Testing caffeic acid as a natural antioxidant in functional fish-fibre restructured products

Isabel Sánchez-Alonso[a*], Mercedes Careche[a], Pilar Moreno[a], María Jesús González[b], Isabel Medina[b]

[a]Instituto del Frío (CSIC), José Antonio Nováis 10, 28040 Madrid, Spain

[b]Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, 36208 Vigo, Spain

The antioxidant effectiveness of caffeic acid addition to minced fish muscle with or without wheat dietary fibre added was studied. Wheat dietary fibre showed a significant prooxidant effect on minced fish muscle during chilled storage that was significantly inhibited in presence of 100 mg/kg caffeic acid. In samples containing caffeic acid and wheat dietary fibre, lipid oxidation was completely inhibited after 10 days. Results obtained from the instrumental texture profile analysis showed that the inclusion of wheat dietary fibre with or without caffeic acid lowered the texture profile analysis parameters. Caffeic acid did not render any changes on the water binding capacity. These results prove that caffeic acid can be successfully used as a natural antioxidant in wheat dietary fibre minced fish restructured products.

Keywords: Fish muscle; dietary fibre; caffeic acid; lipid oxidation; texture properties; water holding capacity.

1. Introduction

Dietary fibres (DFs) are regarded as the most widely used functional ingredient in foods, but they have been hardly applied as such in seafood. The addition of DF to fishery products is of great interest not only as a means to further complement its healthy characteristics, but also as a means of improving the technological properties of the products (Borderías, Sánchez-Alonso & Pérez-Mateos, 2005). The technological feasibility of the use of different DFs (Sánchez-Alonso, Haji-Maleki & Borderías, 2006, 2007a; Sánchez-Alonso, Solas & Borderías, 2007b,c; Sánchez-Alonso & Borderías, 2008; Sánchez-González, Rodríguez-Casado, Careche & Carmona, 2009), has been checked and found, among a range of commercial and non commercial DFs, the best DF types and formulations in terms of maintaining or improving texture, water holding properties and sensory properties of the fish product prototypes. Also in these works, Product Tests have shown that there is an opportunity to develop new seafood products enriched in DFs better adapted to consumer preferences (Careche et al., 2008; Borderías et al., 2008), which can be seen as a new business opportunity for some industries.

Fish muscle from some species is very prone to oxidation which in turn causes the end of the commercial acceptability of these products. Some of the DFs studied, had antioxidant properties which helped in delaying lipid oxidation (Sánchez-Alonso, Borderías, Larsson & Undeland, 2007; Sánchez-Alonso, Jiménez-Escrig, Saura-Calixto & Borderías, 2007, 2008; Sánchez-Alonso & Borderías, 2008). However, these antioxidant DFs conferred a colour to the products not always suitable for certain applications.

In this context, it has been found that natural antioxidants such as hydroxycinnamic acids could successfully delay lipid oxidation in minced fish muscle (Medina, Gallardo, González, Lois & Edges, 2007; Medina, González, Iglesias & Hedges, 2009; Iglesias, Pazos, Andersen, Skibsted & Medina, 2009). Particularly natural extracts from plants and herbs as pears, basil, and coffee,

rich sources of caffeic acid and its derivative caffeic acid phenethyl ester, are being commercialized for their use as nutraceuticals and food supplements. There are some previous papers related to the antioxidant properties of caffeic acid and several studies have shown that the main mechanisms behind the ability to delay the lipid oxidation process are direct free radical scavenging, reducing capacity or transition metal chelation (Frankel, 1998; Rice-Evans, Miller & Paganga, 1996). However, it is well recognized that the antioxidant activity in tissues and foods, especially in muscle based foods, depends significantly on some other factors being the location of the antioxidant into the oxidative sensitive sites one of the most determinant. Also factors as the interaction with muscle membranes, the ratio between the antioxidant/lipid classes or the effect of food processing or ingredients are relevant. Consequently, the feature of strong antioxidant in-vitro ability in aprotic or protic solvents does not always mean a strong ability in muscle foods, especially in processed muscle foods having different ingredients. The antioxidant ability of caffeic acid on fish mince and fillets seems to be related to its reducing capacity and its ability to regenerate endogenous α-tocopherol from the α-tocopheroxyl radical through a redox cycle involving endogenous ascorbic acid. This high antioxidant effectiveness exhibited by caffeic acid on muscle based foods can be of high interest for the development of restructured seafood products. Non antioxidant DFs which are bland in colour and odour could thus be used in combination with caffeic acid in the formulations of restructured seafood products.

This work is aimed to study the antioxidant effectiveness of caffeic acid in chilled minced horse mackerel supplemented with wheat DF The possible changes in texture and water holding properties of the resulting formulations have been also determined. The final goal is to

formulate new seafood products enriched in DF and stabilised by means of an effective phenolic antioxidant.

2. Material and Methods

2.1. Raw material and ingredients

Minced fish muscle (MFM) was prepared from fresh horse mackerel (*Caranx rhonchus*) caught in the Mediterranean Sea (FAO zone 37.1) and obtained from a local market in Madrid. Fish were caught 10-18 hours before arriving to lab. The DF used was a commercial wheat DF Vitacel® WF200 (Campi y Jové, S.L., Barcelona, Spain), consisting of 74 g/100 g cellulose, 26 g/100 g hemicellulose and <0.5 lignin, with average fibre length of 250 μm and wide of 25 μm. According to the manufacturer, this DF contained a maximum moisture and ash contents of 8 and 3 g/100 g respectively, 0.4 g/100 g protein, 0.2 g/100 g fat and a pH value of 6.5 ± 1.5 at a 10 g/100 g suspension, and bulk density (in accordance with DIN 53 468) of 85 g/l.

2.2. Chemicals

All the chemicals used were of analytical grade and were obtained from Panreac Química S.A. (Barcelona, Spain), Sigma-Aldrich Corp. (St. Louis, MO, USA or Merck (Darmstadt, Germany). Caffeic acid was supplied by Sigma-Aldrich and streptomycin by AppliChem GmbH (Darmstadt, Germany).

2.3. Preparation of fish and samples

36 kg of horse mackerel were transported in ice to the IF-Madrid laboratory where it was eviscerated and filleted. The fillets were passed through a Baader model 694 de-boning machine

(Lübeck, Germany) equipped with a drum of 3 mm holes. Four different formulations were prepared (Table 1): minced fish muscle (MFM) with 3 g/100 g wheat DF (named WDF), MFM with 100 mg/kg caffeic acid (named CA), MFM with both 3 g/100 g wheat DF and 100 mg/kg caffeic acid (named WDFCA), and the control (named CO). The amount of caffeic acid used was chosen based on previous studies (Medina et al., 2007; 2009). 200 mg/kg of streptomycin was added to each of the samples in order to avoid microbial growth. Wheat DF was added dispersed in cold water (as per formulation). In all cases moisture was adjusted to the same as in the original fish muscle (78 g/100 g) with water. The ingredients were placed into a mixing machine, model RM-20 (Mainca, Granollers, Spain), and mixed for 4 min. Final temperature was kept below 6 ºC in all cases. Samples were placed in portions of 50 grams into 20 x 10 cm plastic bags (75 bags of 50 grams per formulation) resulting in a sample thickness of ~ 6-7 mm, and were sealed with an air space above the sample. The samples were stored on ice in darkness for up to 10 days. Samples were analyzed at the beginning of the experiment and then every 24 h (6-7 bags per formulation at each storage time point).

2.4. Proximate analyses

For the analysis of moisture content, 5.0 g of minced samples were introduced in an aluminium sample holder and kept in a forced air draft oven at 105 ºC during 24 h until constant weight. Results were expressed in grams of water per 100 g of sample. Protein determination was done by the Dumas combustion method in a Leco CNS 2000 instrument (St. Joseph, MI, USA). Results were expressed in g/100 g protein using 6.25 as protein nitrogen conversion factor (AOAC 1995). Ash content was determined as described in the AOAC (1995) method, and the fat content, by Bligh and Dyer (1959). The pH was measured using a Thermo Orion 3star (Thermo Scientific, Environmental Instruments, Beverly, USA).

116

117 2.5. Peroxide value

118 The peroxide value of fish muscle was determined by the ferric thiocyanate method (Chapman
119 & Mackay, 1949) and was expressed as meq oxygen / kg lipid.

120

121 2.6. Conjugated diene and triene hydroperoxides

122 Lipids were extracted from mackerel muscle (Bligh & Dyer, 1959) and the lipid content was
123 determined gravimetrically in triplicate. Conjugated hydroperoxides were measured from fish
124 oil samples dissolved in hexane, and absorbance was measured at 234 nm and 268 nm.
125 Concentrations of hydroperoxides were calculated as mmol of hydroperoxides per kg of oil as
126 described by Frankel, Huang, Kanner and Bruce-German (1994).

127

128 2.7. Volatiles

129 Fish volatiles were analysed by HS-SPME coupled with GC-MS according to Iglesias and
130 Medina (2008). Briefly, 3 g of fish minced muscle were homogenized for 2 minutes with 8 mL
131 of ultrapure water saturated in NaCl. The mixture was centrifuged (10 minutes, 2850 xg) and 6
132 mL of supernatant were exposed to a CAR-PDMS fibre (75µm Carboxen/polydimethylsiloxane
133 coating (Supelco, Bellefonte, PA) during 30 min at 60 °C. The fibre was then removed from the
134 vial and inserted into the GC injection port for desorption during 10 min to 300 ºC.
135 Determination of volatiles was performed by the method of internal standards using 3-methyl-3-
136 buten-1-ol. GC–MS analysis was performed in a Thermo Finnigan ThermoQuest (San Jose, CA)
137 gas chromatograph equipped with a split/splitless injector and coupled with a Trace quadrupole
138 mass detector (Thermo Finnigan ThermoQuest,San Jose, CA).

139

140 2.8. Surface colour measurements

141 Colour measurements consisted of determining L*, a* and b* using a CIELab scale (Young &
142 Whittle, 1985; Park, 1995) where L* is the parameter that measures lightness, +b* the tendency
143 towards yellow and +a* the tendency towards red. Measurements were done on a Minolta
144 Chroma Meter model CR-400 colorimeter and were standardized with respect to the white
145 calibration plate. Measurements were done in raw samples at least in triplicate.

146

147 2.9. Mechanical properties

148 Samples were cooked in cylindrical metal capsules of 30 ± 1 mm dia. and 30 ± 1 mm height in a
149 steam oven (Rational Combi-Master CM6) for 10 min then tempered before measuring. The
150 instrumental analyses were conducted using a [TA.XTPlus] Texture Analyzer (Texture
151 Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK). **Texture**
152 **profile analysis (TPA)**: Each cylinder was compressed by 50% with a load cell of 25.0 Kg at
153 0.8 mm s^{-1} and a 75 mm dia. cylindrical probe. Hardness, springiness, cohesiveness and
154 chewiness were measured and defined according to Uresti, López-Arias, González-Cabriales,
155 Ramirez and Vázquez (2003). **Stress relaxation test:** The samples were compressed by 10%
156 with a 75 mm dia. cylindrical probe and a load cell of 5.0 Kg at a crosshead speed of 0.8 mm s^{-1},
157 the deformation being kept constant for 600 s. Intitial stress (σ_0) was obtained; relaxation of
158 stress was monitored as a function of time and the curves were fitted to the Maxwell model with
159 two exponentials (Mohsenin, 1970): $\sigma(t)= \sigma_e + \sigma_1 e^{-t/T1} + \sigma_2 e^{-t/T2}$, where $\sigma(t)$ is the decaying
160 stress, σ_e is stress at equilibrium ($t = \infty$), σ_1, and σ_2 are the decay stress of each exponential, and
161 T_1 and T_2, the relaxation times. For each fitting, starting T_i and σ_i values included into the
162 nonlinear regression equation were: T_1=200, T_2=20, and σ_e, σ_1, σ_2 =0.3 times σ_0. The viscous

and elastic moduli (η_i and E_i respectively) were calculated taking into account that $T_i=\eta_i/E_i$, and $\sigma_i= E_i$*Deformation.

2.10. Water binding capacity

Samples (3 g) were placed in centrifuge tubes (Ø=10mm) along with enough filter paper (2 filter Whatman nº1 Ø=110 mm). The muscle was centrifuged in a Heraeus Multifuge 3 L-R centrifuge (Kendro Laboratory Products GmbH, Hanau, Germany) for 15 min at 3000xg at room temperature. Results were expressed as grams of water retained in the sample per 100 g of water present in the sample before centrifugation (WBC).

2.11. Sensory analysis

A group of four panellists composed bytwo women (thirty five years) and two men (sixty and thirty six years old) trained in descriptive analysis of fishy off-flavours sniffed the raw samples that were used for chemical determinations. Approximately 10 g of muscle was placed in separate sterile polystyrene Petri dishes and put on a tray of ice. Panellist concentrated on detecting rancidity/painty odours using a hedonic scale from 8 to ≤ 1, where 8 was the aroma of absolutely fresh and ≤ 1 a putrid aroma (Richards, Kelleher & Hultin, 1998). The odour scores were: 8: fresh seaweedy, 7: low odour, 6: stale, earthy, 5: sour, fishy, rotting orange, 4: slight paint, 3: moderate paint, 2: strong paint, 1: putrid/ammonia.

2.12. Statistical analyses

Each sample type (formulation) was replicate in two independent storage experiments (n=2) using different batches of horse mackerel to confirm results obtained in the first experiment.

The samples were analyzed in triplicate (thus, a=3) for proximate analyses, conjugated diene/trienes hydroperoxides, mechanical properties and water binding capacity. PV and volatiles were performed in duplicate (a=2), colour was measured at least in triplicate (a=3), and at least four persons smelled the samples (a=4). Assays were carried out on single bags for each formulation. An average value of the replicate analyses was used in calculations of sample variation and significance testing.

Two way analysis of variance (ANOVA) was done as a function of treatment and storage time. Then, per each treatment, one-way ANOVA has been performed as a function of time, and per each storage time, one way ANOVA was done as a function of treatment. The Levene test was used to check the equality of variances. Where variances were equal, the difference between means was analyzed by the Bonferroni test. Where equality of variances could not be assumed a Tamhane T2 test was used. Level of significance was set to $P<0.05$. Statistical analyses were performed with SPSS 15.0 (SPSS Inc, Chicago IL. USA). Values are presented as means ± standard deviations (SD).

3. Results and Discussion

3.1. Proximate analyses

Results of proximate analyses are presented in Table 2. Only slight differences were found in moisture content between samples with added wheat DF and without, since formulations aimed at maintaining the final moisture at a similar level. Lower values of protein, lipid, and ash in WDF and WDFCA, are the consequence of the addition of wheat DF by keeping moisture levels constant.

3.2. Oxidation

All samples showed fresh odour during the first day of storage. However, sensory data demonstrated that controls and WDF samples developed rancid off-flavours by the second day of storage at 4ºC (sensory scores =4). Samples supplemented with caffeic acid showed no rancidity during the whole experiment and their sensory scores were round 8 and 7 Therefore, the sensory results suggest that there was an inhibitory effect on the formation of off-flavours in samples supplemented with 100 mg/kg of caffeic acid. The sensory results were also consistent with a reduction in the formation of lipid oxidation by-products. Figure 1 depicts the formation of peroxides (a) and conjugated dienes (b) in the different formulations for 8 days of chilled storage. Formation of 1-penten-3-ol, selected as target volatile in horse mackerel muscle (Iglesias & Medina, 2008), is also shown in Table 3. Less oxidation by-products were formed in CA than in the CO sample over a period of 10 days. Wheat DF showed a significant prooxidant effect on MFM that was significantly inhibited in presence of 100 mg/kg of caffeic acid. In CA and WDFCA samples lipid oxidation was also completely inhibited. The effectiveness of this natural antioxidant in preventing lipid oxidation has been proved by Medina et al. (2007) in

minced chilled horse mackerel. These results show that it is also effective in a more complex formulation. Additionally, the pattern of volatiles formed demonstrated that samples containing wheat DF displayed a significant inhibition of the formation of target compounds associated to microbial spoilage. Therefore, compounds as trimethylamine and 3-hydroxy-2-butanone showed lower levels in samples WDF and WDFCA than in CO and CA samples (data not shown) .

3.3. Colour

Colour parameters (L*, a*, b*) were evaluated to detect whether wheat DF and caffeic acid addition caused changes in raw samples (Figure 2). Addition of wheat DF (WDF and WDFCA) increases significantly the value of L* in raw samples. This is due to the fact that wheat DF is white. The addition of caffeic acid slightly lowered the L* tendency of samples (CA and WDFCA). Throughout the chilled storage slightly changes were found in L* tendency in samples (Figure 2).

Addition of caffeic acid slightly lowered the value of a* (shift towards red) in raw samples at the beginning of the study (Figure 2). During storage, no significant changes in the redness values of samples with caffeic acid added (CA and WDFCA) were observed, while this parameter decreased significantly in the samples without this natural antioxidant. Samples CO and WDF decreased after 9 days from 7.6 to 2.6 and 7.8 to 1.2 respectively. The highest decrease in the control sample was observed at day 5 (42% of decrease in a* value) and in the case of the WDF sample the highest decrease was found at day 2 (60% of decrease). This decrease of redness in raw control sample could be related to haemoglobin oxidation and a build up of brownish met-Hemoglobin (Wetterskog & Undeland, 2004). Decreases in a* have been proposed as an indirect mean of following Hb-mediated lipid oxidation in fish muscle (Undeland, Ekstrand & Lingnert, 1998). This is especially important for species like horse

mackerel whose muscle contains large amounts of haemoglobin, a well-known activator of lipid oxidation (Richards & Hultin, 2000; Richards, Modra & Li, 2002; Undeland, Hultin & Richards, 2002; Undeland et al., 1998). These results are consistent with those obtained in lipid oxidation measurements in which an inhibitory effect of samples supplemented with caffeic acid was detected, reducing oxidation of haemoglobin and the amount of lipid peroxidation products. Yellowness increased significantly in raw samples when wheat DF was added (Figure 2) with and without caffeic acid. The b* values increased significantly during chilled storage in samples without caffeic acid added (CO and WDF); this could be connected with losses of redness given that oxygenated heme proteins produce a bright colour while oxidized pigments are brown (meat heme proteins)Fish samples having high oxidative deterioration could also show yellowness through the formation of tertiary lipid oxidation by-products resulting from interaction of carbonyls and amino groups of muscle proteins or peptides (Pokorny and Kołakowska, 2003). Considering the scarce oxidation of samples containing wheat DF plus caffeic acid (Figure 1, Table 3), the yellowness found cannot be attributed to the formation of these interaction products.

3.4. Mechanical properties

Results obtained from the **TPA** are shown in tables 4 and 5. No major changes were observed in the control as a consequence of storage. On the other hand, significant differences between treatments were observed. The inclusion of wheat DF either in the presence or absence of caffeic acid significantly lowered the hardness, springiness, and chewiness values as compared with the samples with no wheat DF added. This may be a consequence of substituting fish muscle by wheat DF, since the formulation was performed at constant moisture. These results are in agreement with those obtained previously when 3g/100g and 6g/100g of wheat DF were

added to horse mackerel (*Trachurus trachurus*) and hake (*Merluccius capensis*) minced samples (Sánchez-Alonso et al., 2007a). However, no differences were found in cohesiveness between samples (Table 4). In addition, wheat DF containing samples (WDF and WDFCA) showed significant increases in hardness and chewiness along storage (Table 4 and 5). No significant differences were found on TPA parameters when only caffeic acid was added to minced fish muscle. Thus, it seems that caffeic acid does not affect significantly the TPA parameters of the restructured fish muscle.

Studies made on frozen fish fillets have shown no any effect of caffeic acid on myofibrillar protein aggregation/denaturation or water holding capacity in frozen fish muscle (Medina et al., 2009). Such protein modifications largely affect texture of fish muscle. Recent results have also indicated that phenolic compounds as chlorogenic and caffeic acids can enhance their antioxidant capacities through phenol-protein interaction in foods (Tsai & She, 2006). These compounds have been also reported to affect the heat denaturation of globular proteins incrementing food stability (O'Connell &Fox, 1999).

Stress relaxation. Two-way analysis of variance showed significant differences among treatments. The data from time 0 are shown in Figure 3. Control samples, have the highest stress relaxation parameters, and those with wheat DF the lowest, with medium values for CA samples. This trend is similar to the one found in TPA. In combination, results indicate that caffeic acid may alter texture and rheology of minced horse mackerel, and suggests that they should be monitored alongside oxidation parameters.

3.5. Water Binding Capacity (WBC)

The inclusion of wheat DF lowered significantly ($p<0.05$) the water binding capacity of raw samples regardless the addition of caffeic acid (Figure 4). A decrease in WBC in minced fish

297 muscle with added wheat DF has been reported previously (Sánchez-Alonso et al., 2007a). The
298 presence of caffeic acid does not affect the water binding capacity of the formulations since no
299 significant differences were found when comparing CO vs CA or WDF vs WDFCA (Figure 4).
300 No differences in the trends were found at the end of the storage (day 9).
301 The results could be explained as follows: on the one hand, the formulations are done in
302 conditions of substitution of mince by wheat DF, thus keeping the moisture constant. Wheat DF
303 has very high water retention properties, ranging between 7-8 g water/g DF (Sánchez-Alonso et
304 al. 2007c), but the ratio of free to entrapped water in wheat DF suspensions at 75 g/100g
305 moisture, is much higher than this ratio for the fish muscle at this moisture (Low Field NMR
306 results, not shown). Andersen and Jorgensen (2004) found that a centrifuged mince as
307 performed in WBC, contained both entrapped and free water, but with a higher proportion of the
308 former. So the substitution of mince by wheat DF implies a lower WBC in wheat DF containing
309 formulations, due to the fact that there is an enrichment of free water in those samples.
310 Also, wheat DF may retire water from the muscle and cause a lower local moisture and partial
311 denaturation of the muscle proteins, contributing to the total WBC loss in these samples, as has
312 been observed in other systems such as *surimi* gels enriched in wheat DF (Sánchez-González et
313 al., 2009). This lower local moisture in the mince would also explain the effect of the wheat DF
314 on lipid oxidation and microbial growth, which are influenced by a decrease on water activity
315 (proportion of water available for biological and chemical reactions). The rate of lipid oxidation
316 would increase due to the resulting major concentration of solutes (Frankel, 1998).
317 Nevertheless, this oxidation was inhibited when caffeic acid was added to samples with added
318 wheat DF (WDFCA).
319 There were no differences in cooking loss among samples. WBC values were in general lower
320 than the uncooked ones for the samples with no wheat DF added, and the effect of this

ingredient was much less evident than in raw samples (Figure 4). These trends were similar at the beginning and end of the storage time. The lower values in the cooked, non wheat DF containing samples can be explained in terms of protein denaturation and as a consequence, a change of bound to free water. In wheat DF formulations, the free water released during heating by the muscle would be trapped by the wheat DF, thus explaining the low WBC losses in these formulations.

4. Conclusions

Wheat dietary fibre added to horse mackerel minced muscle showed a certain prooxidant effect, whereas the addition of caffeic acid to wheat DF containing formulations, lead to a significant inhibition of lipid oxidation during the whole storage period. Water retention properties are consistent with the previously published decreasing effect of wheat dietary fibre on the water binding capacity of minced fish muscle, and caffeic acid did not modify these properties. These results are very interesting since they prove that caffeic acid can be used successfully as a natural antioxidant in horse mackerel minced muscle with wheat DF added and could be used together with wheat DF in the design of new minced fish restructured products.

Acknowledgement

This work was performed within the Integrated Research Project SEAFOODplus, contract No FOOD-CT-2004-506359 and the research project AGL2006-26016-E/GAN. The [partial] financing of this work by the European Union and the Spanish Ministerio de Educación y Ciencia is gratefully acknowledged.

References

Andersen, C.M., & Jorgensen, Bo.M. (2004). On the relation between water polls and water holding capacity in cod muscle. *Journal of Aquatic Food Product Technology*, 13, 13-23.

Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.

Borderías, J., Sánchez-Alonso, I., Moreno, P., Dopico, D.C., Tudoran, A., Olsen, S.O., & Careche, M. (2008). Connecting consumer preferences with technical product specifications in wheat dietary fibre enriched seafood restructured products.. In B. M. Poli & G. Parisi, Book of Abstracts of the 38th Annual WEFTA meeting, Seafood from catch and aquaculture for a sustainable supply (pp. 8). Firenze: Firenze University Press

Borderías, J., Sánchez-Alonso, I., & Pérez-Mateos, M. (2005). New applications of fibres in foods: Addition to fishery products. *Trends in Food Science and Technology*, 16, 458-465.

Careche M., Luten J., Kole A., Schelvis R., Saura-Calixto F., Scholten O.E., Díaz-Rubio M.E., Toonen M.A.J., Schram M., Borderías A.J., Sánchez-Alonso I., Carmona P., Sánchez-González I., Gormley T.R., Oehlenschläger J., Mierke-Klemeyer S, Elvevoll E., Nunes M.L., Bandarra N., Stoknes I., & Larsen E.H. (2008). Developing functional seafood products: the consumerproducts project. In T. Borresen, Improving seafood products for

the consumer (pp. 331-362. Woodhead Publishing Limited. Technical University of Denmark, Denmark.

Chapman, R. A., & Mackay, K. (1949). The estimation of peroxides in fats and oils by the ferric thiocyanate method. *Journal of the American Oil Chemists´ Society*, 26, 360-363.

Frankel, E. N. (1998). Lipid Oxidation. Scotland: The Oily Press.

Frankel, E.N., Huang, S.W., Kanner, J., & Bruce-German, J.B. (1994). Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils versus Emulsions. *Journal of Agricultural and Food Chemistry*, 42, 1054-1059.

Iglesias, J., & Medina, I. (2008). Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. *Journal of Chromatography*, 1192(1), 9-16.

Iglesias, J., Pazos, M., Andersen, M. L., Skibsted, L. H., & Medina, I. (2009). Caffeic Acid as Antioxidant in Fish Muscle: Mechanism of Synergism with Endogenous Ascorbic Acid and α-Tocopherol. *Journal of Agricultural and Food Chemistry*, 57(2), 675-681.

Medina, I., Gallardo, J.M., González, M.J., Lois, S., & Hedges, N. (2007). Effect of Molecular Structure of Hydroxycinnamic Acids and Catechins on the Antioxidant Effectiveness in Minced Fish Muscle. *Journal of Agricultural and Food Chemistry*, 55(10), 3889-3895.

Medina, I., González, M.J., Iglesias, J., & Hedges, N.D. (2009). Effect of hydroxycinnamic acids on lipid oxidation and protein changes as well as water holding capacity in frozen minced horse mackerel white muscle. *Food Chemistry*, 114, 881-888.

Mohsenin, N. N. (1970). Some basic concepts of rheology. In Physical properties of plants and animal matherials. Structure, physical characteristics and mechanical properties (Vol. 1, pp. 832-855). New York: Gordon and Breanch Science Publishers.

O'Connell J.E., & Fox, P.F. (1999). Proposed mechanism for the effect of polyphenols on the heat stability of milk. *International Dairy Journal*, 9, 523-536.

Park, J.W. (1995). Surimi gel colors as affected by moisture content and physical conditions. *Journal of Food Science*, 60(1), 15-18.

Pokorný, J., & Kołakowska, A. (2003). Lipid – protein and lipid – saccharide interactions. In Z. E. Sikorski, & A. Kołakowska, Chemical and functional properties of food lipids (pp. 345-362). CRC Press LLC.

Rice-Evans, C.A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20, 933-956.

Richards, M.P., & Hultin, H.O. (2000). Effect of pH on lipid oxidation using trout hemolysate as a catalyst: a possible role for deoxyhemoglobin. *Journal of Agricultural and Food Chemistry*, 48, 3141-3147.

Richards, M. P., Kelleher, S. D., & Hultin, H. O. (1998). Effect of washing with or without antioxidants on quality retention of mackerel fillets during refrigerated and frozen storage. *Journal of Agricultural and Food Chemistry*, 46, 4363-4371

Richards, M.P., Modra, A.M., & Li, R. (2002). Role of deoxyhemoglobin in lipid oxidation of washed cod muscle mediated by trout, poultry and beef hemoglobins. *Meat Science*, 62, 157-163.

Sánchez-Alonso, I., & Borderías, A.J. (2008). Technological effect of red grape antioxidant dietary fibre added to minced fish muscle. *International Journal of Food Science and Technology*, 43, 1009-1018.

409 Sánchez-Alonso, I., Borderías, A.J., Larsson, K., & Undeland, I. (2007). Inhibition of
410 haemoglobin-mediated oxidation of regular and lipid-fortified washed cod mince by a
411 white grape dietary fiber. *Journal of Agricultural and Food Chemistry*, 55, 5299-5305.
412 Sánchez-Alonso, I., Haji-Maleki, R., & Borderías, A.J. (2006). Effect of wheat fibre in frozen
413 stored fish muscular gels. *European Food Research and Technology*, 223, 571-576.
414 Sánchez-Alonso, I., Hají-Maleki, R., & Borderías, A.J. (2007a). Wheat fibre as a functional
415 ingredient in restructured fish products. *Food Chemistry*, 100, 1037-1043.
416 Sánchez-Alonso, I., Jiménez-Escrig, A., Saura-Calixto, F., & Borderías, A.J. (2007). Effect of
417 grape antioxidant dietary fibre on the prevention of lipid oxidation in minced fish:
418 Evaluation by different methodologies. *Food Chemistry*, 101, 372-378.
419 Sánchez-Alonso, I., Jiménez-Escrig, A., Saura-Calixto, F., & Borderías, A.J. (2008).
420 Antioxidant protection of white grape pomace on restructured fish products during
421 frozen storage. *LWT-Food Science International*, 41, 42-50.
422 Sánchez-Alonso, I., Solas, M.T., & Borderías, A.J. (2007b). Physical study of minced fish
423 muscle with a white-grape by-product added as ingredient. *Journal of Food Science*,
424 72(2), E94-E101.
425 Sánchez-Alonso, I., Solas, M.T., & Borderías, A. J. (2007c). Technological implications of
426 addition of wheat dietary fibre to giant squid (*Dosidicus gigas*) *surimi* gels. *Journal of*
427 *Food Engineering*, 81, 404-411.
428 Sánchez-González, I., Rodríguez-Casado, A., Careche, M., & Carmona, P. (2009). Raman
429 analysis of *surimi* gelation by addition of wheat dietary fibre. *Food Chemistry*, 112, 162-
430 168.

Tsai, P.J., &She, C.H. (2006). Significance of Phenol−Protein Interactions in Modifying the Antioxidant Capacity of Peas. *Journal of Agricultural and Food Chemistry*, 54, 8491-8494.

Undeland, I., Ekstrand, B., & Lingnert, H. (1998). Lipid oxidation in minced herring (Clupea harengus) during frozen storage: influence of washing and pre-cooking. *Journal of Agricultural and Food Chemistry*, 46, 2319-2328.

Undeland, I., Hultin, H.O., & Richards, M.P. (2002). Added triacylglycerols do not hasten hemoglobin-mediated lipid oxidation in washed minced cod muscle. *Journal of agricultural and food chemistry*, 50(23), 6847-6853.

Uresti, R. M., López-Arias, N., González-Cabriales, J., Ramirez, J., & Vázquez, M. (2003). Use of amidated low methoxyl pectin to produce fish restructured products. *Food Hydrocolloids*, 17,171-176.

Wetterskog, D., & Undeland, I. (2004). Loss of Redness (a*) as a Tool To Follow Hemoglobin-Mediated Lipid Oxidation in Washed Cod Mince. *Journal of Agricultural and Food Chemistry*, 52 (24), 7214–7221.

Young, K.W., & Whittle, J. (1985). Colour measurement of fish minces using Hunter L, a, b values. *Journal of Science of Food and Agriculture*, 36(5), 383-392.

Figure Captions

Figure 1. Formation of peroxides (left) and conjugated dienes (right) in minced fish muscle (named CO, ◆), minced fish muscle plus caffeic acid (named CA, ■), minced fish muscle plus wheat dietary fibre (named WDF, X), and minced fish muscle plus wheat dietary fibre and caffeic acid (named WDFCA, ▲). Samples were stored on ice and in darkness. The points in the graphs are the average value of the replicate analyses (a=2 for PV and a=3 for conjugated dienes). Error bars illustrate the standard deviation of measurements.

Figure 2. Changes in colour measurements in minced fish muscle (named CO, ◆), minced fish muscle plus caffeic acid (named CA, ■), minced fish muscle plus wheat dietary fibre (named WDF, X), and minced fish muscle plus wheat dietary fibre and caffeic acid (named WDFCA, ▲). Samples were stored on ice and in darkness. The points in the graphs are the average value of the replicate analyses (at least a=3). Error bars illustrate the standard deviation of measurements.

Figure 3. Stress relaxation parameters at time 0. The parameters are: stress (kPa), elastic moduli (E1, E2, E0, kPa), viscous moduli (η1*100, η2*10, kPa.s), and relaxation times (T1*10 , T2, s) in minced fish muscle (named CO, black), minced fish muscle plus caffeic acid (named CA, grey), minced fish muscle plus wheat dietary fibre (named WDF, dotted), and minced fish muscle plus wheat dietary fibre and caffeic acid (named WDFCA, striped). Samples were stored on ice and in darkness. The points in the graphs are the average value of the replicate analyses (a=3). Error bars illustrate the standard deviation of measurements.

Figure 4. Changes in water binding capacity (WBC, g/100g) of crude (left) and cooked samples (right) in minced fish muscle (named CO, black), minced fish muscle plus caffeic acid (named CA, grey), minced fish muscle plus wheat dietary fibre (named WDF, dotted), and minced fish muscle plus wheat dietary fibre and caffeic acid (named WDFCA, striped). Samples were stored on ice and in darkness. The points in the graphs are the average value of the replicate analyses (a=3). Different letters indicate significant time differences ($P<0.05$) for each sample. Different numbers at the same day of storage indicate significant differences between samples.