1

2

3 # EFFECT OF PRE-SOAKING WHOLE PELAGIC FISH IN

4 # A PLANT EXTRACT ON SENSORY AND BIOCHEMICAL

5 # CHANGES DURING SUBSEQUENT FROZEN STORAGE

6

7

8

9 **Lugasi, A. [a], Losada, V. [b], Hóvári, J. [a], Lebovics, V. [a], Jakóczi, I. [c] and**

10 **Aubourg, S. [b,*]**

11

12

13

14

15

16 [a] National Institute of Food Hygiene and Nutrition (Budapest, Hungary)

17 [b] Instituto de Investigaciones Marinas (CSIC) (Vigo, Spain)

18 [c] FitoChem Kft (Monor, Hungary)

19

20 [*] Correspondence: c/ E. Cabello, 6. 36208-Vigo (Spain); Fax: +34986292762; e-mail:

21 saubourg@iim.csic.es

22

23

ABSTRACT

Plant extract treatments have largely shown a positive effect on inhibiting the quality loss during the frozen storage of minced and filleted fish products. In the present case, the effect of a plant extract on a whole fish product was checked. For it, whole fresh horse mackerel was soaked in a commercial extract solution during 60 minutes and then kept frozen up to 12 months at –20ºC. Sampling was carried out on the initial material and at months 1, 3, 5, 7, 9 and 12. Two parallel experiments consisting on untreated fish (Blank Control) and water treated fish (Water Control) were carried out in the same conditions. Lipid damage was measured by lipolisis development (free fatty acid formation), rancidity development (conjugated dienes, secondary oxidation compounds, fluorescent compounds and cholesterol oxides) and sensory (odour, firmness and colour) analyses. As a result of the previous plant extract treatment, better odour and colour scores were obtained that led to a larger shelf-life time (7 months) than in the two controls (5 months), according to the sensory analysis. Water treatment of fish (Water Control) also showed some better results in sensory (odour and colour) analysis than the Blank Control, that could be related to the elimination of some prooxidant molecules included in fish. Some biochemical indices (conjugated dienes and free fatty acids) also provided a damage inhibition ($p<0.05$) in the 9-12 months period as a result of the plant treatment and water treatment; however, fluorescence and cholesterol oxide detections did not show differences ($p>0.05$) when compared to the Blank Control. The present experiment provides promising results for soaking a pelagic whole fish in an aqueous plant extract as a previous step to its commercialisation as a frozen product.

Running Title: Plant extract and frozen whole fish

Keywords: Horse mackerel, frozen storage, whole fish, plant extract, rancidity

1

1. INTRODUCTION

2

3 Freezing and frozen storage have largely been employed to retain fish sensory
4 and nutritional properties (Pigott & Tucker, 1987; Erickson, 1997). However, marine
5 species have shown a highly unsaturated lipid composition (Piclet, 1987) and an
6 important presence of prooxidant molecules that lead to rancidity development and
7 quality loss (Harris & Tall 1994; Richards & Hultin 2002). In frozen conditions, lipid
8 oxidation compounds have shown to facilitate protein denaturation (Mackie, 1993;
9 Sikorski & Kolakowska, 1994), nutritional losses (Castrillón, Álvarez-Pontes, García,
10 M. & Navarro, 1996), modification of electrophoretic profiles of proteins (Saeed &
11 Howell, 2002), loss of endogenous antioxidant systems (Undeland & Lingnert, 1999)
12 and fluorescent compound development (Davies & Reece, 1982).
13 Many efforts are being carried out by fish traders and food technologists in being
14 able to store and commercialise frozen fish products in a safely and high quality state.
15 Recent research has been focused on the positive role of antioxidant molecules present
16 in plants (Yanishlieva & Marinova, 1996; Miyake & Shibamoto, 1997). Thus,
17 successful applications of plant extract treatments have been carried out on frozen
18 minced fish (Ramanathan & Das, 1992; Kelleher, Silva, Hultin & Wilhelm, 1992) and
19 fish fillets (Vareltzis, Koufidis, Graviilidou, Papavergou & Vasiliadou, 1997; Saeed &
20 Howell, 2002). However, research focused on whole fish species is scarce (Aubourg,
21 Pérez-Alonso & Gallardo, 2004a) and basically concerned to maintain the colour
22 stability of rockfish species, such as *Sebastolobus alascanus* (Wasson, Reppond &
23 Kandianos, 1991), *Sebastes ruberriumus* and *Sebastes alutus* (Li, Seymour, King &
24 Morrisey, 1998).

1 One such plant extract employed with antioxidant purposes is Rosmol-P. It is a
2 permitted ingredient composed from polyphenol compounds and rosmarinic acid that
3 has been reported to retard lipid oxidation in meat (Lugasi, Blázovics, Hagymási &
4 Jakóczi, 2000) and fish fillet (Aubourg, Lugasi, Hóvári, Piñeiro, Lebovics & Jakóczi,
5 2004b) products when employed as a pre-treatment of a further frozen storage.
6 The present work concerns frozen horse mackerel trading as a whole fish
7 product. This species has recently attracted a great attention as being considered an
8 underutilised fish species showing large captures in the latest years (FAO, 2004). In this
9 experiment, fish is soaked in an aqueous solution of Rosmol-P to enlarge its lipid
10 stability during a subsequent frozen (-20ºC) storage and accordingly, its quality and
11 consumer acceptance. Lipid damage is monitored up to 12 months of storage by sensory
12 and biochemical analyses.
13
14
15 ## 2. MATERIALS AND METHODS
16
17 ### 2.1. Plant extract preparation
18 Rosmol-P plant extract was obtained from FitoChem Kft (Monor, Hungary). To
19 create it, a 100 g mixture of dry hyssop (*Hysoppus officinalis*), brunella (*Prunella*
20 *vulgaris*), lemon balm (*Melissa officinalis*) and rosemary (*Rosmarinus officinalis*) was
21 percolated with 30% hydro-ethanolic solution to give 500 ml of brown percolate. The
22 solution was mixed with 2 g of activated coal, refrigerated and filtered. An excess of
23 maltodextrin was added to the ethanolic plant extract and the mixture was spray dried to
24 get 30 g antioxidant powder. Total polyphenol and rosmarinic acid contents were 6600
25 mg/100 g and 2100 mg/100 g Rosmol-P powder, respectively (Lugasi et al., 2000).

1 Concentration of Rosmol-P employed in the present experiment was chosen according
2 to previous research (Lugasi et al., 2000; Aubourg et al., 2004b).
3
4 **2.2. Raw fish, sampling and processing**
5 Horse mackerel (*Trachurus trachurus*) (114 individual fishes) were captured in
6 November 2003 and kept on ice till arrival to the laboratory (8 hours). Part of the fish
7 (36 individual fishes) was directly packaged in individual polyethylene bags (Blank
8 Control treatment) and immediately frozen at –80ºC. The remaining fish was immersed
9 either in water (Water Control treatment) (36 individual fishes) or in a 0.664g/ 100 ml
10 aq. Rosmol-P solution (RP treatment) (36 individual fishes) in an isothermal room at
11 4ºC for 60 min; then, the fish were removed, packaged in individual polyethylene bags
12 and frozen at –80ºC. After 3 days at –80ºC, all fish were placed at –20ºC. Sampling was
13 undertaken on the initial material (6 individual fishes) and at months 1, 3, 5, 7, 9 and 12
14 of frozen storage at –20ºC of each kind of treatment. In each case, three different
15 batches (n=3) were considered and studied separately along the experiment. Two
16 individual fishes were taken out from each treatment and batch at each storage point.
17 Analysis of frozen material was undertaken after thawing; thawing was carried out by
18 overnight storage in a cool room (4ºC).
19
20 **2.3. Sensory analysis**
21 Sensory analyses were conducted by a taste panel consisting of five experienced
22 judges in fish sensory quality assessment, according to the guidelines presented in Table
23 1 (Council Regulations, 1989). Four categories were ranked: highest quality (E), good
24 quality (A), fair quality (B) and rejectable quality (C). Sensory assessment included the
25 following parameters: external odour, firmness and external colour.

1 Rancid odour and yellowish colour were chosen as being directly related to
2 rancidity development. Sour odour was also determined on the basis of possible
3 autolysis development and enzyme presence in fish that could have been produced by
4 microorganisms before the freezing step. Firmness loss was evaluated according to
5 protein changes produced during frozen storage, specially as a result of interaction with
6 lipid oxidation compounds.
7 At each sampling time, fish were thawed and analysed in the same session. The
8 panel members shared samples tested. The fish were served to the panel members in the
9 individual polyethylene bags where they had been kept frozen. Once the fish were
10 subjected to sensory analysis, the white muscle was separated and homogenised for
11 carrying out the biochemical analyses.
12
13 **2.4. Composition analysis**
14 Water content was determined by weight difference between the homogenised
15 white muscle (1-2g) and after 24 hr at 105ºC. Results were calculated as g water/100 g
16 white muscle.
17 Lipids were extracted by the Folch, Lees and Stanley (1957) method. Results
18 were calculated as g total lipids/100 g white muscle.
19 Protein content was measured by the Kjeldahl method (AOAC, 1984a),
20 employing the 6.25 conversion factor. Results were calculated as g total protein/100 g
21 white muscle.
22
23 **2.5. Lipid damage measurements**
24 Free fatty acids (FFA) content was determined according to Lowry and Tinsley
25 (1976). The method is based on a complex formation between the acid group of FFA

and cupric acetate in the presence of pyridine at pH = 6.1; the resulting chromophore is read at 710 nm. Results are expressed as mg oleic acid/ 100g white muscle.

Conjugated diene (CD) formation was assessed according to the AOAC (1984b) method. For it, 1g fish muscle was homogenised with 10 ml iso-octane and the filtrate measured at 233 nm. Results are expressed according to the formula: CD = B x V / w, where B is the absorbance reading at 233 nm, V denotes the volume (ml) of iso-octane employed and w is the mass (g) of the white muscle employed.

The thiobarbituric acid index (TBA-i) (mg malondialdehyde/kg white muscle) was determined according to Ramanathan and Das (1992). The method is based on reaction between a trichloracetic acid extract of the fish muscle and thiobarbituric acid at high temperature (95-97ºC); the resulting chromophore is read at 532 nm.

Fluorescence formation (Perkin-Elmer LS 3B) at 327/415 nm and 393/463 nm was studied as described elsewhere (Aubourg, Sotelo & Pérez-Martín, 1998) on the aqueous phase resulting from the lipid fraction extraction. Fluorescence measurements of samples were normalised with quinine sulphate measurements, so that relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission pair, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 μg/ml in 0.05 M H_2SO_4) at the corresponding wavelength. Results are expressed as the fluorescence ratio (FR) obtained, according to the following calculation: $FR = RF_{393/463nm} / RF_{327/415nm}$.

2.6. Cholesterol oxide analysis

Cholesterol oxide standards (1-cholestan-3β, 5α, 6β-triol-hydroxycholesterol; 7α-cholesterol; 7β-cholesterol; 7-keto-cholesterol; cholesterol-5α, 6α-epoxide; 25-

1 hydroxycholesterol; 20α-hydroxycholesterol) and cholesterol were purchased from
2 Sigma (St Louis, MO,USA).

3 Saponification of total lipids and isolation of cholesterol and cholesterol
4 oxidation products from the non-saponifiable fraction were carried out according to
5 Missler, Wasilchuk and Merrit (1985). The separation of cholesterol and the individual
6 cholesterol oxidation compounds was performed by thin layer chromatography (TLC)
7 as described previously (Lebovics, Gaàl, Somogyi & Farkas, 1992). Detection and
8 quantification (mg/ kg white muscle) was carried out by enzymatic (cholesterol oxidase)
9 assay combined to the TLC analysis (Lebovics, Antal & Gaàl, 1996).

10

11 **2.7. Statistical analysis**

12 According to the sampling procedure, data from the different biochemical
13 measurements were subjected to the one-way ANOVA method ($p<0.05$); comparison of
14 means was performed using a least-squares difference (LSD) method (Statsoft, 1994).
15 Linear and non-linear (exponential and logarithmic) correlation analyses and Spearman
16 test for nonparametric correlations were performed (Statsoft, 1994).

17

18

19 **3. RESULTS AND DISCUSSION**

20

21 **3.1. Composition analysis**

22 Lipid contents of white muscle ranged in all cases in the present experiment
23 between 5.0 and 9.0 g/ 100g white muscle, showing relatively large differences that can
24 be explained as a result of fish-to-fish variation and not arising from storage time or

treatment. The lipid content range is as expected for a medium-fat fish that is captured in the period of the highest lipid content (Bandarra, Batista, Nunes & Empis, 2001).

Water contents of the different fish samples were in the range 74-77 g/ 100g white muscle. This range is due to variations in individual fish and not arising from antioxidant treatment or frozen storage time. Present results were lower than for leaner fish species (blue whiting, cod and haddock) (Aubourg, 1999; Aubourg & Medina, 1999) and higher than for fattier fish species (sardine) (Aubourg et al., 1998), in accordance with an inverse ratio between water and lipid matter (Piclet, 1987).

Total protein content was included in the range 18-21 g/ 100g white muscle, according to referred data concerning protein-rich foods such as fish species (Piclet, 1987).

As for lipid matter, variations in water and protein contents are attributed to fish-to-fish variation and not to antioxidant treatment or frozen storage time.

3.2. Lipid hydrolysis

FFA content in the initial fish (0.19±0.07 mg/ 100g white muscle) was similar to that of fatty fish species (tuna, sardine) (Aubourg et al., 1998; Medina, Sacchi & Aubourg, 1995) and lower than that of lean fish species (blue whiting, haddock, cod) (Aubourg, 1999; Aubourg & Medina, 1999). A gradual increase in FFA content was observed in all treatments as a result of the frozen storage time (Figure 1); good linear and nonlinear correlation values with time were obtained in all cases ($r^2 = 0.90$ to $r^2 = 0.95$). A higher ($p<0.05$) FFA value for Blank Control was obtained at months 9 and 12 than in the two other treatments. No differences ($p>0.05$) were observed between Water Control and RP treatments.

The formation of FFA itself does not lead to nutritional losses. However, examining the extent of lipid hydrolysis was deemed important to the study because of the high lipid hydrolysis development previously observed in horse mackerel during frozen storage (Simeonidou, Govaris & Vareltzis, 1997; Aubourg, Piñerio & González, 2004c) and also because of the reported influence of lipid hydrolysis on lipid oxidation (Han & Liston, 1988; Aubourg, 2001).

3.3. Lipid oxidation

Primary oxidation compounds as measured by the CD formation (Figure 2) did not show a clear trend as a result of the frozen storage in the three treatments. However, the plant extract treatment led to lower ($p<0.05$) values when compared to Blank Control at months 3, 5, 9 and 12 and Water Control at months 3 and 5. A lower ($p<0.05$) CD formation was observed for Water Control in the 9-12 months period when compared to the Blank Control, although the opposite result was obtained at month 5.

Formation of thiobarbituric acid reactive substances did not show a continuous tendency during storage for any of the treatments studied (Figure 3), so that very irregular pattern distributions were obtained and a clear effect of the antioxidant treatment can not be inferred.

The fluorescence development provided no significant ($p>0.05$) changes in the 1-9 months period for each kind of sample (Figure 4) when compared to the starting fish material. At the end of the experiment, a FR increase ($p<0.05$) was observed in all cases, although no significant ($p>0.05$) differences could be observed among treatments. Exponential correlation values in the r^2 = 0.80-0.92 range were obtained with the storage time for the three treatments. Formation of fluorescent products as a result of interaction compounds between lipid oxidation compounds and nucleophilic molecules

1 (proteins, peptides, etc.) has been reported (Pokorný, 1981; Howell, 1995) to be
2 dependent on the formation of lipid oxidation products (peroxides and carbonyls). Thus,
3 in the present experiment fluorescent compounds developed only at the end of the
4 storage, according to the previous primary and secondary oxidation formation.
5 Cholesterol oxidation was studied during the experiment in the three sample
6 groups. According to the Materials and Methods section, the presence of a wide range
7 of cholesterol oxides was checked. Thin layer chromatography coupled to an enzymatic
8 assay showed no formation of cholesterol oxides as a result of the frozen storage in any
9 of the samples. Figure 5 shows the results obtained at month 12, where only cholesterol
10 could be detected in the three kinds of samples (lanes A, B and C) when compared to
11 the cholesterol oxide mixture (lane D).
12
13 **3.4. Sensory analysis**
14 Progressive score decreases were observed with time for the three attributes
15 considered (Table 2), so that very good correlation values (r^2 = 0.93 to r^2 = 0.97) were
16 obtained with the storage time for all the parameters in all the samples. When compared
17 to biochemical indices, sensory analysis showed the best correlation values with the
18 FFA content. Thus, fair values were obtained for FFA content when compared to odour,
19 firmness and colour (r^2 = 0.82, r^2 = 0.83 and r^2 = 0.83, respectively). FFA formation has
20 been reported to be strongly correlated to lack of acceptability (Refsgaard, Brockhoff &
21 Jensen, 2000), according to previous research where interaction with proteins leading to
22 toughening and enhancement of lipid oxidation development have been proposed
23 (Mackie, 1993; Aubourg, 2001).
24 According to sensory acceptance scores, RP treatment provided a shelf-life time
25 of 7 months, while both controls led to a corresponding 5 months time. In all cases, the

1 limiting factor was the rancid external odour. Comparison between both controls
2 showed a longer time of good quality (categories E and A) for Water Control when
3 considering the external odour and colour, being both attributes related to oxidation
4 development; no difference was obtained for the firmness attribute.
5
6
7

4. FINAL REMARKS

8
9 In the present research, a plant extract treatment was applied to whole fresh
10 horse mackerel prior to frozen storage. According to the sensory analysis on the frozen
11 product, quality loss inhibition was observed when compared to the untreated (Blank
12 Control) and water treated (Water Control) fish samples, so that a longer shelf-life was
13 obtained. Antioxidant treatment showed a bigger effect on the external part of the fish
14 body (sensory assessment) than in the white muscle of the fish (biochemical analyses).
15 Thus, biochemical analyses showed an inhibitory effect of antioxidant treatment when
16 compared to the untreated samples, although no differences were concluded by
17 comparison with the water treated fish. Larger differences in muscle quality may have
18 been found for the antioxidant activity if the entire edible part (white and dark muscles)
19 had been considered in the study.
20 The water treatment of fish (Water Control) also showed some advantages when
21 compared to Blank Control, since better results were obtained in the sensory (odour and
22 colour attributes) and biochemical (CD and FFA assessments) analyses. A lipid
23 oxidation inhibitory effect by a water treatment had already been observed and
24 explained in previous studies on fillet products (Undeland, Ekstrand & Lingnert, 1998;
25 Richards & Hultin, 2002) as a result of removal of hemoproteins and metal ions

included in the fish body, specially in blood. In the case of whole fish products, the washing effect did not lead to sensory differences when studying the chilled storage of horse mackerel (Inácio, Bernardo & Vaz-Pires, 2003).

Bibliography accounts for a large number of experiments where frozen pelagic fish species prepared as minced and fillet products (Kelleher et al., 1992; Vareltzis et al., 1997; Saeed & Howell, 2002; Aubourg et al., 2004b) are successfully treated with natural antioxidants leading to a marked inhibitory effect on quality loss. Although whole fish products have shown a larger shelf-life time than fillet products (Aubourg et al., 2004c), antioxidant addition to whole fish has been found less effective (Aubourg et al., 2004a), since direct contact of antioxidant molecules is more difficult than in the case of fillet fish product. Because of the great industrial interest on the frozen commercialisation of whole pelagic fish species, the present experiment provides promising results for fish soaking in an aqueous plant extract as a previous step.

Acknowledgments

The authors thank Mr. *Marcos Trigo* for technical assistance and the *Academy of Sciences* (Hungary)–*CSIC* (Spain) Program (Project 2003 HU 0003), the Hungarian Ministry of Education (NKFP 1/016/2001, "Széchenyi" Project and the *Comisión Interministerial de Ciencia y Tecnología* (CICyT; Spain) (Project ALI 99-0869) for financial support.

REFERENCES

AOAC (1984a). Official Methods of Analysis (24.027) (14th edition). Arlington, VA (USA).

AOAC (1984b). Official Methods of Analysis (28.054) (14th edition). Arlington, VA (USA).

Aubourg, S. & Medina, I. (1999). Influence of storage time and temperature on lipid deterioration during cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) frozen storage. *Journal of the Science of Food and Agriculture,* 79, 1943-1948.

Aubourg, S. (1999). Lipid damage detection during the frozen storage of an underutilized fish species. *Food Research International,* 32, 497-502.

Aubourg, S. (2001). Fluorescence study of the prooxidant activity of free fatty acids on marine lipids. *Journal of the Science of Food and Agriculture*, 81, 385-390.

Aubourg, S., Lugasi, A., Hóvári, J., Piñeiro, C., Lebovics, V. & Jakóczi, I. (2004b). Damage inhibition during frozen storage of horse mackerel (*Trachurus trachurus*) fillets by a previous plant extract treatment. *Journal of Food Science*, 69, 136-141.

Aubourg, S., Pérez-Alonso, F. & Gallardo, J. (2004a). Studies on rancidity inhibition in frozen horse mackerel (*Trachurus trachurus*) by citric and ascorbic acids. *European Journal of Lipid Science and Technology*, 106, 232-240.

Aubourg, S., Piñeiro, C. & González, Mª J. (2004c). Quality loss related to rancidity development during frozen storage of horse mackerel (*Trachurus trachurus*). *Journal of the American Oil Chemists' Society*, 81, 671-678.

1 Aubourg, S., Sotelo, C. & Pérez-Martín, R. (1998). Assessment of quality changes in
2 frozen sardine (*Sardina pilchardus*) by fluorescence detection. *Journal of the*
3 *American Oil Chemists' Society,* 75, 575-580.
4 Bandarra, N., Batista, I., Nunes, M. & Empis J. (2001). Seasonal variation in the
5 chemical composition of horse mackerel (*Trachurus trachurus*). *European*
6 *Journal of Lipid Science and Technology,* 212, 535-539.
7 Castrillón, A., Álvarez-Pontes, E., García, M. & Navarro P. (1996). Influence of frozen
8 storage and defrosting on the chemical and nutritional quality of sardine (*Clupea*
9 *pilchardus*). *Journal of the Science of Food and Agriculture,* 70, 29-34.
10 Council Regulations (1989). Baremo de Clasificación de Frescura. In *Diario Oficial de*
11 *las Comunidades Europeas*. European Commission. Brussels (Belgium), No. L
12 5/21 (07.01.1989), pp. 5-6.
13 Davies, H. & Reece, P. (1982). Fluorescence of fish muscle: Causes of change
14 occurring during frozen storage. *Journal of the Science of Food and Agriculture*,
15 33, 1143-1151.
16 Erickson, M. (1997). Lipid oxidation: Flavor and Nutritional Quality Deterioration in
17 Frozen Foods. In M. Erickson & Y.-C. Hung, Quality in Frozen Food (pp. 141-
18 173). New York (USA): Chapman & Hall.
19 FAO (2004). Fishery statistics. Capture production. *Food and Agriculture Organization*
20 *of the United Nations,* Rome (Italy). Yearbook 2002. Vol. 94/1, p. 256.
21 Folch, I., Lees, M. & Stanley, G. (1957). A simple method for the isolation and
22 purification of total lipids from animal tissue. *Journal of Biological Chemistry*,
23 726, 497-509.
24 Han, T. & Liston, J. (1988). Correlation between lipid peroxidation and phospholipid
25 hydrolysis in frozen fish muscle. *Journal of Food Science*, 53, 1917, 1919.

Harris, P. & Tall, J. (1994). Rancidity in fish. In J. Allen & R. Hamilton, Rancidity in foods (pp. 256-272). London (UK): Chapman and Hall.

Howell, N. (1995). Interaction of proteins with small molecules. In A. Gaonkar, Ingredient Interactions- Effects on Food Quality (pp. 269-289). New York (USA): Marcel Dekker.

Inácio, P., Bernardo, F. & Vaz-Pires, P. (2003). Effect of washing with tap and treated seawater on the quality of whole scad (*Trachurus trachurus*). *European Food Research Technology*, 217, 406-411.

Kelleher, S., Silva, L., Hultin, H. & Wilhelm, K. (1992). Inhibition of lipid oxidation during processing of washed, minced Atlantic mackerel. *Journal of Food Sci*ence, 57, 1103-1108.

Lebovics, V., Antal, M. & Gaàl, Ö. (1996). Enzymatic determination of cholesterol oxides. *Journal of the Science of Food and Agriculture*, 71, 22-26.

Lebovics, V., Gaàl, Ö., Somogyi, L. & Farkas, J. (1992). Cholesterol oxides in γ-irradiated spray-dried egg powder. *Journal of the Science of Food and Agriculture*, 60, 251-254.

Li, S., Seymour, T., King, A. & Morrisey, M. (1998). Color stability and lipid oxidation of rockfish as affected by antioxidant from shrimp shell waste. *Journal of Food Sci*ence, 63, 438-441.

Lowry, R. & Tinsley, I. (1976). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists' Society*, 53, 470-472.

Lugasi, A., Blázovics, A., Hagymási, K. & Jakóczi, I. (2000). Application of a natural antioxidant as food ingredient. In 10th Biennale Meeting of the International Society for Free Radical Research. Kyoto (Japan), October 16-20. Abstract book (p. 223).

1 Mackie, I. (1993). The effects of freezing on flesh proteins. *Food Reviews International,*
2 9, 575-610.
3 Medina, I., Sacchi, R. & Aubourg, S. (1995). A ^{13}C-NMR study of lipid alterations
4 during fish canning: Effect of filling medium. *Journal of the Science of Food*
5 *and Agriculture,* 69, 445-450 (1995).
6 Missler, S., Wasilchuk, B. & Merrit, C. (1985). Separation and identification of
7 cholesterol oxidation products in dried egg preparations. *Journal of Food*
8 *Science*, 50, 595-598.
9 Miyake, T. & Shibamoto, T. (1997). Antioxidative activities of natural compounds
10 found in plants. *Journal of the Agricultural Food Chemistry,* 45, 1819-1822.
11 Piclet, G. (1987). Le poisson aliment. Composition et intérêt nutritionnel. *Cahiers de*
12 *Nutrition et Diététique,* XXII, 317-335.
13 Pigott, G. & Tucker, B. (1987). Science opens new horizons for marine lipids in human
14 nutrition. *Food Reviews International*, 3, 105-138.
15 Pokorný, J. (1981). Browning from lipid-protein interactions. *Progress in Food*
16 *Nutrition and Science*, 5, 421-428.
17 Ramanathan, L. & Das, N. (1992). Studies on the control of lipid oxidation in ground
18 fish by some polyphenolic natural products. *Journal of the Agricultural and*
19 *Food Chemistry*, 40, 17-21.
20 Refsgaard, H., Brockhoff, P. & Jensen, B. (2000). Free polyunsaturated fatty acids
21 cause taste deterioration of salmon during frozen storage. *Journal of the*
22 *Agricultural and Food Chemistry*, 48, 3280-3285.
23 Richards, M. & Hultin, H. (2002). Contributions of blood and blood components to
24 lipid oxidation in fish muscle. *Journal of the Agricultural and Food Chemistry,*
25 50, 555-564.

1 Saeed, S. & Howell, N. (2002). Effect of lipid oxidation and frozen storage on muscle
2 proteins of Atlantic mackerel (*Scomber scombrus*). *Journal of the Science of*
3 *Food and Agriculture,* 82, 579-586.
4 Sikorski, Z. & Kolakowska, A. (1994). Changes in protein in frozen stored fish. In Z.
5 Sikorski, B. Sun Pan & F. Shahidi, Seafood proteins (pp. 99-112). New York
6 (USA): Chapman and Hall.
7 Simeonidou, S., Govaris, A., & Vareltzis, K. (1997). Effect of frozen storage on the
8 quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and
9 Mediterranean hake (*Merluccius mediterranean*). *Zeitschrift für Lebensmittel*
10 *Untersuchung und Forschung,* 204, 405-410.
11 Statsoft (1994). Statistica for Macintosh. Statsoft and its licensors. Tulsa, Ok (USA).
12 Undeland, I. & Lingnert, H. (1999). Lipid oxidation in fillets of herring (*Clupea*
13 *harengus*) during frozen storage. Influence of prefreezing storage. *Journal of the*
14 *Agricultural and Food Chemistry,* 47, 2075-2081.
15 Undeland, I., Ekstrand, B. & Lingnert, H. (1998). Lipid oxidation in minced herring
16 (*Clupea harengus*) during frozen storage. Effect of washing and precooking.
17 *Journal of Agricultural and Food Chemistry,* 46, 2319-2328.
18 Vareltzis, K., Koufidis, D., Graviilidou, E., Papavergou, E. & Vasiliadou, S. (1997).
19 Effectiveness of a natural Rosemary (*Rosmarinus officinalis*) extract on the
20 stability of filleted and minced fish during frozen storage. *Zeitschrift für*
21 *Lebensmittel-Untersuchung und-Forschung,* 205, 93-96.
22 Wasson, D., Reppond, K. & Kandianis, T. (1991). Antioxidants to preserve rockfish
23 color. *Journal of Food Science*, 56, 1564-1566.

1 Yanishlieva, N. & Marinova, E. (1996). Antioxidative effectiveness of some natural
2 antioxidants in sunflower oil. *Zeitschrift für Lebensmittel-Untersuchung und-*
3 *Forschung,* 203, 220-223.
4

1 **FIGURE LEGENDS**

2

3

4

5 **Figure 1**: Free fatty acid determination (mg oleic acid/ 100g white muscle)* during

6 frozen storage of horse mackerel that was pre-treated under different conditions**

7

8 * Mean values (n = 3) of three independent determinations. Standard deviations are

9 indicated in brackets.

10 ** Treatment names: Blank Control (untreated), Water Control (water treated)

11 and RP (Rosmol-P treated).

12

13

14 **Figure 2**: Conjugated diene formation (absorbance reading x volume (ml) iso-octane/

15 mass (g) white muscle)* during frozen storage of horse mackerel that was pre-treated

16 under different conditions**

17

18 * Mean values (n = 3) of three independent determinations. Standard deviations are

19 indicated in brackets.

** Treatment names: Blank Control (untreated), Water Control (water treated)

and RP (Rosmol-P treated).

20

21

22 **Figure 3**: Thiobarbituric acid index determination (mg malondialdehyde/ kg white

23 muscle)* during frozen storage of horse mackerel that was pre-treated under different

24 conditions**

25

1 * Mean values (n = 3) of three independent determinations. Standard deviations are
2 indicated in brackets.

** Treatment names: —◆— Blank Control (untreated), —■— Water Control (water treated) and —▲— RP (Rosmol-P treated).

3

4

5 **Figure 4**: Fluorescence formation assessment (fluorescence ratio)* during frozen

6 storage of horse mackerel that was pre-treated under different conditions**

7

8 * Mean values (n = 3) of three independent determinations. Standard deviations are
9 indicated in brackets.

** Treatment names: —◆— Blank Control (untreated), —■— Water Control (water treated) and —▲— RP (Rosmol-P treated).

10

11

12 **Figure 5**: Cholesterol oxide formation* after 12 months of frozen storage of horse

13 mackerel that was pre-treated under different conditions**

14

15 * Cholesterol oxides tested: **1** (1-cholestan-3β, 5α, 6β-triol-hydroxycholesterol), **2** (7α-
16 cholesterol), **3** (7β-cholesterol), **4** (7-keto-cholesterol), **5** (cholesterol-5α, 6α-
17 epoxide), **6** (25-hydroxycholesterol) and **7** (20α-hydroxycholesterol).
18 Cholesterol is compound **8**.
19 ** Lanes identification: lane A (Blank Control treatment), lane B (Water Control
20 treatment), lane C (RP treatment), lane D (cholesterol oxide mixture).
21

22

1 **TABLE 1: Scale employed for evaluating quality of frozen horse mackerel***
2
3
4

Attribute	QUALITY CATEGORIES			
	E (highest quality)	**A (good quality)**	**B (fair quality)**	**C (rejectable quality)**
External Odour	Shellfish	Weakly shellfish	Slightly sour and incipiently rancid	Sharply sour and rancid
Firmness	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes due to mechanical factors
External Colour	Very intense pigmentation; absence of yellowish spots	Insignificant pigmentation losses; absence of yellowish spots	Pigmentation discoloured and without shine; incipient yellowish spots	Important pigmentation losses; presence of yellowish spots

5
6
7
8
9
10
11 * Analysis of frozen fish was undertaken after thawing (overnight at 4ºC). At each
12 sampling time, fish were thawed and analysed in the same session. The panel
13 members shared samples tested. The fish were served to the panel members in
14 the individual polyethylene bags where they had been kept frozen.
15