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ORIGINAL PAPER

Effect of a Polyphenol–Vacuum Packaging on Lipid Deterioration During an 18-Month Frozen Storage of Coho Salmon (*Oncorhynchus kisutch*)

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Abstract A packaging system combining a polyphenol-rich film and vacuum (PPRF–VP) was applied to farmed coho salmon (*Oncorhynchus kisutch*) muscle for an 18-month storage (−18 °C). For it, two different concentrations of polyphenol compounds (namely, *p*-coumaric and ferulic acids) obtained from a barley husk extract were applied (PPRF–VP conditions) and compared to vacuum packaging without polyphenol presence (vacuum control; VP condition) and to packaging in the absence of vacuum and polyphenols (control; CP condition). The study was addressed to lipid hydrolysis and oxidation development and to lipid changes related to nutritional value. Both PPRF–VP conditions provided an inhibitory effect ($p<0.05$) on conjugated diene and fluorescent compound formation in frozen salmon. Compared to CP condition, vacuum packaging (PPRF–VP and VP conditions) led to lower ($p<0.05$) peroxide and anisidine values and to an inhibitory effect ($p<0.05$) on α- and γ-tocopherol losses. No effect ($p>0.05$) of polyphenol presence and vacuum packaging could be inferred on free fatty acid formation (hydrolysis development) and on polyunsaturated fatty acid retention (polyene index assessment). A low rancid odour development was observed in all kinds of fish samples, this being lower ($p<0.05$) in fish kept under vacuum (PPRF–VP and VP) conditions.

Keywords Coho salmon · Barley husk · Polyphenols · Vacuum packaging · Frozen storage · Lipid damage

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Introduction

Freezing and frozen storage have largely been employed for the preservation of fish species. Although most damage pathways are partially inhibited by such processes, undesirable reactions have shown to occur, leading to detrimental changes in nutritional and sensory properties. Such quality losses have shown to be especially important when fatty fish is concerned, its shelf life being markedly short as a result of the rancidity development of the highly unsaturated lipid composition (Erickson 1997; Kolakowska 2003).

Many efforts have been carried out by fish traders and food technologists in order to commercialise frozen fish products in a safe and high-quality state. To retard lipid oxidation as long as possible and, accordingly, extend the shelf life, a wide number of advanced and traditional strategies to be combined to freezing and frozen storage have been tested such as hydrostatic high pressure, vacuum packaging, brining, glazing including protecting chemicals and active and intelligent packaging (Ozen and Floros 2001; Aubourg and Ugliano 2002; López-Rubio et al. 2006; Tironi et al. 2009).

Among such complementary technologies, the employment of antioxidants has obtained most relevance. Since replacement of synthetic antioxidants by natural ones has become imperative, recent efforts are widely focused on the positive role of antioxidants present in plant extracts and residual sources (Moure et al. 2001; Yanishlieva et al. 2006; Maqsood and Benjakul 2011). Thus, successful applications of plant extracts have been carried out on frozen minced fish, fish fillets and whole fish (Medina et al. 2009; Tironi et al. 2010). Among the different food fitochemicals tested, polyphenol compounds (caffeic acid, ferulic acid, coumaric acids) have provided an important positive role in inhibiting rancidity development.

The present work focuses on the lipid deterioration during the frozen storage of coho salmon (*Oncorhynchus kisutch*). This fatty fish species was chosen because of its increasing farming production and accordingly, its increasing economical significance. A packaging system combining a polyphenol-rich film and vacuum was applied for an 18-month storage period at −18 °C. The study was addressed to hydrolytic and oxidative changes related to rancidity development and nutritional value.

Materials and Methods

Packaging System

A packaging system consisting of polyethylene bags combining vacuum and an antioxidant-rich film was tested. For it, a barley husk extract including polyphenol compounds (namely, *p*-coumaric and ferulic acids) was obtained from the lignin fraction and quantified according to previous research (Cruz et al. 2007). Barley husk was subjected to hydrolysis with a solution of 3% H_2SO_4 for 15 min at 130 °C at a liquid/solid ratio of 8:1 g/g. The solid residue was then delignified with a 6.5% solution of NaOH for 60 min at 130 °C at a liquid/solid ratio of 10:1 g/g. Phenolic compounds were extracted from the liquid phase obtained in the delignification step (water phase/organic phase ratio, 1:3) with ethyl acetate. The organic phase was separated by settlement in a vertical evaporator and removed. The dried extract was then dissolved in methanol to obtain solutions of 50 and 100 g of extract per litre.

The low-density polyethylene film used to elaborate the packaging bags (15×18 cm; 0.140 mm thickness) had a water vapour transmission rate of 3.62 g/m^2/24 h when measured at 38 °C and 90% relative humidity. Both extract solutions mentioned (50 and 100 g/L) were used to prepare the active packaging by a coating process. As a result, polyethylene films coated with the two antioxidants (29 μg *p*-coumaric acid and 19 μg ferulic acid dm^{-2} film; 88 μg *p*-coumaric acid and 40 μg ferulic acid dm^{-2} film) and combined to vacuum packaging (P1 and P2 conditions, respectively) were obtained. Once the coating process was finished, the film was-dried at room temperature until methanol was completely evaporated.

Both antioxidant concentrations were chosen according to the results obtained in previous research related to application to frozen fish (Pereira de Abreu et al. 2010). Comparison to vacuum packaging without polyphenol extract presence (vacuum control; VP condition) and to packaging in the absence of both vacuum and polyphenol extracts (control; CP condition) was carried out.

Raw Fish, Processing and Sampling

Coho salmon specimens (51 individuals; 50–52 cm length; 2.8–3.0 kg weight) were obtained from an aquaculture facility (AQUACHILE, S. A., Puerto Montt, X Región, Chile). Individuals were sacrificed in the plant, the gills cut, bled in a water–ice mixture, beheaded, gutted and transported to the laboratory during 24 h under slurry ice condition (40% ice and 60% water; –1.0 °C) at a 1:1 fish to ice ratio.

Each individual fish was processed and analysed independently throughout the whole experiment. Thus, each fish was filleted and cut into pieces (weight range 100–125 g), each fish piece individually packed. Fish pieces corresponding to 48 fishes were distributed among the four above-mentioned packaging conditions (CP, VP, P1 and P2; 12 individuals in each condition). Then, packed fish was placed in a freezer at −40 °C. After 24 h, all frozen fish samples were placed in a monitored freezer room at −18 °C.

Sampling was carried out on the starting raw fish (fish pieces corresponding to three individuals) and in frozen fish after 3, 9, 15 and 18 months of storage at −18 °C. Analyses were carried out on the white muscle of salmon. For all kinds of samples (starting fish and frozen fish related to each processing condition), fish pieces corresponding to three different individuals (n=3) were considered and analysed separately at each sampling point.

Lipid Composition Analyses

Lipids were extracted by the Bligh and Dyer (1959) method, by employing a single-phase solubilisation of the lipids using a chloroform–methanol (1:1) mixture. Quantification results were expressed as grams of lipid per kilogram of muscle.

Lipid extracts were converted into fatty acid methyl esters (FAME) by employing acetyl chloride and then analysed by gas chromatography, according to Aubourg et al. (1996). FAME were analysed by means of a PerkinElmer 8700 chromatograph (PerkinElmer, Waltham, MS, USA) employ-

ing a fused silica capillary column SP-2330 (0.25 mm i.d. × 30 m, Supelco Inc., Bellefonte, PA, USA). Nitrogen at 10 psi as carrier gas and flame ionisation detector at 250 °C were used. Peaks corresponding to fatty acids were identified by comparison of their retention times with standard mixtures (Larodan, Qualmix Fish, Malmo, Sweden; FAME Mix, Supelco Inc.). Peak areas were automatically integrated, 19:0 fatty acid being used as internal standard for quantitative analysis. The polyene index (PI) was calculated as the following fatty acid content ratio: PI=(C 20/5ω3+C 22/6ω3)/C 16:0.

Tocopherols were analysed according to Cabrini et al. (1992). For it, lipophilic antioxidants were extracted from the muscle with hexane, carried out to dryness under nitrogen flux, dissolved in isopropanol and injected in the HPLC system. An ultrasphere ODS column (15×0.46 cm i.d.; Waters, Milford, MA, USA) was employed, by applying a gradient from 0% to 50% of isopropanol. Flow rate was 1.5 ml min^{-1}. Detection was achieved at 280 nm. Different kinds of tocopherol isomers (α, γ and δ) were detected in farmed salmon samples, their contents expressed as milligrams per kilogram of muscle.

Chemical Assessment of Lipid Damage

Conjugated diene (CD) formation was spectrophotometrically (Beckman Coulter DU 640, London, UK) measured in the lipid extract at 233 nm (Kim and Labella 1987). Results are expressed according to the following formula $CD=B\times V/w$, where B is the absorbance reading at 233 nm, V is the volume (millilitres) and w is the mass (milligrams) of the lipid extract measured.

The peroxide value was determined in the lipid extract by peroxide reduction with ferric thiocyanate, according to the Chapman and McKay (1949) method. Results were expressed as meq active oxygen per kilogram of lipids.

The anisidine value (AV) was determined in fish muscle according to the AOCS (1993) method, based on the reaction between α- and β-unsaturated aldehydes (primarily 2-alkenals) and *p*-anisidine reagent. AV was expressed as 100 times the absorbance measured at 350 nm (Beckman Coulter DU 640) in a 1-cm path length cuvette from a solution containing 10 mg lipid ml^{-1} reaction medium.

Formation of fluorescent compounds (PerkinElmer LS 45) was determined by measurements at 393/463 and 327/415 nm (Aubourg 1999). The relative fluorescence (RF) was calculated as follows: $RF=F/F_{st}$, where F is the fluorescence measured at each excitation/ emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 μg ml^{-1} in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR=RF_{393/463}$ nm/$RF_{327/415}$ nm. The FR value was determined in the aqueous phase resulting from the lipid extraction (Bligh and Dyer 1959).

Free fatty acid (FFA) content was determined in the lipid extract of the fish muscle by the Lowry and Tinsley (1976) method based on complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment (Beckman Coulter DU 640). Results were calculated as gram of FFA per kilogram of muscle and as gram of FFA per kilogram of lipids.

Rancid Odour Assessment

The development of rancid odour was conducted by a sensory panel consisting of ten experienced judges. Panellists were selected and trained according to International Standards (ISO 3972 1991); before the present experiment, a special training was carried out concerning the assessment of rancid odour in frozen salmon.

At each sampling time, the fish muscle portions were presented to panellists in individual trays and were scored individually. The panel members shared samples tested. Rancid odour development was evaluated using the Quantitative Descriptive Analysis method. A non-structured linear scale with numerical scores from 0 (stage of no rancidity at all) to 10 (stage where no increase in rancidity is possible) was applied; 5.0 was considered the borderline of fish acceptability. Scores among panellists were averaged.

Statistical Analysis

Data ($n=3$; three independent determinations at each sampling time for each processing condition) obtained from the different analyses were subjected to the ANOVA method ($p<0.05$) to explore differences by two different ways: packaging system and frozen storage time (Statsoft, Statistica, version 6.0, 2001); comparison of means was performed using a least-squares difference method. Correlation analysis among parameters (frozen time, chemical lipid indices and odour assessment) was also carried out. In them, linear fittings are expressed; otherwise, the kind of fitting is mentioned.

Results and Discussion

Assessment of Lipid Oxidation

Different and complementary chemical indices were assessed to evaluate the rancidity development in the present experiment.

Fish samples corresponding to CP and VP conditions showed a progressive increase formation of CD throughout

the storage time (Table 1; r^2=0.90–0.92, quadratic fitting). Meantime, a different pattern could be outlined for its counterpart fish corresponding to P1 and P2 systems; under such conditions, mean values showed a decreasing tendency during the 0–9-month period, followed by a marked increase at month 15. Comparison among packaging conditions showed in all cases lower mean values for fish packaged with films including polyphenol compounds; such differences were significant in the 3–9-month period. However, no effect of the polyphenol concentration could be depicted on CD formation. Lower mean values were obtained in most cases in VP-treated fish when compared to its counterpart from control group (CP condition), although significant differences were only observed at month 9.

A marked inhibitory effect on peroxide formation could be concluded for vacuum packaging (VP, P1 and P2 conditions) throughout the frozen storage period (9–18-month period; Table 1). Previous research has also shown an important effect of oxygen accessibility on peroxide formation during the frozen storage of a related salmonid species (rainbow trout, *Salmo gairdneri*) (Christophersen et al. 1992). However, present data did not provide differences as a result of the polyphenol presence and content in the packaging film. In all packaging conditions including vacuum (VP, P1 and P2), a progressive peroxide formation was observed with frozen time (r^2=0.89–0.93). In the case of control fish (CP system), a marked peroxide content decrease (p<0.05) was produced at month 18 that could be explained as a result of peroxide breakdown. Previous research has already shown that in advanced oxidation stages, peroxides are capable of breakdown and/or react with other constituents, so that their values would decrease in spite of the fact that fish damage increases (Christophersen et al. 1992; Ortiz et al. 2009).

Secondary lipid oxidation was measured by the AV. In such index, comparison among packaging conditions led to similar conclusions to the case of peroxide formation (Fig. 1). Thus, an inhibitory effect (p<0.05) on α- and β-unsaturated aldehyde formation could be concluded as a result of vacuum packaging employment (VP, P1 and P2 conditions) during the 9–18-month period; however, no effect (p>0.05) could be accorded to the polyphenol extract presence and content in the film.

Compared to starting raw fish, an AV increase (p<0.05) was produced in all kinds of samples at month 3. At month 9, a marked increase in control fish (CP condition) could be observed; however, no other difference (p>0.05) could be assessed throughout the storage time (3–18 months) for any of the packaging conditions tested. This result could be explained by the fact that molecules susceptible to be measured under this index (α-unsaturated aldehydes, namely) are known to be more reactive than saturated aldehydes, according to the possibility of under-

Table 1 Assessment of conjugated diene formation and peroxide value in salmon muscle kept frozen under different packaging conditions

Frozen storage time (months)	Conjugated diene formation				Peroxide value (meq active oxygen kg^{-1} lipids)			
	CP	VP	P1	P2	CP	VP	P1	P2
0[a]	*z* 6.74 (0.90)	*z* 6.74 (0.90)	*zy* 6.74 (0.90)	*z* 6.74 (0.90)	*z* 3.81 (1.67)	*z* 3.81 (1.67)	*z* 3.81 (1.67)	*z* 3.81 (1.67)
3	*z* 7.40b (0.48)	*z* 7.24b (0.32)	*z* 5.37a (1.13)	*z* 5.31a (0.67)	*z* 5.97b (0.60)	*z* 4.03ab (1.44)	*z* 2.86a (0.70)	*z* 4.29ab (1.58)
9	*y* 9.64c (1.40)	*z* 7.07b (0.53)	*z* 4.96a (1.36)	*z* 4.90a (0.37)	*y* 25.69b (8.03)	*zy* 5.72a (4.54)	*z* 4.48a (0.47)	*z* 4.64a (1.23)
15	*y* 10.84 (0.91)	*y* 11.27 (2.21)	*x* 10.18 (1.04)	*y* 10.00 (1.57)	*x* 46.24b (7.04)	*y* 11.21a (4.07)	*y* 11.05a (3.36)	*y* 8.44a (1.62)
18	*x* 14.78c (2.60)	*y* 12.44bc (2.66)	*yx* 7.52a (0.93)	*y* 9.37ab (1.11)	*y* 25.73b (9.58)	*x* 22.90a (4.38)	*y* 16.60a (2.82)	*x* 16.27a (3.10)

Average values of three (n=3) independent determinations. Standard deviations are indicated in parenthesis. Conjugated diene calculations as expressed in the "Materials and Methods" section. For each parameter and for each frozen storage time, mean values followed by different letters (a, b, c) indicate significant differences (p<0.05) as a result of the packaging system. For each parameter and for each packaging condition, mean values preceded by different letters (*z*, *y*, *x*) denote significant differences (p<0.05) as a result of the frozen time

CP control packaging, *VP* vacuum packaging, *P1* vacuum packaging combined with polyphenol extract addtiation including 29 μg *p*-coumaric acid and 19 μg ferulic acid dm^{-2} film, *P2* vacuum packaging combined with polyphenol extract addtiation including 88 μg *p*-coumaric acid and 40 μg ferulic acid dm^{-2} film

[a] Data shown at time 0 correspond to the starting raw fish

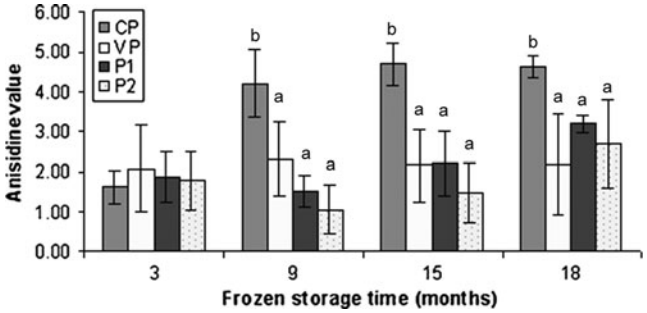

Fig. 1 Assessment of anisidine value in salmon muscle kept frozen under different packaging conditions. Average values of three (n=3) independent determinations; standard deviations are indicated by *bars*. Starting raw fish value, 0.84±0.18. For each frozen storage time, values accompanied by different letters (*a*, *b*) indicate significant differences (p<0.05) as a result of the packaging system. *CP* control packaging, *VP* vacuum packaging, *P1* vacuum packaging combined with polyphenol extract addition including 29 μg *p*-coumaric acid and 19 μg ferulic acid dm^{-2} film, *P2* vacuum packaging combined with polyphenol extract addition including 88 μg *p*-coumaric acid and 40 μg ferulic acid dm^{-2} film

going a Michael-type reaction (McMurry 1992). Such a reaction would consist of a β-attack on the unsaturated aldehyde by a nucleophile compound. As a result of an increased damage in frozen fish corresponding to 3–18 months of frozen storage, a higher nucleophile compound content would be expected to exist, this favouring the Michael attack process; consequently, the α-unsaturated aldehyde content would not be likely to increase throughout the mentioned period.

Interaction compound formation between primary and secondary lipid oxidation compounds and nucleophilic-type molecules (namely, aminated) was measured by means of their fluorescence properties (FR value). All kinds of fish samples provided a progressive increase with time (r^2=0.89–0.95, quadratic fitting), this being bigger in the case of fish corresponding to CP and VP conditions (Table 2). Comparison among packaging systems did not provide differences in the 0–9-month storage period. However, an inhibitory effect on fluorescent compound formation by means of polyphenol compound presence in the packaging films could be observed at the end of the storage time, while no effect could be inferred from the polyphenol compound content (P1 and P2 conditions comparison). Meantime, no differences could be observed between fish corresponding to CP and VP conditions throughout the whole experiment.

Increasing interaction compound formation agrees with the above-mentioned results on primary and secondary lipid oxidation compounds formation. The electrophilic character of most lipid oxidation compounds leads them to interact with food constituents possessing nucleophilic functions (Aubourg 1999; Tironi et al. 2002). Such interactions are very important secondary reactions of oxidized lipids, particularly in protein-rich foodstuffs such as marine

Table 2 Assessment of fluorescent compound formation and polyene index in salmon muscle kept frozen under different packaging conditions

Frozen storage time (months)	Fluorescent compound formation				Polyene index			
	CP	VP	P1	P2	CP	VP	P1	P2
0[a]	*z* 0.29 (0.06)	*z* 0.29 (0.06)	*z* 0.29 (0.06)	*z* 0.29 (0.06)	*y* 1.29 (0.04)	*y* 1.29 (0.04)	*y* 1.29 (0.04)	*y* 1.29 (0.04)
3	*zy* 0.36 (0.08)	*z* 0.31 (0.04)	*z* 0.31 (0.04)	*zy* 0.36 (0.06)	*y* 1.32 (0.04)	*y* 1.32 (0.05)	*y* 1.28 (0.03)	*y* 1.26 (0.11)
9	*y* 0.51 (0.10)	*y* 0.49 (0.05)	*z* 0.45 (0.12)	*y* 0.48 (0.05)	*y* 1.17 (0.10)	*y* 1.33 (0.09)	*y* 1.23 (0.04)	*y* 1.30 (0.03)
15	*x* 1.06b (0.13)	*x* 1.03ab (0.41)	*y* 0.88ab (0.27)	*x* 0.79a (0.11)	*z* 0.97 (0.02)	*z* 0.96 (0.05)	*zy* 1.08 (0.16)	*z* 1.00 (0.04)
18	*w* 2.14b (0.16)	*w* 2.35b (0.20)	*y* 1.01a (0.10)	*x* 1.08a (0.18)	*z* 0.96 (0.05)	*z* 0.99 (0.10)	*z* 0.89 (0.03)	*z* 0.95 (0.12)

Average values of three (n=3) independent determinations. Standard deviations are indicated in parenthesis. For each parameter and for each frozen storage time, mean values followed by different letters (a, b) indicate significant differences (p<0.05) as a result of the packaging system. For each parameter and for each packaging condition, mean values preceded by different letters (*z*, *y*, *x*, *w*) denote significant differences (p<0.05) as a result of the frozen time. Packaging condition abbreviations as expressed in Table 1

[a] Data shown at time 0 correspond to the starting raw fish

sources, which are reported to have high proportions of essential and reactive amino acids such as lysine and methionine (Simopoulos 1997). Lipid oxidation development has been recognized as a complex process where different kinds of molecules are produced, most of them unstable, susceptible of breakdown and originate lower weight compounds, or react with other molecules (nucleophilic-type, mostly) present in the fish muscle, so that the determination of each kind of compound cannot always provide an accurate method for the quality assessment. In the present research, reliability of the secondary lipid oxidation compounds as quality indices decreased with advanced frozen time. However, primary and tertiary (interaction compounds) lipid oxidation compounds turned out to be helpful in order to assess the lipid oxidation development throughout the whole frozen-storage period.

Lipid Hydrolysis Analysis

Although enzymatic lipid hydrolysis has been shown to occur during fish frozen storage, the formation of FFA itself does not lead to nutritional losses (Aubourg 2001). However, accumulation of FFA has been related to some extent to lack of acceptability, because FFA are known to have detrimental effects on protein properties (Sikorski and Kolakowska 1994) and oxidize faster than higher molecular weight lipid classes (namely, triacylglycerols and phospholipids) by providing a greater accessibility (lower steric hindrance) to oxygen and other pro-oxidant molecules (Labuza 1971). According to this, examining the extent of lipid hydrolysis was deemed important to the present research.

Thus, a marked FFA formation ($p<0.05$) was observed (Fig. 2) with time in all kinds of samples ($r^2=0.91–0.94$), showing that enzymes responsible for lipid hydrolysis (lipases and phospholipases, namely) are still active during the frozen storage of salmon coho at −18 °C. As in the present research, a linear FFA formation with time was already obtained for the same frozen species (Ortiz et al. 2009). However, most previous researches (Ingemansson et al. 1995; Aubourg 1999; Rodríguez et al. 2007) have shown a logarithmic fitting between FFA content and frozen time so that a marked hydrolysis increase during the first period (0–3 months at −18 °C, approximately) was found, this being explained by a maximal lipase release from liposomes during this period, which then facilitates closer proximity between enzyme and substrate (Sikorski and Kolakowski 2000). Some differences among present salmon samples can be outlined as a result of the packaging system employed (Fig. 2); however, a clear pattern cannot be observed ($p>0.05$) so that no conclusion can be carried out concerning the effect of packaging system on the lipid hydrolysis development.

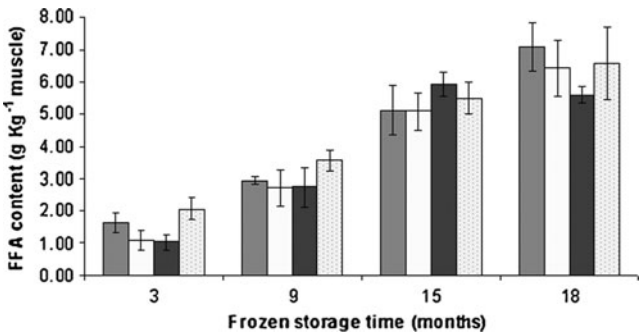

Fig. 2 FFA formation in salmon muscle kept frozen under different packaging conditions. Average values of three ($n=3$) independent determinations; standard deviations are indicated by *bars*. Starting raw fish value, 0.69±0.16 g kg^{-1} muscle. For each frozen storage time, values accompanied by different letters (*a*, *b*) indicate significant differences ($p<0.05$) as a result of the packaging system. Packaging condition abbreviations as expressed in Fig. 1

An inverse ratio between lipid content and FFA proportion in fish lipids is well known. Present research provided a lipid content ranged between 55.0 and 87.5 g kg^{-1} flesh muscle. As expected, a relatively low FFA value (16.0±3.8 g FFA kg^{-1} lipids) was observed for starting raw fish that increased sharply during the frozen storage, reaching at month 18 a range value of 62–85 g FFA kg^{-1} lipids. As in the case of considering the FFA content on muscle basis (Fig. 2), FFA assessment on lipid basis did not provide differences ($p>0.05$) as a result of the packaging condition.

Good correlation values were obtained between the FFA formation and the FR value ($r^2=0.86–0.93$). Such a result has already been observed in previous research on fatty fish species (Ortiz et al. 2009). In this sense, it has been pointed out that the release of FFA from a triacylglycerol matrix may accelerate their interaction with oxidative catalysts and hence accelerate the rate of lipid oxidation (Sista et al. 1997). This pro-oxidant effect has been explained on the basis of a catalytic effect of the carboxyl group on the formation of free radicals by the decomposition of hydroperoxides (Aubourg 2001).

Lipid Changes Related to Nutritional Value

Fish species have attracted a great attention from consumers as a source of important nutritional polyunsaturated fatty acid (PUFA) components for the human diet (Simopoulos 1997). Damage undergone by PUFA during the present study was measured by means of the PI analysis. A PI of 1.29±0.04 for starting fish was obtained, which agreed to previous data (Rodríguez et al. 2007), but was found lower than that reported for the same species obtained from wild conditions (Gruger et al. 1964). Fatty acid composition of fish species has shown an important effect of fatty acid composition of diet; particularly, a higher PUFA content has been reported for wild fish species than for their

corresponding species obtained from farming conditions (Alasalvar et al. 2002).

A slow PI decrease could be assessed in all cases throughout the frozen storage time (Table 2; r^2=0.91–0.98, quadratic fitting). Previous research has also shown an important effect of lipid oxidation development on the partial loss of PUFA content (PI decrease) during the frozen storage of the same farmed species (Rodríguez et al. 2007; Ortiz et al. 2009) and for related salmon species such as farmed Atlantic salmon (Refsgaard et al. 1998) and wild sea salmon (Tironi et al. 2010). However, in the present research, the PI did not provide good correlation values with the different lipid oxidation parameters; instead, a fair correlation was obtained with the FFA formation (r^2=0.85–0.90).

No significant PI differences could be assessed throughout the frozen storage time among the different kinds of samples, so that no effect of the vacuum and the polyphenol compound presence and content could be inferred (Table 2). In a previous research (Tironi et al. 2010), rosemary extract addition to minced sea salmon (*Pseudopercis semifasciata*) and further frozen storage at −11 °C up to 4 months proved to inhibit the loss of DHA and EPA. Also contrary to the present results, a decrease inhibition of PI was found in frozen farmed coho salmon that was previously fed with diets including additional natural antioxidants (rosemary extracts and tocopherol isomers) (Ortiz et al. 2009).

Concerning tocopherol isomer analysis, three of them (α-, γ- and δ-tocopherol) were detected in the different salmon coho samples. However, δ isomer was found in very low concentrations (<1.0 mg kg^{-1} muscle) and could not be quantified satisfactorily. Content values obtained in starting fish for α and γ isomers were 11.41±0.18 and 8.04±1.20 mg kg^{-1} muscle, respectively. As a result of feed composition provided, previous research shows varying values for the different tocopherol isomer content in farmed salmonid species (Gordon Bell et al. 1998); such differences are also related to fish-to-fish variations and varying deposition ability in different parts of the fish body (Parazo et al. 1998).

Assessment of α- and γ-tocopherol provided in most cases a progressive content decrease throughout the storage time (Table 3), this decrease being specially marked in the case of the α isomer. According to this, γ isomer content showed poor correlation values with frozen time, while α-isomer provided fair acceptable values (r^2=0.77–0.90, quadratic fitting). Tocopherols are known as endogenous antioxidants that can act as scavengers of radicals, so that protection against the very early stages of lipid oxidation would be favoured. According to this, the lipid damage index that best correlated the α- and γ-tocopherol losses was the CD formation (r^2=0.81–0.89 and r^2=0.83–0.90, respectively). Present tocopherol isomer losses during frozen storage agree to previous research on rainbow trout

Table 3 Tocopherol isomer content in salmon muscle kept frozen under different packaging conditions

Frozen storage time (months)	α-Tocopherol content (mg kg^{-1} muscle)				γ-Tocopherol content (mg kg^{-1} muscle)			
	CP	VP	P1	P2	CP	VP	P1	P2
0[a]	*x* 11.41 (0.98)	*y* 11.41 (0.98)	*x* 11.41 (0.98)	*zy* 11.41 (0.98)	*y* 8.04 (1.20)	*zy* 8.04 (1.20)	*zy* 8.04 (1.20)	*zy* 8.04 (1.20)
3	*yx* 8.37 (2.33)	*zy* 10.33 (0.85)	*yx* 11.61 (2.02)	*zy* 12.74 (3.29)	*zy* 6.36a (1.52)	*zy* 9.47bc (2.27)	*y* 8.92b (0.67)	*x* 11.75c (1.15)
9	*y* 7.19a (1.76)	*z* 9.70b (0.65)	*yx* 11.88bc (2.08)	*y* 13.78c (2.52)	*zy* 5.42a (1.65)	*y* 9.25b (0.73)	*y* 9.94b (1.93)	*yx* 10.49b (1.41)
15	*y* 6.58 (1.11)	*zy* 8.78 (2.06)	*zy* 7.88 (1.77)	*zy* 9.17 (2.19)	*y* 6.20 (1.03)	*z* 7.13 (1.16)	*z* 6.43 (1.23)	*z* 7.05 (1.69)
18	*z* 3.23a (1.54)	*z* 8.36b (2.02)	*z* 7.27b (1.98)	*z* 9.63b (0.72)	*z* 3.30a (1.60)	*z* 6.93b (1.22)	*z* 6.28b (1.38)	*z* 6.49b (1.35)

Average values of three (n=3) independent determinations. Standard deviations are indicated in parenthesis. For each parameter and for each frozen storage time, mean values followed by different letters (a, b, c) indicate significant differences (p<0.05) as a result of the packaging system. For each parameter and for each packaging condition, mean values preceded by different letters (z, y, x) denote significant differences (p<0.05) as a result of the frozen time. Packaging condition abbreviations as expressed in Table 1

[a] Data shown at time 0 correspond to the starting raw fish

(Baron et al. 2007) and farmed channel catfish (Brannan and Erickson 1996).

Lower mean values for both isomers were obtained in all cases for fish samples corresponding to control condition when compared to its counterpart fish corresponding to all packaging systems including vacuum. Further, higher α-tocopherol mean values were obtained for fish corresponding to P2 condition when compared to its counterpart from VP system. Accordingly, a partial inhibitory effect on tocopherol loss by vacuum and also by polyphenol content is inferred. Inhibition of depletion of endogenous α-tocopherol by grape polyphenols (rich in procyanidin-type compounds) has already been proven during minced fish frozen storage (Pazos et al. 2005a); in such experiment, α-tocopherol depletion proved to correlate with the evolution of lipid oxidation. In addition, presence of caffeic acid, hydroxytyrosol or propyl gallate showed to regenerate endogenous α-tocopherol from its oxidised forms resulting in an antioxidant synergy consistent with the reduction of lipid oxidation during the frozen storage of minced horse mackerel (*Trachurus trachurus*) (Pazos et al. 2005b).

Rancid Odour Assessment

A low rancid odour development was detected in all samples (Fig. 3), so that values were included in all cases below 0.5 score. Such a result agrees with previous research where beheaded coho salmon was kept frozen at −18 °C up to 15 months (Rodríguez et al. 2007). Rancid odour development has proven to be the limiting factor of acceptability for most wild fatty fish species kept under frozen conditions (Erickson 1997; Kolakowska 2003). However, according to the present results, rancid odour development was not found to be markedly relevant as demonstrated to be during the frozen storage of other farmed salmonid species such as rainbow trout (Christophersen et al. 1992) and Atlantic salmon (Refsgaard et al. 1998).

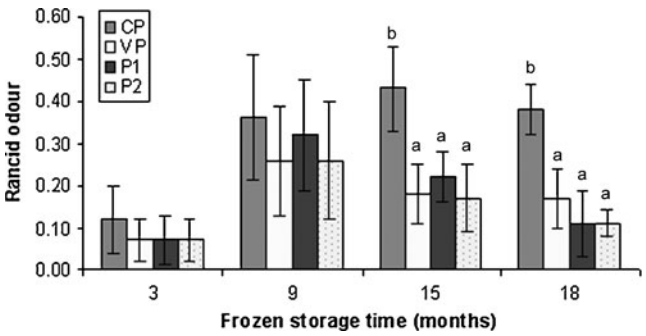

Fig. 3 Rancid odour assessment in salmon muscle kept frozen under different packaging conditions. Average values of three (n=3) independent determinations; standard deviations are indicated by *bars*. Starting raw fish value, 0.04±0.01. For each frozen storage time, values accompanied by different letters (*a*, *b*) indicate significant differences (p<0.05) as a result of the packaging system. Packaging condition abbreviations as expressed in Fig. 1

Rancid odour values increased (p<0.05) in all kinds of samples during the 0–9-month period, according to previous research (Rodríguez et al. 2007). Later on, a decreasing score was obtained in fish kept under packaging conditions including vacuum (VP, P1 and P2) and remained fairly constant in salmon corresponding to CP condition. Throughout the whole experiment, rancid odour assessment showed higher mean values for fish kept under CP conditions than in its counterpart where vacuum packaging was included (Fig. 3); differences were found significant (p<0.05) during the 15–18-month period. However, no effect could be inferred by the presence and content of polyphenol compounds in the packaging films. According to present results, sensory quality was also retained in frozen (−18 °C) rainbow trout by employment of packaging with low oxygen transmission rates (Bjerkeng and Johnsen 1995).

Among the different kinds of molecules produced as a result of lipid damage, secondary lipid oxidation compounds are considered the main compounds responsible for oxidized flavours (White 1994). However, in the present research, quite poor correlation values were obtained between rancid odour and AV assessments. Previous research has shown a strong incidence of FFA accumulation on fish quality loss (Ingemansson et al. 1995), their presence associated to some extent to the lack of sensory acceptability and strongly interrelated with off-odour development (Refsgaard et al. 2000). However, poor correlation values were obtained in the present research between rancid odour development and FFA content.

Conclusions

The present study provides a first approach to the employment of a novel packaging condition focussed on fatty fish storage under frozen conditions. In it, a combination of vacuum and polyphenol extracts from barley husk was applied to farmed coho salmon. According to data concerning primary (CD formation) and tertiary (FR value) lipid oxidation compounds, a partial inhibitory effect on rancidity development was concluded for the employment of this combined packaging. In addition, all vacuum packaging conditions tested (VP, P1 and P2) led to lower peroxide and anisidine values, to a lower rancid odour development and to an inhibitory effect on α- and γ-tocopherol losses when compared to fish corresponding to CP condition. However, no profitable effect of polyphenol presence and vacuum packaging could be inferred on the polyene index, and accordingly, on PUFA retention.

Additional quality advantages obtained as a result of the polyphenol presence in the coating medium can be

considered scarce when compared to those advantages attained by employing vacuum packaging alone. Further research is envisaged in order to optimise the qualitative and quantitative polyphenol composition present in the films to be further employed during the frozen storage of coho salmon.

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