decimal reduction of non-proteolytic *Clostridium botulinum* for pasteurised refrigerated products with long shelf life. Reference temperature for *Clostridium botulinum* type E and B is commonly set to 90°C, $D_{90}$-value of 1.6 minutes and a $z$-value of 7.5 to 10°C. For practical use it has been recommended to use a $z$-value of 7.5°C below 90°C and 10°C above 90°C (European Commission, 1999), as this represent the worst case values reported on each side of 90°C. A change in $z$-value would complicate the comparison of the processes and not reflect a realistic profile, and therefore a constant $z$-value has been chosen. These values are general recommendations and are not specific for fish mince based products. In the Norwegian industry another criteria has been used for fish mince and through decades proved to be safe, using 80°C in the core of the product for 30 minutes ($P_{80}^{30}$). Hence, in this study the pasteurization value is calculated using the following expressions:

$$P_{90}^{10} = \int_0^t 10^{\frac{T(t) - 90}{10}} \, dt$$

$$P_{80}^{10} = \int_0^t 10^{\frac{T(t) - 80}{10}} \, dt$$

The results shown in Table 1 and Fig. 1 showed that an extended time in the baking line (B), and subsequently an extended holding time, gave a lower pasteurization value ($P_{90}^{10} = 3.1$) compared to baking and pasteurization (BP) ($P_{90}^{10} = 4.3$), but still in the same order of magnitude.

Reorganised production layout

There is an increased risk of bacterial growth caused by recontamination when the products are packaged after heat processing with no secondary heating. Vacuum packaging at high temperatures (>70°C) with a steam flushing process was therefore tested. A survey of microbial contamination on equipment, conveyor belts and surrounding air was carried out to evaluate contamination routes and the requirement that had to be met with a new hygienic production design. From these requirements a new hygienic layout was planned with hot fill packaging in a superhygienic sone (Fig. 2, C2) compared to packaging after chilling (Fig. 2, C1).
Shelf life and quality

Several shelf life studies were carried out with fish pudding processed at different temperatures and time regimes. During a storage period of 80-90 days, samples for microbiological analyses were collected both in the centre of the product, to confirm surviving spore forming bacteria, and on the surface to conform recontamination. The aerobic plate counts were similar to the modified baking process compared to baking and pasteurisation (Fig. 4). Stiffness and gel strength were also comparable, but varied with the different heat processes. Colour, stiffness and gel strength were measured and used for process optimisation (Fig. 3).
Conclusions

- Comparable pasteurisation values were obtained with one heating step (baking) in stead of two (baking and pasteurisation).
- Microbiological analyses, stiffness and gel strength were comparable for baking compared to baking and pasteurisation.
- The new process design is more cost effective and will simplify the production process considerably.

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