Published in **Journal of the Science of Food and Agriculture**
MARCH 2005; 85(10) : 1750-1756
http://dx.doi.org/10.1002/jsfa.2145

Development of lipid oxidation during manufacturing of horse mackerel surimi

Sylvie Eymard [1], Elodie Carcouët [1], Marie-Joëlle Rochet [2], Justine Dumay [1], Christine Chopin [1*], Claude Genot [3]

[1]IFREMER, Laboratoire Génie Alimentaire, BP 21105, F-44311 Nantes cedex 3, France
[2]IFREMER, Mathématiques Appliquées à l'Exploitation des Ressources Halieutiques et Aquacoles, BP 21105, F-44311 Nantes cedex 3, France
[3]INRA, Laboratoire d'Etude des Interactions des Molécules Alimentaires, BP 71627, F 44316 Nantes cedex 3, France

*: Corresponding author : Christine.Chopin@ifremer.fr

Abstract:

When fatty fish are transformed into surimi, lipid oxidation takes place, decreasing the quality of the product. This study was aimed to identify the critical stages of the process in terms of the development of lipid oxidation. Horse mackerels were transformed into surimi on a pilot line and samples taken (hand-skinned fillets = minced fillets, mince, washed and refined minces, paste, surimi and washing water). Most of the lipids were removed during the process and neutral lipids were lost in higher proportion than polar lipids. As a consequence, total lipids of surimi contained more polyunsaturated fatty acids (338 ± 19 g kg^{-1}) than total lipids of the minced fillets (220 ± 8 g kg^{-1}). Thiobarbituric acid reactive substances (TBARS) was higher in the minced fillets than in the mince because less subcutaneous fat and dark muscle were removed during hand-mincing, indicating that the settings of the skinning-deboning machine can strongly influence the final quality of the product. Concentrations of lipid oxidation products increased significantly during the next stages of surimi processing. The increase was more pronounced for TBARS than hydroperoxides. Concentrations in hydroperoxides were similar in mince and washed mince (15.3 ± 2.8 and 16.6 ± 2.8 mmoles kg^{-1} lipid) and increased in refined mince (29.6 ± 2.8 mmoles kg^{-1} lipid). TBARS accounted for 2.7 ± 1.0 mg kg^{-1} lipid in mince, 40.4 ± 2.3 mg kg^{-1} lipid in washed mince and 237 ± 7 mg kg^{-1} lipid in refined mince. Hydroperoxides and TBARS were found in appreciable amounts in washing water (76.9 ± 4.7 mmoles kg^{-1} lipid and 479 ± 8 mg kg^{-1} lipid respectively), when they decreased in surimi (27.3 ± 3.8 mmoles kg^{-1} lipid and 44.2 ± 0.8 mg kg^{-1} lipid respectively) compared with refined mince. This shows that the last dewatering stage is crucial to ensure surimi quality.

Keywords: lipid oxidation • polyunsaturated fatty acids • surimi • fatty fish • process

INTRODUCTION

Surimi is a concentrate of fish myofibrillar proteins obtained by successive washing of fish mince to remove blood, lipids, enzymes and sarcoplasmic proteins. Surimi is an intermediate product which requires a second stage of transformation during which NaCl, texturing agents and aroma are added, and thermal treatment is applied, to obtain seafood analogues.

The demand for fish proteins and surimi throughout the world is increasing faster than the supply in traditional resources. This great demand has led to overfishing of many of traditional lean fish species as Alaska pollock (*Theragra chalcogramma*). In contrast, fatty fish species as horse mackerel, sardine, mackerel, are under exploited in spite of their availability, low price and good nutritional value. Fatty fish are difficult to process because they exhibit a great seasonal variability, particularly in lipid contents[1,2]. Moreover, due to their high content in dark muscle and fat they are very sensitive material which rapidly losses its freshness and the functional properties of its proteins Lipids of fatty fish are rich in long-chain polyunsaturated fatty acids[3] of excellent nutritional value. However, they are very prone to oxidation that is also favoured because myoglobin and oxidizing enzymes present in dark fish muscle are efficient pro-oxidants[4,5]. Lipid oxidation induces formation of an array of products decreasing directly or indirectly the sensory quality of fish and fish products[6]. It produces unstable intermediary compounds such as free radicals and hydroperoxides and leads to formation of volatile compounds involved in development of off-flavors[7]. Oxidized lipids also interact with proteins inducing modification of textural properties[8,9].

It is difficult to maintain the quality of the product during processing of surimi from fatty fish. First, during mincing and mixing, cell membranes are disrupted and membrane lipids are blended with pro-oxidants[10]. Oxygen is also incorporated and temperature tends to increase, promoting lipid oxidation. Then both water-soluble and hydrophilic pro-oxidants and

antioxidants are removed during the extensive washing stages. Thus, the material progressively losses its natural protections and becomes very sensitive to oxidation.

To optimize the process that enables the transformation of fatty fish, as horse mackerel, in surimi, a pilot production line has been set up at Ifremer (Nantes, France). The aim of this paper was to identify the stages of the process that were critical to the development of lipid oxidation during manufacturing of horse mackerel surimi.

EXPERIMENTAL

Reagents and Standards

HPLC-grade methanol, ethanol, HPLC-grade dichloromethane, chloroform and analytical grade pentane were purchased from *Carlo Erba* (Val de Reuil, France). Phosphatidylcholine extracted from eggs was obtained from *Larodan fine chemical* (Malmö, Sweeden). Butylated hydroxytoluene, ferric chloride and ammonium thiocyanate were obtained from *Sigma-Aldrich* (Saint Quentin Fallavier, France). Sulfuric acid 970 g L^{-1} was obtained from *Panreac Quimica* (Barcelona, Spain). Analytical grade thiobarbituric acid (99 % purity) and trichloroacetic acid (99.5 % purity) were obtained from *Grosseron* (Saint-Herblain, France). Distilled, deionized water (*Millipore system*) was used throughout.

Raw material, processing and sampling

Horse mackerels (*Trachurus trachurus*) were caught in July 2001 off the coast of Brittany by a local fisher (La Turballe, France). They were kept on ice and received in our laboratory in less than 12 hours after catching. They were then beheaded, eviscerated, filleted and washed.

Five fillets were sampled for raw material analysis. They were hand skinned and minced in a mincer (*Moulinex HV3, France*) in a temperature-controlled room (4 °C). The hand prepared mince (minced fillets) was put in air-tight plastic bags and stored at -80 °C before analysis.

Surimi was manufactured with about 40 kg of horse mackerel fillets stored on ice (Figure 1). To obtain mince (mince), skin and bones of iced fish were first removed with a skinning and deboning machine (*Baader, Type 694, Germany*) at ambient temperature. The mince was successively washed and dewatered two times (washed mince) with chilled tap water at about 10°C. Mince was refined (refined mince) in a refiner (*Robot Coupe C120, France*) to remove undesirable particles, such as bone and connective tissues. Then it was dewatered in a decanter centrifuge (*Sharples, PENNWALT, England*) (washing water) until a paste was obtained. Cryoprotectants [sorbitol/saccharose/polyphosphate : 4/4/0.3 ; w/w/w] were finally incorporated to the paste (80 g kg^{-1} paste) with a mixer (*Stephan UM 12,Germany*) to obtain horse mackerel surimi. At each stages of processing, samples (mince, washed mince, refined mince, rejected washing water, paste and surimi) were collected. Solid samples were stored in air-tight plastic bags at -80 °C until analysis. Liquid samples were stored in 100 mL flasks at -80 °C.

Water and lipid contents

Water content was determined by weight difference of the sample (5 g) before and after 18 hours at 100 °C. Analysis were performed in triplicate and results were expressed as g water per kg wet sample.

Total lipids (TL) were extracted in triplicate from 5 g of sample with methanol/dichloromethane (1/2; v/v) containing butylated hydroxytoluene (25 mg L^{-1}), according to the Folch *et al* method[11]. The solvent (chloroform) used in the original method was replaced by dichloromethane with no differences in total lipid content and fatty acid composition of the lipid fraction extracted by the one or the other solvent (results not shown). The lipid extracts were dried under vacuum on a rotary evaporator (temperature ≤ 35 °C).

Lipids were weighed and results were expressed as g kg^{-1} wet or dry sample. Lipid extracts were kept in 5 mL chloroform and stored at -80 °C.

Phospholipid content of total lipid extracts

Phospholipid content of lipid extracts was measured by the colorimetric method of Stewart [12] based on the formation of a complex between phospholipids and ammonium ferrothiocyanate. Thiocyanate reagent was made of ferric chloride (27 g L^{-1}) and ammonium thiocyanate (30 g L^{-1}) in distilled water. One mL of thiocyanate reagent was added to 2 mL of lipid extract in chloroform (0.25 mg/mL). The mixture was homogenized and centrifuged for 10 min at 2000 rpm (750 *g*). The absorbance of the lower phase was read at 488 nm against the blank on a dual beam spectrophotometer (*SPECTRO ATI UNICAM SP890, United Kingdom).* A standard curve was made with standard phosphatidylcholine in chloroform (5 to 50 µg/mL) and results were expressed as g phosphatidylcholine equivalents per kg of total lipid extract (g PC Eq kg^{-1} TL).

Fatty acid composition of total lipids

Fatty acid composition of lipids was determined by gas chromatography of fatty acid methyl esters (FAMEs). Lipids (1 mg) were transesterified by a solution of 20 mL L^{-1} sulfuric acid in anhydrous methanol and the reaction was carried out overnight in tightly closed tubes at 50 °C[13]. Then, 1 mL of double distilled water and 2 mL of pentane were added. After mixing and decantation, FAMEs were collected in the organic upper-phase. FAMEs (1.5 µg) in pentane were injected in the gas chromatograph (*Auto System Gas Chromatography, Perkin Elmer*) equipped with an auto-sampler and fitted with a split/splitless injector (liner : 8.5 cm x 2 mm i.d.) and a flame ionisation detector. The separation was carried out in a capillary column (60 m long, 0.25 mm internal diameter, 0.25 µm film thickness), containing a polar

stationary phase (*BPX70, SGE, France*). The carrier gas was helium with an inlet pressure of 25 psi. The injector was in splitless mode and its temperature set at 55 °C for 2 min, then increased to 350 °C in 1.48 min, held at 350 °C for 8 min and finally decreased to 70 °C. The oven temperature was held at 50 °C for 2 min, increased to 150 °C at 20 °C min^{-1}, maintained at 150 °C for 5 min, increased to 230 °C at 1.5 °C min^{-1}, then maintained at 230 °C during 45 min, and then decreased to 70 °C until the end of the analysis; the total duration of the analysis was 60 min. The detector temperature was set at 300 °C. Methyl esters were identified by comparison of the retention times of authentic standards (*Sigma-Aldrich*, Saint Quentin Fallavier, France). The peaks were integrated with Diamir (*Varian*) software. Identified fatty acids were quantified by comparison of integrated areas with calibration curves built with forty-five standard fatty acids. Fatty acid compositions were expressed as g of fatty acid methyl ester per kg of total identified fatty acid methyl esters (g kg^{-1} total FAMEs).

Hydroperoxide measurement

Hydroperoxides are primary products of lipid oxidation and their decomposition leads to secondary compounds. Hydroperoxide content was determined according to Eymard & Genot method[14]. Results were expressed as mmoles of cumene hydroperoxide equivalents (CuOOH Eq) per kg of total lipids (mmol kg^{-1} TL) and mmoles of cumene hydroperoxide equivalents per kg of sample (mmol kg^{-1}).

TBARS determination

Aldehydes are secondary compounds of lipid oxidation; they are very reactive and easily react with proteins. Thiobarbituric acid reactive substances (TBARS) test is based on spectrophotometric quantification of the pink complex formed after reaction of secondary

compounds of lipid oxidation, such as 1- and 2-alkenal, with thiobarbituric acid. TBARS were determined with a method adapted from Salih *et al*[15] and Bostoglou *et al*[16] by Genot[17]. Two grams of sample were mixed with 100 µL of butylated hydroxy toluene (BHT) in ethanol (1 g L^{-1}) and 16 mL of trichloroacetic acid (TCA 50 g L^{-1}). Samples were homogenized for 15 sec at 20,000 rpm with an Ultra-Turrax (*Janke & Kunkel, IKA-Werk*, Staufen, Germany) and then filtered. Two mL of filtrate (or 2 mL of TCA for blank) were added to 2 mL thiobarbituric acid solution (20 mol L^{-1}). The tightly closed tubes were heated at 70 °C for 30 min and rapidly cooled in ice. Absorbance was read against the blank at 508 (A_{508nm}), 532 (A_{532nm}) and 600 (A_{600nm}) nm with a double beam spectrophotometer (*SPECTRO ATI UNICAM SP890*, United Kingdom). The absorbance measured at the maximum ($A_{532\ nm}$) was corrected for the baseline drift as follows:

$$A_{532\ nm}\ \text{corrected} = A_{532nm} - [(A_{508nm}-A_{600nm})x(600-532)/(600/508)]-A_{600nm}$$

Results were expressed as mg of malonaldehyde equivalents (MDA Eq) per kg of total lipids (mg kg^{-1} TL) and mg of malonaldehyde equivalents per kg of sample (mg kg^{-1}) using the molar extinction coefficient of MDA-TBA adduct at 532 nm ($1.56x10^5$ M^{-1} cm^{-1})[18].

Statistical analyses

Equality of variances in the set of samples is an important precondition for classical variance analysis (ANOVA)[19]. Therefore ANOVA was not appropriate to compare lipid contents and concentrations of lipid oxidation products between the samples, which had very different variances. As the within-sample variance increased with concentration of lipid oxidation products, an extended linear model with heteroscedastic errors was used: in this model, the variance increases linearly with the estimated concentrations[20]. The estimated sample concentrations do not differ from the classical ANOVA ones, but the variances do. The latter were used to construct confidence intervals for the concentrations. This test was performed

for all results with the exception of fatty acid compositions. To compare fatty acid compositions of lipids extracted from minced filets or surimi, the student test was used.

RESULTS

Water content was similar in minced fillets, mince and surimi; it was higher in paste, in washed and refined mince and in washing water (Table 1). The lower water content in surimi than in paste was due to the incorporation of cryoprotectants to the paste, increasing dry weight of surimi. Water contents of washed mince, refined mince and washing water were the highest since they were taken during washing processes.

Lipid content regularly decreased during surimi manufacturing. It accounted from 205 g kg^{-1} dry weight in raw material to 44 g kg^{-1} dry weight in surimi (Table 1). Lipid content expressed per wet weight was lower in washed mince (11.9 g kg^{-1} wet weight), refined mince (6.4 g kg^{-1} wet weight), paste (12.1 g kg^{-1} wet weight) and surimi (10.0 g kg^{-1} wet weight) than in mince (36.0 g kg^{-1} wet weight). Lipid content of washing water sampled during the last dewatering stage was low (3.7 g kg^{-1} wet weight) but this lipid fraction represented about 20 % of the dry weight of the sample (Table 1). High standard deviations observed for lipid content of samples taken during intermediary stages of processing, were explained by heterogeneity of samples composed with mince's fragments in water.

Phospholipid content of total lipid extract was lower in minced fillets (113 g kg^{-1} TL) than in mince (140 g kg^{-1}TL). It was the highest in surimi (209 g kg^{-1}TL) (Table 1). In the washing water sampled at the end of the process, phospholipids represented 12 % of total lipids.

A wide variety of fatty acids were identified in total lipids from minced fillets and surimi (Table 2). Proportion of saturated fatty acid were similar in minced fillets (379 g kg^{-1} total FAMEs) and in surimi (375 g kg^{-1} total FAMEs); C16:0 was the major saturated fatty acid. Monounsaturated fatty acids C16:1 and C18:1 were prominent in minced fillets (401 g kg^{-1}

total FAMEs) and significantly less weighty in surimi (286 g kg^{-1} total FAMEs) whereas polyunsaturated fatty acids (PUFAs), mainly represented by eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3), were significantly more abundant in surimi (338 g kg^{-1} total FAMEs) than in minced fillets (220 g kg^{-1} total FAMEs) (Table 2).

Concentrations in lipid oxidation products were expressed on different bases. Results expressed per kg lipids give information about the degree of lipid oxidation (Table 3). Concentrations in primary (hydroperoxides) and secondary (TBARS) products of lipid oxidation in lipid fraction were low in raw material and increased in transformed and intermediary products (Table 3) showing that lipid oxidation took place during processing. After the first washing-dewatering stage, TBARS level in the washed mince reached more than 40 mg kg^{-1} TL whereas in mince it was only 2.7 mg kg^{-1} TL (Table 3). Hydroperoxides and TBARS reached the highest levels in refined mince (29.6 mmoles CuOOH Eq kg^{-1} TL and 235 mg MDA Eq kg^{-1} TL) and then decreased in paste (Table 3). In parallel, very high concentration of lipid oxidation products were measured in the washing water rejected during the last stage of dewatering (76.9 mmoles CuOOH Eq kg^{-1} TL and 479 mg MDA Eq kg^{-1} TL) (Table 3). Concentrations of hydroperoxides and TBARS in the lipid fraction were also significantly higher in surimi than in paste.

Samples taken during surimi manufacturing were very different in terms of dry matter and lipid contents. Thus, express lipid oxidation amounts per dry sample provide information on total amounts of lipid oxidation products in the samples and on the overall quality of these samples. The highest contents in lipid oxidation products were measured in the rejected washing water (Figure 2). It was also noticeable that concentrations of lipid oxidation products, hydroperoxides and TBARS, were higher in minced fillets than in mince (Figure 2).

In spite of the small content in lipid and high content in water of rejected washing water, the concentrations of TBARS in the samples sharply increased in the order: washed mince <

refined mince < washing water, and then decreased in the paste (Figure 2). As TBARS, hydroperoxide concentration decreased between refined mince and paste (Figure 2). Hydroperoxide concentration was similar in paste and surimi but TBARS content was significantly higher in surimi than in paste.

DISCUSSION

Most of the lipids were removed and phospholipid fraction, rich in polyunsaturated fatty acids, was less easily removed than neutral lipids during processing

Major part of lipids present in fillets was removed during processing. Most of lipid losses occurred during the washing stages (Table 1). Indeed, lipid losses during surimi manufacturing could not really be calculated as soluble proteins were also lost in the washing water and mass balance of constituents could not be calculated. Difference in lipid contents of minced fillets and mince was attributed to hand and mechanical skinning procedures. In hand skinned fillets only the epidermal coat was removed. In contrast, to obtain the mince, the skin was mechanically removed and the main part of the subcutaneous fat as well as a part of lipid-rich red muscle were withdrawn. Subcutaneous fat is rich in triglycerides. This explains the lower phospholipid proportion in total lipid extract of minced fillets than in total lipids of mince. The higher phospholipid content in surimi than in mince could be explained by the fact that membrane polar lipids such as phospholipids, interact with proteins and consequently are less easily removed than neutral lipids during processing. Similar results were obtained during manufacturing of sardines (*Sardina pilchardus*) surimi[21]. However, when muscle fibers were highly damaged, significant proportions of phospholipids were extracted in the washing water, probably together with membrane proteins (Table 1). Higher levels of polyunsaturated fatty acids were also found in surimi than in minced fillets (Table 2). This increase was due to higher proportions of eicosatetraenoic acid (C20:4ω3) and docosahexaenoic acid (C22:6ω3)

when other PUFAs such as eicosapentaenoic acid (C20:5ω3) remained steady (Table 2). The higher proportion of docosahexaenoic acid in surimi can be attributed to the easiest elimination, during surimi processing, of the neutral lipids as compared to the polar lipids, richer in docosahexaenoic acid. Indeed, we have found, in accordance with Bandarra *et al*[22] and Passi *et al*[23], that phospholipid fraction of horse mackerel muscle contains more polyunsaturated fatty acids and less monounsaturated fatty acids than neutral lipids (results not shown). Due to their interactions with proteins, phospholipids were less easily removed during processing than neutral lipids, and proportions of polyunsaturated fatty acids, such as DHA, increased in surimi.

Skinning can be critical for the quality of the end-product

In the raw material, higher amounts of hydroperoxides and TBARS (Figure 2) and higher concentration of TBARS expressed per kg lipids (Table 3) were found in the minced fillets than in mince. This can be explained by the skinning process: compared to the mince preparation with the skinning and deboning machine, dark muscle and subcutaneous fat were not removed during hand-skinning. Therefore, lipid content was higher in hand-minced fillets, and the abundance of hemoproteins and free metals in the dark muscle and under the skin layer would favor and promote lipid oxidation[24] in the minced fillets. Accordingly, after catch, fatty fish muscle is very prone to lipid oxidation and good storage conditions including storage in ice and the shortest duration of storage are required to maintain fish quality. For instance, significant increase in amounts of lipid oxidation products was detected in the fillets of horse mackerel as soon as after 6 hours at 17 °C and 36 hours in ice during storage of whole fish[25]. In such a sensitive material, the presence of more or less lipids and pro-oxidant substances will be crucial for development of lipid oxidation. As good quality of the raw material and the lowest initial level of oxidation are key conditions to control concentration of

lipid oxidation products in surimi[25, 26], the present results evidence the interest to control the settings of the skinning and deboning conditions. In one way, the machine should be set up so that most of the dark muscle and subcutaneous fat are removed. In the other way, it should be adjusted to minimize losses and insure maximum final yield and economically effectiveness of the whole process. As it is crucial to minimize oxidation reactions during processing, in order to insure the quality of the final product, only careful and precise adjustment of the skinning and deboning conditions is susceptible to satisfy these opposite conditions.

Lipid oxidation initiated during washing developed during refining

During surimi processing, concentrations of primary and secondary products of lipid oxidation in the lipid phase increased, showing that lipid oxidation took place (Table 3). The increase was more pronounced for TBARS than hydroperoxides. Lipid hydroperoxides, especially long-chain polyunsaturated fatty acid hydroperoxydes, are intermediary compounds quickly decomposed into secondary compounds that were evaluated by TBARS determination. In the course of fatty fish processing, amount of secondary compounds, which accumulate, increased while hydroperoxides, which were broken down, remained at a fairly constant level.

Depending on expression mode (mg kg^{-1} TL or mg kg^{-1} dry weight) TBARS was fifteen to twenty fold higher in washed mince than in mince (Table 3, Figure 2). During the washing stage, the minced fish muscle was thoroughly mixed with water favoring contact between lipids, pro-oxidant substances such as heme proteins present in blood, and oxygen[10]. Therefore, lipid oxidation was strongly favoured during the washing stages. Then, the reaction propagated during refining, and the hydroperoxyde and TBARS contents of the total lipid fraction were very high in refined mince. The lipid fraction of washing water also contained very high amounts of lipid oxidation products whereas the lipid fraction of resulting

paste had remarkably lower content of lipid oxidation products than refined mince. These results indicate that the last dewatering stage removed a major part of the primary and secondary products of lipid oxidation that were present in the refined mince. This result should be highlighted as it indicates that significant proportions of lipid oxidation products can be swept along by the washing water. The last washing stage made possible to limit the concentration of lipid oxidation products in paste and surimi. As a consequence, even if the level of lipid oxidation was not negligible in surimi (Table 3), as the product contained very small amounts of lipids, total amounts of secondary oxidation products (expressed per kg of surimi) were equivalent to the amounts measured in the minced fillets (Figure 2).

Both TBARS and hydroperoxides were higher in surimi than in paste (Table 3). This increase can be attributed to a technical problem, which occurred when the cryoprotectants were incorporated to the paste: the process was performed without vacuum and without refrigeration, which favored lipid oxidation. Indeed, the control of this processing stage is also required to insure the quality of the product. The pilot line has already been optimized to limit the development of lipid oxidation reactions at this stage of the process.

CONCLUSION

This paper shows that, initiated during fish storage and during the first stages of surimi manufacturing, lipid oxidation proceeds with processing while lipid content decreases. Phospholipids, rich in omega-3 fatty acids such as docosahexaenoic acid (DHA, C22:6ω3) are less removed than neutral lipids and polyunsaturated fatty acids proportions in lipid fraction are higher in surimi than in raw material.

Even though most of pro-oxidant substances were presumably removed during skinning and washing stages, lipid oxidation developed during these stages that should be carefully controlled. Then, dewatering enables to prepare the concentrate of fish myofibrillar protein

and to remove significant proportions of the lipid oxidation products generated during previous stages.

To limit oxidation reactions during surimi manufacturing, assays should be carried to better evaluate the influence of several processing factors such as temperature, number of washing cycles, protection against light or ambient air. The pilot line will be optimized according to the relevant factors. In addition, antioxidants could be added. However, the nature of the antioxidants and the way of addition should be studied.

ACKNOWLEDGEMENTS

This work was supported by a grant from the "Ministère de la Culture, de l'Enseignement Supérieur et de la Recherche" of the Grand-Duché du Luxembourg (Sylvie Eymard's doctoral fellowship). Authors acknowledge Euro Seafood Trading Society for its technical and financing support.

REFERENCES

1 Shimizu Y, Toyohara H and Lanier TC, Surimi production from fatty and dark-fleshed fish species, in *Surimi Technology*, Ed by Lanier TC and Lee CM, Marcel Decker Inc, New York, US, pp 181-207 (1992).

2 Hultin HO and Kelleher SD, Surimi processing from dark muscle fish, in *Surimi and surimi seafood*, Ed by Park JW, Marcel Decker Inc, New York, US, pp 59-77 (2000).

3 Ackman R, Fatty acids, in *Marine biogenic lipids, fats and oils*, Vol 1, Ed by Ackman R, CRC Press, Boca Raton, FL, pp 103-137 (1989).

4 Hultin HO, Oxidation of lipids in seafoods, in *Seafoods: Chemistry, Processing, Technology and Quality*, Ed by Shahidi F and Botta JR, Blackie Academic and

Professional Inc, Glasgow, UK, pp 49-74 (1994).

5 Richards MP and Hultin HO, Contributions of blood components to lipid oxidation in fish muscle. *J Agric Food Chem* **50 :**555-564 (2002).

6 Jacobsen C, Sensory impact of lipid oxidation in a complex food systems. *Fett/Lipid* **101:**484-492 (1999).

7 Hsieh RJ and Kinsella JE, Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. *Adv Food Nutr Res* **33:**233-341 (1989).

8 Pokorny J, Interactions of oxidized lipids with proteins *Riv Ital Sostanze Grasse* **4 :**389-393 (1977).

9 Saeed S and Howell NK, Effect of lipid oxidation and frozen storage on muscle protein of Atlantic mackerel *(Scomber scombrus). J Sci Food Agric* **82:**579-586 (2002).

10 Huang CH, Hultin HO and Jafar SS, Some aspects of Fe^{2+}-catalysed oxidation in fish sarcoplasmic reticular lipid. *J Agric Food Chem* **41:**1886-1892 (1993).

11 Folch J, Lees M and Sloane Stanley GHS, A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226:**497-509 (1957).

12 Stewart JC, Colorimetric determination of phospholipids with ammonium ferrothiocyanate. *Anal Biochem* **104:**10-14 (1980).

13 Christie WW, Preparation of ester derivatives of fatty acids for chromatographic analysis, in *Advances in lipid methodology*, Ed by Christie WW, Oily Press Inc, Dundy, UK, Vol **2** pp 69-111 (1993).

14 Eymard S and Genot C, A modified xylenol orange method to evaluate formation of lipid

hydroperoxides during storage and processing of small pelagic fish. *Eur J Lipid Sci Technol* **105:**497-501 (2003).

15 Salih AM, Smith DM, Price JF and Dawson LE, Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poult Sci* **66:**1483-1488 (1987).

16 Bostoglou NA, Fletouris DJ, Papageorgiou GE, Vassilopoulos VN, Mantis AJ and Trakatellis AG, Rapid sensitive and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food and feedstuff samples. *J Agric Food Chem* **42:**1931-1937 (1994).

17 Genot C, Some factors influencing TBA test, *Annual report of the Vth PCRD EU project : Dietary treatment and oxidative stability of muscle and meat products : nutritive value, sensory quality and safety (Diet-ox),* AIR III-CT-92-1577 15 pp (1996).

18 Buedge JA and Aust SD, Microsomal lipid peroxidation, in *Biomembranes (Part C : Biological Oxidation) Methods in Enzymology,* Ed by Fleisher SF and Packer L, London Academic Press, London, UK, **52:**302-309 (1978).

19 Sokal RR and Rohlf FJ, *Biometry*, W.H. Freeman and Company, New York, US, 887 pp (1995).

20 Pinheiro JC and Bates DM, *Mixed-effects models in S and S-PLUS. Statistics and computing*, Springer New York, US, 528 pp (2000).

21 Nunes ML, Cardinal M, Mendes R, Campos RM, Bandarra NM, Lourenço H and Jerome M, Effect of season and storage on proteins and lipids of sardine (*Sardina pilchardus*) minces and surimi, in *Quality Assurance in the Fish Industry,* Ed by Huss HH, Jakobsen M and Liston J, Elsevier Science Publishers B.V, Amsterdam, NL, pp 73-

79 (1992).

22 Bandarra NM, Batista I, Nunes ML and Empis JM, Seasonal variation in the chemical composition of horse-mackerel *(Trachurus trachurus). Eur Food Res Technol* **212:**535-539 (2001).

23 Passi S, Cataudella S, Di Marco P, De Simone F and Rastrelli L, Fatty acid composition and antioxidant levels in muscle tissue of different Mediterranean marine species of fish and shellfish. *J Agric Food Chem* **50:**7314-7322 (2002).

24 Undeland I, Stading M and Lingnert H, Influence of skinning on lipid oxidation in different horizontal layers of herring (*Clupea harengus*) during frozen storage. *J Sci Food Agric* **78:**441-450 (1998).

25 Eymard S, Mise en évidence et suivi de l'oxydation des lipides au cours de la conservation et de la transformation du chinchard (*Trachurus trachurus*) : choix des procédés. Thèse de l'Université de Nantes, France, N°ED 0367-089, 143 p, (2003).

26 Genot C, Eymard S, Viau M, Comment protéger les acides gras polyinsaturés à longues chaînes oméga 3 (AGPI-LC ω3) vis à vis de l'oxydation ? OCL, **11**, 2 : 133-141.

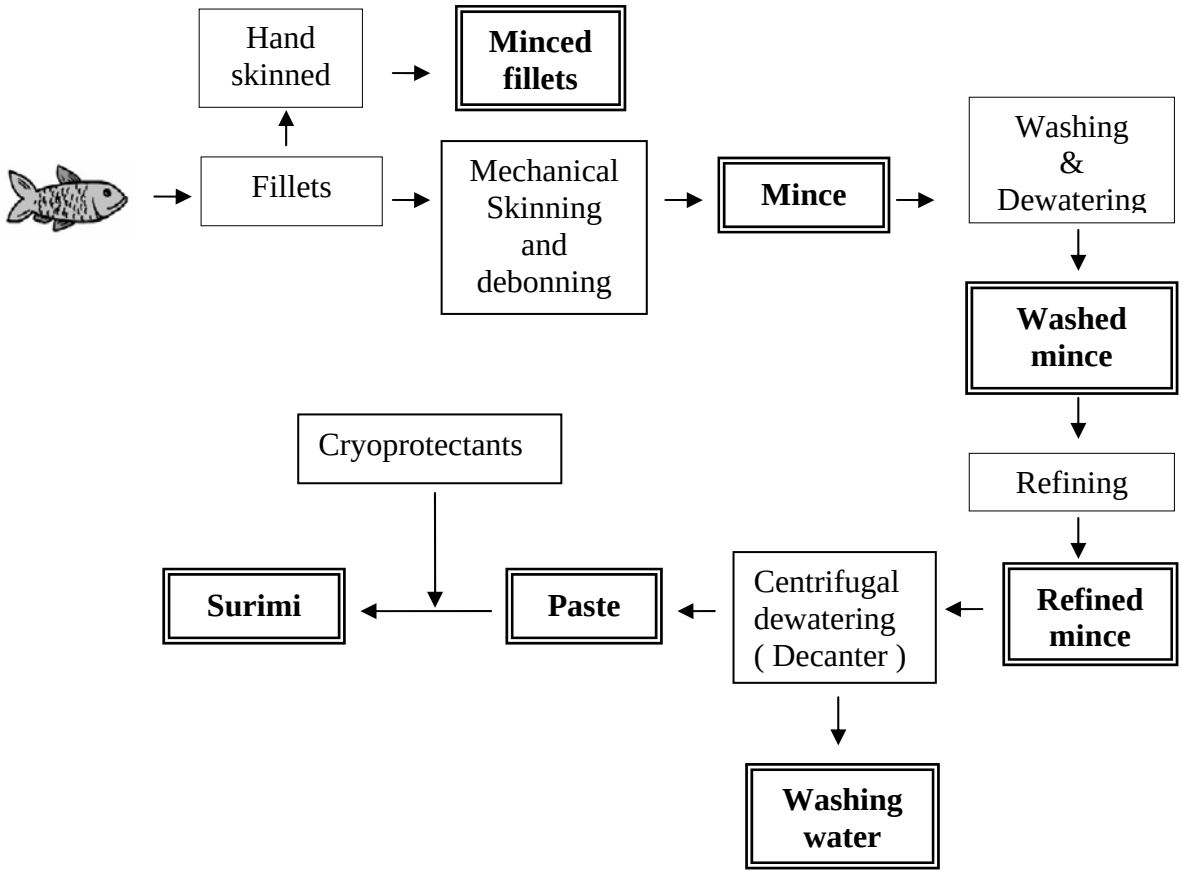

Figure 1. Schematic representation of the pilot production line for manufacturing of horse mackerel surimi. Samples were presented in double-framed boxes.

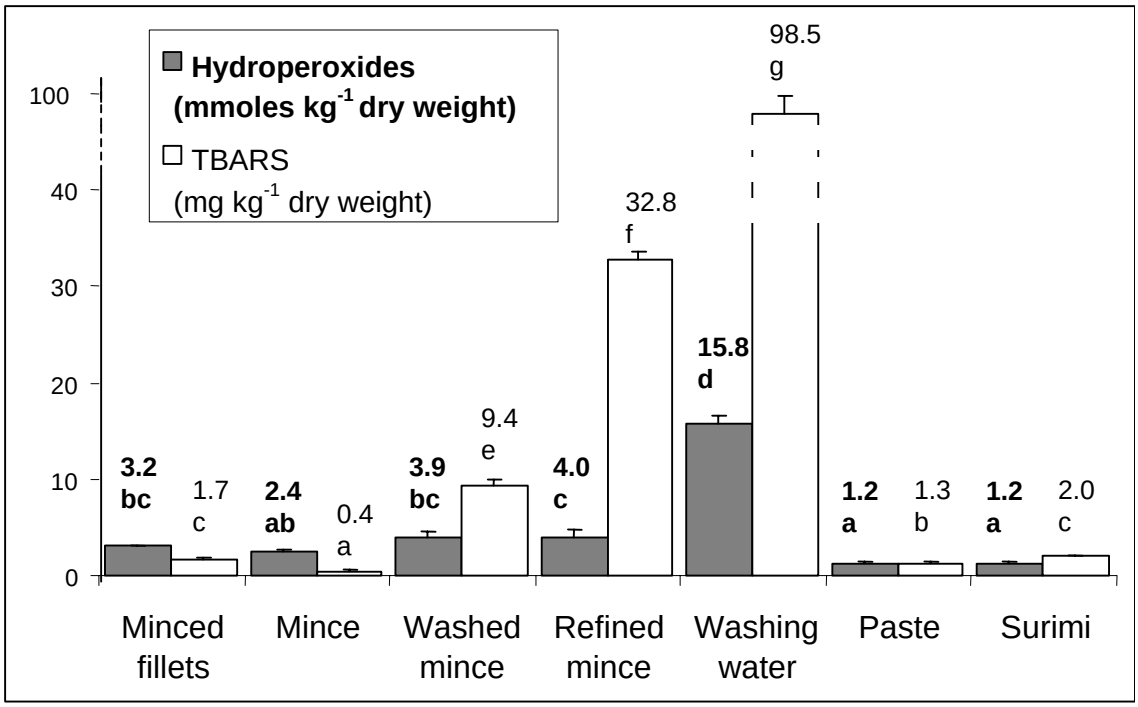

Figure 2. Evolution of concentrations in hydroperoxides and TBARS expressed per kg of dry weight, for samples taken during processing of horse mackerel surimi. Error bars correspond to standard deviations. For the same analysis, hydroperoxide concentration (n=3) or TBARS content (n=5), different letters indicate significantly different results ($P < 0.05$).

Table 1. Lipid, water and phospholipid contents (mean +/- standard deviation) of the samples taken during surimi manufacturing (n=3). Different letters in the same column indicate significantly different results ($P < 0.05$).

	Water content		Total lipid content				Phospholipid content	
	g kg^{-1}		g kg^{-1} wet weight		g kg^{-1} dry weight		g Eq PC kg^{-1} TL	
Minced fillets	763 +/- 1	a	49.1 +/- 0.2	c	205 +/- 1	c	113 +/- 2	c
Mince	772 +/- 1	b	36.0 +/- 1.6	b	158 +/- 7	cd	140 +/- 3	d
Washed mince	949 +/- 7	d	11.9 +/- 3.3	a	233 +/- 64	cd	134 +/- 10	bd
Refined mince	954 +/- 3	d	6.4 +/- 0.3	a	138 +/- 7	bd	111 +/-12	bc
Washing water	*982 +/- 1*	*e*	*3.7 +/- 1.8*	*a*	*204 +/- 102*	*abcd*	*121 +/- 42*	*bcd*
Paste	818 +/- 1	c	12.1 +/- 0.4	a	66 +/- 2	ba	192 +/- 18	a
Surimi	768 +/- 1	ab	10.0 +/- 0.6	a	44 +/- 2	a	209 +/- 11	a

Table 2. Fatty acid composition (g kg^{-1} total FAMEs) of lipid extracts from horse mackerel minced fillets and surimi (n=3). On the same line, stars indicate significantly different results at (*) 5 %, (**) 1 % and (***) 0.1 % determined by Student test.

Fatty acids (g kg^{-1})	**Minced fillets**	**Surimi**	
C14:0	55 +/- 2	60 +/- 8	
C16:0	250 +/- 4	241 +/- 12	
C18:0	74 +/- 3	74 +/- 1	
Σ Saturated	**379 +/- 3**	**375 +/- 8**	
C16:1	78 +/- 1	51 +/- 4.6	**
C18:1	323 +/- 3	235 +/- 13	**
Σ Monounsaturated	**401 +/- 4**	**286 +/-11**	***
C18:2 ω6	8 +/- 1	10 +/- 1	
C18:3 ω3	5 +/- 1	7 +/- 2	
C18:4 ω3	32 +/- 5	57 +/-10	
C20:3 ω3	6 +/- 1	9 +/- 1	*
C20:4 ω3	7 +/- 1	48 +/- 5	**
C20:5 ω3	59 +/- 2	63 +/- 2	
C22:5 ω3	12 +/- 1	13 +/- 1	
C22:6 ω3	91 +/- 1	131 +/- 11	*
Σ Polyunsaturated	**220 +/- 8**	**338 +/-19**	**

(means +/- standard deviations)

Table 3. Development of lipid oxidation during manufacturing of horse mackerel surimi. Hydroperoxide concentrations were expressed in mmoles of cumen hydroperoxide equivalent per kg of total lipids (n=3) and TBARS contents in mg MDA equivalent per kg of total lipids (n=5). Different letters in a same column indicate significantly different results ($P < 0.05$).

	Hydroperoxide concentration mmoles kg^{-1} TL	TBARS content mg kg^{-1} TL
Minced fillets	15.3 +/- 2.1 a	8.4 +/- 1.1 b
Mince	15.3 +/-2.8 a	2.7 +/-1.0 a
Washed mince	16.6 +/- 2.8 a	40.4 +/- 2.3 d
Refined mince	29.6 +/- 2.8 b	235 +/- 7 f
Washing water	*76.9 +/- 4.7 d*	*479 +/- 8 g*
Paste	17.1 +/- 4.5 a	19.5 +/- 1.7 c
Surimi	27.3 +/- 3.8 b	44.2 +/- 0.8 e

(means +/- standard deviations)