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Relation between types of packaging, frozen storage and grilling on cholesterol and fatty acids oxidation in Atlantic hake fillets (*Merluccius hubbsi*)

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Abstract

Two different commercial samples of frozen and packaged, in low and high-oxygen permeability packaging, Atlantic hake fillets were stored at −18 °C for 4 months and the intensity of lipid oxidation, as well as the formation of cholesterol oxidation products (COP), during storage and subsequent grilling were studied. Raw fillets at the initial time of storage showed low total COP levels, however, after 120 days of storage the concentrations were raised significantly, under both packed conditions. During freezing and subsequent grilling there was a significant decrease ($p < 0.02$) in the contents of the cholesterol and polyunsaturated fatty acids in all the hake samples. Correlations were found between the cholesterol and fatty acid parameters and cholesterol oxides formation during storage and heat treatment. The commercial frozen storage with a low-oxygen permeability packaging was more effective in preventing lipid oxidation than high-oxygen permeability packaging, with less accented cholesterol degradation as well as cholesterol oxides formation.
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Keywords: Atlantic hake fillets; Frozen storage; Grilling; Cholesterol oxidation; Fatty acids; HPLC

1. Introduction

The consumption of fish has been linked to health benefits, a reduced risk of coronary heart disease, arterial hypertension, human breast cancer growth, inflammatory diseases, asthma and disorders of the immune system (Uauy & Valenzuela, 2000), which are largely attributed to the polyunsaturated fatty acids (PUFA) in fish oils (Burr, 1992). Moreover, fish products are rich in cholesterol, and it is well recognised that oxidation of the lipid fraction of fish muscle is a major cause of deterioration in fatty fish (Brannan & Erickson, 1996) due to the high degree of fatty acid unsaturation (May, Shimp, Weihrauch, & Kinsella, 1978). Polyunsaturated fatty acids are very susceptible to oxidation even under mild ambient conditions and are easily incorporated into the chain mechanism of lipid peroxidation, to yield free and peroxy radicals, which may accelerate cholesterol oxidation (Hsieh & Kinsella, 1989). A continuous increase in the amount of cholesterol oxides was accompanied by a remarkable concurrent decrease in the total amount of PUFA (Ohshima, Li, & Koizumi, 1993).

The consumption of processed and frozen food has increased due to the impositions of modern life, where food preparation time is the main factor. The temperature and time required to process food, as also the transport and storage conditions, are some of the factors which can contribute to fatty acid and cholesterol degradation. Other conditions, including pH, light, oxygen, water activity or a combination of such factors, are also important in oxidative degradation, although heat processing is the most effective factor in the formation of cholesterol oxidation products (COP) (Li, Cherian, Ahn, Hardin, & Sim, 1996; Osada, Kodama, Cui, Yamada, & Sugano, 1993; Smith, 1987).

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Freezing is considered to be an excellent process for preserving fish quality for prolonged periods of time (Santos-Yap, 1995) because quality deterioration due to micro-organisms and some biochemical processes decreases during the frozen storage of fish (Chevalier, Sequeira-Munoz, Le Bail, Simpson, & Ghoul, 2000). Frozen fish products are normally sold in light- and gas-permeable packages of 0.5–1.0 kg (Bak, Andersen, Andersen, & Bertelsen, 1999), made of polyethylene and given a shelf life of 12 months at −18 °C.

On the other hand, it is well established that during freezing and storage of frozen products, lipid hydrolysis occurs and PUFA contents decrease, with a consequent increase in the content of peroxides, important factors in the deterioration of fish meat quality (Bonnel, 1989). Some authors have found changes in the lipid composition of meat (Kowale, Rao, Babu, Sharma, & Bisht, 1996; Pie, Spahis, & Seillan, 1991; Rao, Kowale, Babu, & Bisht, 1996) and fish (Boran, Karaçam, & Boran, 2006; Tokur, Ozkütük, Atici, Ozyurt, & Ozyurt, 2006) during frozen storage. To our knowledge, studies on lipid changes during the shelf life of frozen fish and their relation to the formation of COP are not available in the literature.

The segments of the industrial fish are concentrated in captures of the main resources in volume or value of production and the hake is a species of fish in great demand by the Brazilian consumer and the volume of captures is very large in Brazil. In the present study, the effect of different types of packing during frozen storage of the Atlantic hake fillets and subsequent grilling, on the lipid composition and cholesterol oxidation, were studied.

2. Materials and methods

2.1. Samples and samples preparation

Two different commercial brands of frozen Atlantic hake fillets were obtained from a local store in Sao Paulo, Brazil, soon after distribution of the same samples by retailers. The samples represented the 2 most consumed brands in the city of Sao Paulo. The fillets of brand A were packed in polyethylene with low-oxygen permeability (0.1 mm film thickness) and for the other one, brand B, just wrapped in polyethylene film (0.6 μm film thickness). Two batches of each brand with different expiring dates were analysed. Each batch consisted of 10 packages of 500 g of the hake fillets. Two packs of each batch were analysed on the same day of acquisition of the fish fillets, corresponding to zero time. The other samples were stored at −18 °C in a domestic freezer and removed for analysis after 30, 60, 90 and 120 days. The fillets from the two packages were thawed and cut lengthwise, one half being grilled and the other analysed raw. The samples were grilled at 165 °C for 2 min on each side, until reaching an internal temperature of 75 ± 1 °C. The internal temperature was monitored using a digital calibrated thermometer (Traceable Long-Stem, VWR, Friendswood, TX, USA). The fillets were ground and homogenised in a multi-processor to obtain a homogeneous mass. Convenient aliquots were taken for the analyses, which were carried out in triplicate.

2.2. Chemicals and reagents

Cholesterol and COP standards, including 19-hydroxycholesterol (19-OH), 20α-hydroxycholesterol (20α-OH), 22(S)-hydroxycholesterol (22(S)-OH), 22(R)-hydroxycholesterol (22(R)-OH), 25-hydroxycholesterol (25-OH), 7-ketocholesterol (7-keto), 7β-hydroxycholesterol (7β-OH), 5,6α-epoxycholesterol (5,6α-Ep) and 5,6β-epoxycholesterol (5,6β-Ep) were from Sigma (Milford, MA, USA). The other COP standards, 24(S)-hydroxycholesterol (24(S)-OH), 25(R)-hydroxycholesterol (25(R)-OH) and 7α-hydroxycholesterol (7α-OH) were obtained from Steraloids (Newport, RI, USA). A total of 37 saturated, monounsaturated and polyunsaturated fatty acids standards (Supelco™ FAME Mix 18919, Bellefonte, PA, USA) and nonadecanoic methyl ester (Sigma, St. Louis, MO, USA) were used. The purities of the standards ranged from 95% to 99%.

2.3. Analytical procedure

Moisture was measured as described by A.O.A.C (2002). The lipids were extracted and determined according to Bligh and Dyer (1959).

2.3.1. Fatty acid composition

Fish oil (25 mg) was submitted to saponification and methylation using BF_3 in methanol (Joseph & Ackman, 1992). The GC instrument (3400CX) from Varian (Walnut Creek, CA, USA) was used, equipped with a split injector (1:50), fused silica CP-SIL 88 capillary column 100 m x 0.25 mm i.d., 0.20 μm film thickness (Chrompack, Middelburg, The Netherlands), flame ionisation detector and workstation. The temperature program was: initial temperature, 120 °C (8 min) followed by 15 °C/min to 160 °C (zero min), 4 °C/min to 195 °C (12 min), and 10 °C/min to final temperature of 230 °C (25 min); injector and detector temperatures 280 °C. The carrier gas was hydrogen at a flow rate of 1 ml/min, and nitrogen was used as the make-up gas at 30 ml/min. Fatty acid identification was achieved by comparison of the retention times of the sample with those of the standards and by spiking. Quantification was done using nonadecanoic methyl ester as the internal standard. Factors for conversion of fatty acids methyl esters to their corresponding triglycerides were utilised (Carpenter, Ngeh-Ngwainbi, & Lee, 1993).

2.3.2. Simultaneous determination of cholesterol and cholesterol oxides by HPLC

Cholesterol and cholesterol oxides were extracted by direct saponification (2 g of the hake fillets, 4 ml of a 50% aqueous solution of KOH and 6 ml of ethanol) at 20 °C for 22 h in the dark and the non-saponifiable matter

extracted 4 times with hexane. The hexane extract was dried, diluted with 1 ml of mobile phase and injected into the HPLC system (Saldanha, Sawaya, Eberlin, & Bragagnolo, 2006).

For HPLC, a Shimadzu liquid chromatograph (Kyoto, Japan) equipped with on-line UV–visible (SPD-10 AV_{VP}) and refractive index (RID-10AV_{VP}) detectors, rheodyne injector with a 20 µl loop, a tertiary solvent delivery system (LC-10_{VP}), oven heated column at 32 °C (CTO-10_{VP}) and software (CLASS LC-10) was used. The analytical column used was a Nova Pack CN HP 300 mm x 3.9 mm column, 4 µm (Waters, Milford, MA, USA), preceded by a Hypersil BDS CN 7.5 mm × 4.6 mm, 4 µm guard column (Alltech, Deerfield, IL, USA). The mobile-phase was *n*-hexane: 2-propanol (97:3, v/v) at a flow rate of 1 ml/min and an analysis time of 30 min. The HPLC solvents were filtered through a 22 µm Millipore filter (Bedford, MA) under vacuum prior to use. Quantification was done by external standardisation, with a concentration range from 0.3 to 70 µg/ml for the oxides and from 0.2 to 1.8 mg/ml to cholesterol. HPLC-APCI-MS was used to confirm the identity of cholesterol and cholesterol oxides in the fish samples (Saldanha et al., 2006). Cholesterol and epimeric 5,6 epoxides were quantified using a refractive index detector, the cholesterol because it is better separated and the epoxides because they do not absorb at UV wavelengths. The other oxides were quantified using the UV detector at 210 nm. The detection limits (S/N = 3) were 0.04 µg/g for 19-OH and 20α-OH; 0.06 µg/g for 22(R)-OH, 24(S)-OH, 25(R)-OH and 25-OH; 0.07 µg/g for 22(S)-OH, 7β-OH and 7α-OH; 0.01 µg/g for 7-keto and cholesterol and 0.18 µg/g for epimeric 5,6 epoxides. The recovery varied between 95% and 103% for all the studied cholesterol oxides.

2.4. Statistical analysis

A one-way analysis of variance (ANOVA) was applied to the data. The means of the different storage periods and preparation forms (raw and grilled) were compared using the Tukey multiple comparisons test, with $p < 0.02$. Software used was Origin 5.0 for Windows.

3. Results and discussion

3.1. Moisture, fat and cholesterol contents

Moisture, fat and cholesterol contents in different commercial hake low-oxygen permeability packed samples (brand A) and high-oxygen permeability packed samples (brand B) are presented in Table 1.

Considering all samples (brands A and B), the average moisture content during frozen storage varied from 78.6 ± 1.9 to 83.8 ± 0.3 g/100 g in the raw hake, and from 69.9 ± 0.7 to 74.5 ± 0.3 g/100g in the grilled hake. No significant differences were found in the moisture contents

Table 1
Moisture (g/100 g), total lipid (g/100 g, dry basis) and cholesterol (mg/100 g, dry basis) levels in low-oxygen (brand A) and high-oxygen (brand B) permeability packing hake fillets

	Time (days)									
	Zero		30		60		90		120	
	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled
Brand A										
Moisture	83.8 ± 0.3^{A}	74.5 ± 0.3^{a}	83.4 ± 0.4^{A}	74.2 ± 0.3^{a}	82.3 ± 0.2^{A}	72.3 ± 0.4^{ab}	81.6 ± 0.5^{A}	71.3 ± 0.4^{b}	80.2 ± 0.3^{A}	70.6 ± 0.2^{b}
Total lipids	8.6 ± 0.2^{E}	7.9 ± 0.3^{e}	9.0 ± 0.3^{D}	8.5 ± 0.3^{d}	9.4 ± 0.3^{CD}	8.7 ± 0.3^{c}	9.9 ± 0.1^{B}	9.3 ± 0.2^{b}	10.4 ± 0.2^{A}	9.6 ± 0.2^{a}
Cholesterol	360 ± 1^{A}	345 ± 2^{a}	350 ± 2^{B}	316 ± 2^{b}	323 ± 1^{C}	290 ± 3^{c}	292 ± 5^{D}	243 ± 2^{d}	243 ± 2^{E}	230 ± 1^{e}
Brand B										
Moisture	82.5 ± 1.6^{A}	73.4 ± 0.7^{a}	81.1 ± 0.5^{A}	72.6 ± 0.4^{a}	80.5 ± 0.8^{A}	71.8 ± 1.3^{a}	79.9 ± 0.9^{A}	70.8 ± 0.1^{a}	78.6 ± 1.9^{A}	69.9 ± 0.7^{a}
Total lipids	8.3 ± 0.4^{E}	7.7 ± 0.3^{e}	9.0 ± 0.6^{D}	8.5 ± 0.4^{d}	10.0 ± 0.2^{C}	9.4 ± 0.3^{c}	10.9 ± 0.4^{B}	10.1 ± 0.6^{b}	11.7 ± 0.5^{A}	11.0 ± 0.8^{a}
Cholesterol	337 ± 1^{A}	320 ± 3^{a}	307 ± 4^{B}	275 ± 3^{b}	283 ± 3^{C}	244 ± 1^{c}	254 ± 1^{D}	214 ± 2^{d}	223 ± 1^{E}	202 ± 2^{e}

Values are means ± standard deviation of the six samples (two lots analysed in triplicates). All the raw and grilled samples are significantly different ($p < 0.02$). Values bearing and different letters (capital letters) are significantly differences ($p < 0.02$) at storage time in the raw samples. Values bearing and different letters (small letters) are significantly differences ($p < 0.02$) at storage time in the grilled samples.

between the raw hake samples analysed. As expected, the moisture levels in the grilled samples were significantly lower ($p < 0.02$) than in the raw samples, with a decrease between 11% and 13%, due to a loss of water during the heat treatment. The loss of the moisture during grilling is time, heating temperature, size and thickness of the fillets dependent.

The lipid levels (dry basis) in the thawed raw and grilled hake samples ranged from 7.9 ± 0.3 to 10.4 ± 0.2 g/100 g in the brand A samples and from 7.7 ± 0.3 to 11.7 ± 0.5 g/100 g in the brand B samples (Table 1). No significant differences were found between low- and high-oxygen permeability packed hake with respect to fat contents. The values obtained in the present study were similar to the values obtained by Méndez and González (1997) with the same specie of fish, however, the lipid components of fish can vary according to the month of capture, the season and the particular area of sea (Gámez-Meza et al., 1999).

The lipid contents increased 21% in brand A and 41% in brand B during frozen storage. Freezing probably causes weakening of the protein–lipid linkages and more complete extraction by solvents, leading to an increase in total lipids during storage (Béltran & Moral, 1990). The difference between the samples could be explained by the different packing conditions.

The amount of lipids decreased after grilling, the losses varying from 6% to 8% for samples from batch A, and from 6 to 7% for samples from batch B. In another study (Shozen, Ohshima, Ushio, & Koizumi, 1995), the content of lipids decreased from 4% to 16% after grilling in most of the marine processed products, however, significant differences had not been observed after grilling in other evaluated samples. There is consensus that during grilling a loss in fat occurs due to dripping, although the amount lost depends on the temperature and time of grilling.

The cholesterol levels in the raw and grilled hake fillets (Table 1) varied between 230 ± 1 and 360 ± 1 mg/100 g for the brand A samples and between 202 ± 2 and 337 ± 1 mg/100 g for the brand B samples. Significant differences ($p < 0.02$) were found between the two brands, the levels in brand A being higher than the levels brand B. The cholesterol content of the fish is influenced by factors such as the geographic region, season of the year and maturity (Osman, Suriah, & Law, 2001).

During frozen storage, the level of cholesterol decreased in all the samples evaluated. After 120 days, the losses in raw samples were in the order of 33% in brand A and

Table 2
Saturated fatty acids composition (g/100 g oil) in low-oxygen (brand A) and high-oxygen (brand B) permeability packing hake fillets

Fatty acids	Zero time		30 days		60 days		90 days		120 days	
	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled
Brand A										
Lauric C12:0	0.50 ± 0.01^A	0.43 ± 0.02^a	0.49 ± 0.03^A	0.41 ± 0.02^a	0.48 ± 0.02^A	0.40 ± 0.02^a	0.50 ± 0.01^A	0.41 ± 0.01^a	0.48 ± 0.03^A	0.40 ± 0.01^a
Myristic C14:0	3.35 ± 0.2^A	3.16 ± 0.2^a	3.29 ± 0.1^A	3.15 ± 0.2^a	3.31 ± 0.1^A	3.17 ± 0.2^a	3.30 ± 0.3^A	3.13 ± 0.2^a	3.32 ± 0.1^A	3.16 ± 0.2^a
Pentadecanoic C15:0	0.75 ± 0.03^A	0.67 ± 0.1^a	0.71 ± 0.04^A	0.65 ± 0.03^a	0.73 ± 0.01^A	0.62 ± 0.1^a	0.75 ± 0.05^A	0.61 ± 0.05^a	0.72 ± 0.02^A	0.63 ± 0.01^a
Palmitic C16:0	12.2 ± 0.4^A	11.9 ± 0.4^a	12.2 ± 0.2^A	11.8 ± 0.4^a	12.2 ± 0.3^A	11.8 ± 0.2^a	12.2 ± 0.4^A	11.8 ± 0.2^a	12.2 ± 0.5^A	11.8 ± 0.3^a
Heptadecanoic C17:0	0.25 ± 0.05^A	0.19 ± 0.03^a	0.22 ± 0.01^A	0.16 ± 0.05^a	0.23 ± 0.02^A	0.17 ± 0.02^a	0.27 ± 0.01^A	0.18 ± 0.01^a	0.25 ± 0.03^A	0.19 ± 0.01^a
Stearic C18:0	2.86 ± 0.6^A	2.37 ± 0.2^a	2.89 ± 0.2^A	2.31 ± 0.3^a	2.84 ± 0.01^A	2.39 ± 0.3^a	2.85 ± 0.2^A	2.35 ± 0.3^a	2.88 ± 0.2^A	2.32 ± 0.1^a
Arachidic C20:0	0.93 ± 0.02^A	0.76 ± 0.03^a	0.89 ± 0.01^A	0.75 ± 0.01^a	0.86 ± 0.05^A	0.74 ± 0.02^a	0.87 ± 0.01^A	0.70 ± 0.01^a	0.91 ± 0.01^A	0.72 ± 0.01^a
Behenic C22:0	0.51 ± 0.05^A	0.42 ± 0.01^a	0.46 ± 0.02^A	0.40 ± 0.02^a	0.45 ± 0.01^A	0.39 ± 0.01^a	0.48 ± 0.02^A	0.39 ± 0.02^a	0.49 ± 0.03^A	0.33 ± 0.01^a
Lignoceric C24:0	0.37 ± 0.08^A	0.28 ± 0.02^a	0.34 ± 0.01^A	0.26 ± 0.01^a	0.31 ± 0.02^A	0.22 ± 0.02^a	0.38 ± 0.01^A	0.26 ± 0.01^a	0.35 ± 0.02^A	0.24 ± 0.02^a
$\sum$ SFA	21.8 ± 0.1^A	20.2 ± 0.1^a	21.5 ± 0.1^B	19.9 ± 0.09^b	21.4 ± 0.06^B	20.0 ± 0.09^b	21.6 ± 0.08^C	19.8 ± 0.08^c	21.6 ± 0.1^C	19.8 ± 0.07^c
Brand B										
Lauric C12:0	1.20 ± 0.1^A	1.13 ± 0.2^a	1.18 ± 0.4^A	1.09 ± 0.3^a	1.15 ± 0.1^A	1.03 ± 0.09^a	1.17 ± 0.2^A	1.07 ± 0.05^a	1.19 ± 0.1^A	1.09 ± 0.02^a
Myristic C14:0	3.27 ± 0.3^A	3.15 ± 0.4^a	3.25 ± 0.5^A	3.11 ± 0.1^a	3.22 ± 0.4^A	3.09 ± 0.2^a	3.29 ± 0.2^A	3.12 ± 0.5^a	3.24 ± 0.3^A	3.15 ± 0.4^a
Pentadecanoic C15:0	0.60 ± 0.02^A	0.55 ± 0.02^a	0.58 ± 0.01^A	0.52 ± 0.01^a	0.56 ± 0.02^A	0.49 ± 0.05^a	0.55 ± 0.02^A	0.48 ± 0.05^a	0.55 ± 0.05^A	0.46 ± 0.04^a
Palmitic C16:0	10.9 ± 0.8^A	10.2 ± 0.5^a	10.8 ± 0.7^A	10.2 ± 0.3^a	10.8 ± 0.6^A	10.2 ± 0.6^a	10.9 ± 0.3^A	10.1 ± 0.3^a	10.8 ± 0.2^A	10.3 ± 0.1^a
Heptadecanoic C17:0	0.16 ± 0.02^A	0.15 ± 0.03^a	0.15 ± 0.02^A	0.13 ± 0.01^a	0.15 ± 0.03^A	0.14 ± 0.02^a	0.14 ± 0.03^A	0.12 ± 0.02^a	0.13 ± 0.01^A	0.11 ± 0.02^a
Stearic C18:0	2.46 ± 0.2^A	2.29 ± 0.3^a	2.41 ± 0.1^A	2.23 ± 0.2^a	2.38 ± 0.2^A	2.26 ± 0.3^a	2.40 ± 0.2^A	2.30 ± 0.2^a	2.42 ± 0.1^A	2.29 ± 0.1^a
Arachidic C20:0	0.15 ± 0.01^A	0.14 ± 0.01^a	0.15 ± 0.02^A	0.14 ± 0.01^a	0.14 ± 0.02^A	0.12 ± 0.01^a	0.13 ± 0.03^A	0.12 ± 0.01^a	0.13 ± 0.01^A	0.11 ± 0.01^a
Lignoceric C24:0	0.34 ± 0.01^A	0.29 ± 0.03^a	0.33 ± 0.03^A	0.29 ± 0.01^a	0.32 ± 0.04^A	0.25 ± 0.01^a	0.31 ± 0.05^A	0.26 ± 0.01^a	0.34 ± 0.01^A	0.24 ± 0.01^a
$\sum$ SFA	19.0 ± 0.2^A	17.9 ± 0.2^a	18.8 ± 0.2^B	17.7 ± 0.1^b	18.8 ± 0.1^B	17.6 ± 0.1^c	18.9 ± 0.1^C	17.6 ± 0.2^c	18.8 ± 0.00^C	17.7 ± 0.09^d

Values are means ± standard deviation of the six analysis (two lots analysed in triplicates). All the raw and grilled samples are significantly different ($p < 0.02$). Values bearing and different letters (small letters) are significantly differences ($p < 0.02$) at storage time in the raw samples. Values bearing and different letters (capital letters) are significantly differences ($p < 0.02$) at storage time in the grilled samples.

34% in brand B. The cholesterol content after grilling was significantly ($p < 0.02$) lower than in the raw samples. Other authors have found similar effects in cooked and fried fish (Candela, Astiasarán, & Bello, 1997; May et al., 1978). The decrease in cholesterol content during storage and after grilling could be attributed to oxidative processes. On the contrary, Shozen et al. (1995) did not observe remarkable changes in the cholesterol contents after grilling of fish samples.

3.2. Fatty acids contents

The effects of freezing and subsequent grilling on the individual fatty acids of Atlantic hake fillets are presented in Tables 2–5 expressed in g/100 g of oil. The main fatty acids were oleic, docosahexaenoic (DHA), eicosapentaenoic (EPA), palmitic and palmitoleic acid in both samples, however, not in the same amounts. Brand A samples contained higher amounts of oleic, palmitoleic and palmitic acid and lesser amounts of EPA and DHA than the brand B samples. The same fatty acids were found for both brands with the exception of behenic acid (C22:0), which was present only in brand A. Significant differences ($p < 0.02$) were determined in the content of fatty acids for both brands. Brand B showed higher total PUFA amounts than brand A. The lipid components and contents of fish vary according to the species, age, location, species origin characteristics, such as spawning and migration seasons, and also some environmental conditions, such as temperature (Huss, 1988).

After 120 days of storage, a significant decrease ($p < 0.02$) in the total amounts of the polyunsaturated fatty acids (PUFA) were observed with losses of 41% for brand A and 50% for brand B. The decrease in the PUFA concentrations is commonly attributable to oxidation and it is normally accepted that the oxidation rate increases dramatically with the degree of unsaturation (Hsieh & Kinsella, 1989).

After grilling a significant decrease ($p < 0.02$) was observed in the total MUFA and PUFA contents. The maximum losses found, in the MUFA and PUFA levels, were from 20% and 30% in brand A and from 18% and 36% in brand B samples. Unsaturated fatty acids are much more susceptible to oxidation than their saturated counterparts and in aquatic species it was demonstrated that PUFA contents decrease during storage and after cooking (Candela, Astiasáran, & Bello, 1998; Ohshima, Shozen, Ushio, & Koizumi, 1996; Shozen et al., 1995), behavior also observed in the present study.

It is known that EPA and DHA have an essential role in human diet to prevent diseases. Since these compounds are typical of seafood and hake fillets contain higher amounts of these fatty acids, the losses observed in the EPA and DHA contents are especially important. Grilling produced reduction from 20% to 39% for EPA and 17% to 33% for DHA, in both hake brands. The losses of these products were probably related to autoxidation of the lipids. These results are in agreement with other researches (Ohshima et al., 1996; Shozen et al., 1995), which reported a decrease of the EPA and DHA levels during grilling in fish samples.

Trans fatty acid (C18:1, 9*t* and C18:2, 9*t*, 12*t*) levels in the hake fillets varied between 0.34 ± 0.01 and 1.70 ± 0.1 g/100 g in raw and between 0.46 ± 0.02 and 2.07 ± 0.05 g/100 g of oil in the grilled samples, considering both brands. These fatty acids were significantly reduced during frozen storage ($p < 0.02$), similar trend to the PUFA

Table 3
Monounsaturated fatty acids composition (g/100 g oil) in low-oxygen (brand A) and high-oxygen (brand B) permeability packing hake fillets

Fatty acids	Zero time		30 days		60 days		90 days		120 days	
	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled
Brand A										
Myristoleic C14:1 ω5	0.68 ± 0.01^{A}	0.49 ± 0.02^{a}	0.62 ± 0.01^{B}	0.37 ± 0.03^{b}	0.55 ± 0.01^{C}	0.30 ± 0.01^{c}	0.50 ± 0.02^{D}	0.28 ± 0.05^{c}	0.46 ± 0.04^{D}	0.21 ± 0.01^{d}
Pentadecenoic C15:1 ω6	0.17 ± 0.02^{A}	0.13 ± 0.01^{a}	0.14 ± 0.02^{AB}	0.10 ± 0.01^{ab}	0.13 ± 0.02^{AB}	0.09 ± 0.00^{bc}	0.11 ± 0.06^{B}	0.08 ± 0.00^{bc}	0.10 ± 0.02^{B}	0.06 ± 0.00^{cd}
Palmitoleic C16:1 ω7	12.4 ± 0.8^{A}	10.2 ± 0.6^{a}	11.7 ± 0.7^{B}	10.0 ± 0.6^{b}	11.0 ± 0.8^{C}	9.00 ± 0.6^{c}	10.2 ± 0.5^{D}	8.27 ± 0.5^{d}	9.86 ± 0.3^{E}	7.96 ± 0.7^{e}
Heptadecenoic C17:1 ω7	2.43 ± 0.4^{A}	1.75 ± 0.1^{a}	2.27 ± 0.4^{AB}	1.46 ± 0.4^{b}	2.04 ± 0.6^{B}	1.15 ± 0.2^{c}	1.92 ± 0.1^{BC}	0.89 ± 0.02^{d}	1.85 ± 0.5^{C}	0.74 ± 0.05^{e}
Oleic C18:1 ω9	28.9 ± 1.0^{A}	26.5 ± 1.0^{a}	27.3 ± 0.9^{B}	24.9 ± 0.5^{b}	25.7 ± 0.8^{C}	22.9 ± 0.7^{c}	24.8 ± 1.0^{D}	21.4 ± 0.9^{d}	24.0 ± 0.8^{E}	20.0 ± 1.0^{e}
Eicosenoic C20:1 ω11	0.25 ± 0.02^{A}	0.18 ± 0.03^{a}	0.21 ± 0.01^{AB}	0.16 ± 0.01^{ab}	0.18 ± 0.02^{B}	0.13 ± 0.02^{bc}	0.15 ± 0.02^{BC}	0.10 ± 0.01^{e}	0.14 ± 0.05^{BC}	0.09 ± 0.00^{c}
Erucic C22:1 ω9	0.17 ± 0.01^{A}	0.14 ± 0.05^{a}	0.16 ± 0.02^{A}	0.13 ± 0.01^{ab}	0.14 ± 0.01^{AB}	0.10 ± 0.03^{bc}	0.13 ± 0.01^{B}	0.08 ± 0.02^{cd}	0.12 ± 0.01^{B}	0.06 ± 0.00^{d}
Nervonic C24:1 ω9	0.31 ± 0.02^{A}	0.24 ± 0.02^{a}	0.27 ± 0.02^{AB}	0.22 ± 0.01^{ab}	0.26 ± 0.01^{B}	0.19 ± 0.01^{bc}	0.22 ± 0.01^{BC}	0.15 ± 0.01^{c}	0.20 ± 0.01^{C}	0.10 ± 0.01^{d}
∑ MUFA	45.2 ± 0.2^{A}	39.6 ± 0.2^{a}	42.6 ± 0.1^{B}	37.3 ± 0.2^{b}	40.0 ± 0.3^{C}	33.9 ± 0.2^{c}	37.8 ± 0.2^{D}	31.3 ± 0.2^{d}	36.7 ± 0.2^{E}	29.2 ± 0.2^{e}
Brand B										
Myristoleic C14:1 ω5	0.90 ± 0.01^{A}	0.74 ± 0.02^{a}	0.82 ± 0.05^{B}	0.62 ± 0.02^{b}	0.74 ± 0.01^{C}	0.51 ± 0.02^{c}	0.66 ± 0.01^{D}	0.43 ± 0.02^{d}	0.60 ± 0.03^{E}	0.36 ± 0.02^{e}
Pentadecenoic C15:1 ω6	0.23 ± 0.02^{A}	0.16 ± 0.01^{a}	0.20 ± 0.03^{AB}	0.12 ± 0.01^{b}	0.16 ± 0.03^{B}	0.09 ± 0.00^{c}	0.15 ± 0.01^{B}	0.09 ± 0.00^{cd}	0.14 ± 0.01^{B}	0.07 ± 0.00^{d}
Palmitoleic C16:1 ω7	8.70 ± 0.5^{A}	7.89 ± 0.4^{a}	8.19 ± 0.2^{B}	7.26 ± 0.6^{b}	7.68 ± 0.3^{C}	7.08 ± 0.2^{c}	7.16 ± 0.1^{D}	6.67 ± 0.5^{d}	6.80 ± 0.3^{E}	6.21 ± 0.3^{e}
Heptadecenoic C17:1 ω7	0.97 ± 0.03^{A}	0.82 ± 0.05^{a}	0.81 ± 0.03^{B}	0.73 ± 0.02^{b}	0.69 ± 0.02^{C}	0.50 ± 0.02^{c}	0.53 ± 0.01^{D}	0.38 ± 0.02^{d}	0.45 ± 0.01^{E}	0.29 ± 0.01^{e}
Oleic C18:1 ω9	27.0 ± 1.0^{A}	22.5 ± 0.9^{a}	25.4 ± 0.6^{AB}	21.2 ± 0.8^{ab}	24.5 ± 0.5^{B}	20.3 ± 0.6^{b}	23.4 ± 0.5^{BC}	19.5 ± 0.9^{bc}	22.6 ± 0.1^{C}	18.4 ± 0.7^{cd}
Eicosenoic C20:1 ω11	0.58 ± 0.05^{A}	0.46 ± 0.06^{a}	0.51 ± 0.04^{B}	0.40 ± 0.01^{b}	0.45 ± 0.01^{C}	0.33 ± 0.01^{c}	0.41 ± 0.01^{CD}	0.28 ± 0.02^{d}	0.39 ± 0.01^{D}	0.24 ± 0.02^{d}
Erucic C22:1 ω9	0.46 ± 0.02^{A}	0.39 ± 0.04^{a}	0.40 ± 0.01^{B}	0.33 ± 0.02^{b}	0.35 ± 0.01^{C}	0.26 ± 0.01^{c}	0.30 ± 0.01^{D}	0.22 ± 0.01^{c}	0.27 ± 0.01^{D}	0.17 ± 0.03^{d}
Nervonic C24:1 ω9	5.30 ± 0.2^{A}	4.83 ± 0.1^{a}	5.16 ± 0.3^{AB}	4.49 ± 0.2^{b}	4.85 ± 0.1^{B}	4.16 ± 0.4^{c}	4.43 ± 0.3^{C}	3.78 ± 0.2^{d}	4.25 ± 0.1^{C}	3.51 ± 0.4^{e}
∑ MUFA	44.1 ± 0.2^{A}	37.8 ± 0.2^{a}	41.5 ± 0.1^{B}	35.1 ± 0.2^{b}	39.4 ± 0.1^{C}	33.2 ± 0.1^{c}	37.0 ± 0.1^{D}	31.4 ± 0.2^{d}	35.5 ± 0.07^{E}	29.2 ± 0.1^{e}

Values are means ± standard deviation of the six analysis (two lots analysed in triplicates). All the raw and grilled samples are significantly different ($p < 0.02$). Values bearing and different letters (capital letters) are significantly differences ($p < 0.02$) at storage time in the raw samples. Values bearing and different letters (small letters) are significantly differences ($p < 0.02$) at storage time in the grilled samples.

Table 4
Polyunsaturated fatty acids composition (g/100 g oil) in low-oxygen (brand A) and high-oxygen (brand B) permeability packing hake fillets

Fatty acids	Zero time		30 days		60 days		90 days		120 days	
	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled
Brand A										
Linoleic C18:2 ω6	0.06 ± 0.00^{A}	0.04 ± 0.00^{a}	0.05 ± 0.00^{AB}	0.03 ± 0.00^{a}	0.04 ± 0.00^{AB}	0.03 ± 0.00^{a}	0.03 ± 0.00^{BC}	0.01 ± 0.00^{b}	0.02 ± 0.00^{C}	0.009 ± 0.00^{c}
Linolenic C18:3 ω3	0.07 ± 0.00^{A}	0.05 ± 0.00^{a}	0.06 ± 0.00^{A}	0.04 ± 0.00^{a}	0.05 ± 0.00^{AB}	0.03 ± 0.00^{ab}	0.04 ± 0.00^{BC}	0.02 ± 0.00^{bc}	0.03 ± 0.00^{C}	0.01 ± 0.00^{c}
γ-Linolenic C18:3 ω6	2.64 ± 0.3^{A}	2.15 ± 0.1^{a}	2.29 ± 0.4^{B}	1.84 ± 0.1^{b}	1.98 ± 0.3^{C}	1.42 ± 0.2^{c}	1.75 ± 0.4^{D}	1.27 ± 0.1^{d}	1.53 ± 0.2^{E}	1.00 ± 0.1^{e}
Arachidonic C20:4 ω6	0.89 ± 0.05^{A}	0.65 ± 0.01^{a}	0.71 ± 0.02^{B}	0.52 ± 0.01^{b}	0.63 ± 0.02^{C}	0.44 ± 0.02^{c}	0.54 ± 0.05^{D}	0.38 ± 0.01^{d}	0.42 ± 0.02^{E}	0.29 ± 0.01^{e}
EPA C20:5 ω3	6.79 ± 0.3^{A}	4.92 ± 0.3^{a}	5.94 ± 0.1^{B}	4.35 ± 0.5^{b}	5.12 ± 0.3^{C}	3.89 ± 0.3^{c}	4.75 ± 0.6^{D}	3.26 ± 0.2^{d}	3.97 ± 0.1^{E}	2.43 ± 0.1^{e}
DHA C22:6 ω3	13.0 ± 0.8^{A}	10.5 ± 0.7^{a}	11.6 ± 0.5^{B}	9.54 ± 0.6^{b}	9.98 ± 0.4^{C}	7.29 ± 0.8^{c}	8.74 ± 0.7^{D}	6.69 ± 0.3^{d}	7.89 ± 0.1^{E}	5.95 ± 0.2^{e}
∑ PUFA	23.4 ± 0.2^{A}	18.3 ± 0.2^{a}	20.7 ± 0.3^{B}	16.3 ± 0.3^{b}	17.8 ± 0.2^{C}	13.1 ± 0.2^{c}	15.9 ± 0.35^{D}	11.6 ± 0.1^{d}	13.9 ± 0.1^{E}	9.68 ± 0.06^{e}
Brand B										
Linoleic C18:2 ω6	0.37 ± 0.00^{A}	0.25 ± 0.03^{a}	0.32 ± 0.01^{B}	0.20 ± 0.01^{b}	0.26 ± 0.01^{C}	0.16 ± 0.03^{bc}	0.20 ± 0.01^{D}	0.13 ± 0.01^{c}	0.15 ± 0.01^{E}	0.08 ± 0.00^{d}
Linolenic C18:3 ω3	0.71 ± 0.01^{A}	0.60 ± 0.01^{a}	0.60 ± 0.02^{B}	0.49 ± 0.02^{b}	0.52 ± 0.03^{C}	0.36 ± 0.02^{c}	0.43 ± 0.02D	0.24 ± 0.01^{d}	0.34 ± 0.01^{E}	0.19 ± 0.02^{d}
γ-Linolenic C18:3 ω6	2.46 ± 0.1^{A}	2.11 ± 0.2^{a}	2.10 ± 0.1^{B}	1.85 ± 0.3^{b}	1.87 ± 0.4^{C}	1.31 ± 0.2^{c}	1.54 ± 0.3D	1.12 ± 0.1^{d}	1.28 ± 0.2^{E}	0.86 ± 0.02^{e}
Arachidonic C20:4 ω6	1.13 ± 0.2^{A}	0.90 ± 0.01^{a}	0.99 ± 0.01^{B}	0.72 ± 0.03^{b}	0.84 ± 0.04^{C}	0.53 ± 0.01^{c}	0.73 ± 0.01^{D}	0.36 ± 0.01^{d}	0.64 ± 0.01^{E}	0.27 ± 0.01^{e}
EPA C20:5 ω3	10.6 ± 0.6^{A}	8.49 ± 0.3^{a}	8.27 ± 0.5^{B}	6.32 ± 0.3^{b}	6.84 ± 0.1^{C}	4.28 ± 0.2^{c}	5.16 ± 0.1^{D}	3.89 ± 0.3^{c}	4.32 ± 0.2^{E}	2.72 ± 0.1^{d}
DHA C22:6 ω3	14.3 ± 0.4^{A}	11.9 ± 0.5^{a}	12.43 ± 0.6^{B}	9.43 ± 0.2^{b}	10.7 ± 0.1^{C}	8.67 ± 0.2^{c}	9.00 ± 0.4^{D}	7.45 ± 0.1^{d}	7.95 ± 0.2^{E}	5.32 ± 0.1^{e}
∑ PUFA	29.5 ± 0.2^{A}	24.2 ± 0.2^{a}	24.7 ± 0.2^{B}	19.0 ± 0.1^{b}	21.0 ± 0.1^{C}	15.3 ± 0.1^{c}	17.1 ± 0.1^{D}	13.2 ± 0.1^{c}	14.7 ± 0.1^{E}	9.44 ± 0.04^{e}

Values are means ± standard deviation of the six analysis (two lots analysed in triplicates). All the raw and grilled samples are significantly different ($p < 0.02$). Values bearing and different letters (capital letters) are significantly differences ($p < 0.02$) at storage time in the raw samples. Values bearing and different letters (small letters) are significantly differences ($p < 0.02$) at storage time in the grilled samples.

Table 5
Trans fatty acids (g/100 g oil), ∑ ω6, ∑ ω3 and ω6/ ω3 in low-oxygen (brand A) and high-oxygen (brand B) permeability packing hake fillets

Fatty acids	Zero time		30 days		60 days		90 days		120 days	
	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled
Brand A										
Elaidic C18:1 ω9	0.39 ± 0.00^{A}	0.43 ± 0.00^{a}	0.33 ± 0.00^{B}	0.40 ± 0.00a	0.28 ± 0.00^{C}	0.32 ± 0.01^{bc}	0.23 ± 0.05^{D}	0.28 ± 0.01^{c}	0.21 ± 0.01^{D}	0.27 ± 0.03^{c}
Linolelaidic C18:2 ω6*	0.23 ± 0.02^{A}	0.36 ± 0.01^{a}	0.20 ± 0.03^{A}	0.31 ± 0.01^{b}	0.16 ± 0.03^{AB}	0.27 ± 0.01^{b}	0.13 ± 0.02^{B}	0.20 ± 0.02^{c}	0.13 ± 0.02^{B}	0.19 ± 0.01^{c}
∑ trans	0.62 ± 0.01^{A}	0.79 ± 0.00^{a}	0.53 ± 0.01^{B}	0.71 ± 0.00^{b}	0.44 ± 0.01^{C}	0.59 ± 0.01^{c}	0.39 ± 0.03^{C}	0.48 ± 0.01^{d}	0.34 ± 0.01^{CD}	0.46 ± 0.02^{d}
∑ ω6	3.76 ± 0.1^{A}	2.97 ± 0.02^{a}	3.19 ± 0.1^{B}	2.49 ± 0.02^{b}	2.78 ± 0.08^{C}	1.98 ± 0.05^{c}	2.43 ± 0.1^{D}	1.74 ± 0.02^{d}	2.07 ± 0.06^{E}	1.36 ± 0.02^{e}
∑ ω3	19.8 ± 0.4^{A}	15.4 ± 0.3^{a}	17.6 ± 0.2^{B}	13.9 ± 0.4^{b}	15.2 ± 0.2^{C}	11.2 ± 0.4^{c}	13.5 ± 0.4^{D}	9.97 ± 0.2^{d}	11.9 ± 0.0^{E}	8.39 ± 0.1^{d}
ω3/ω6	5.27 ± 0.2^{E}	5.19 ± 0.2^{e}	5.52 ± 0.1^{D}	5.59 ± 0.3^{d}	5.44 ± 0.1^{C}	5.66 ± 0.2^{C}	5.56 ± 0.2^{B}	5.72 ± 0.1^{b}	5.74 ± 0.00^{A}	6.17 ± 0.00^{a}
Brand B										
Elaidic C18:1 ω9	0.62 ± 0.01A	0.76 ± 0.01a	0.55 ± 0.01B	0.67 ± 0.02b	0.47 ± 0.01C	0.58 ± 0.02c	0.39 ± 0.01D	0.50 ± 0.02d	0.28 ± 0.01E	0.41 ± 0.02e
Linolelaidic C18:2 ω6	1.08 ± 0.2A	1.31 ± 0.1a	0.95 ± 0.01B	1.16 ± 0.2b	0.86 ± 0.01C	0.92 ± 0.4c	0.74 ± 0.01D	0.85 ± 0.02d	0.60 ± 0.03E	0.74 ± 0.03e
∑ trans	1.70 ± 0.1A	2.07 ± 0.05a	1.50 ± 0.01B	1.83 ± 0.1b	1.33 ± 0.01C	1.50 ± 0.2c	1.13 ± 0.01D	1.35 ± 0.02c	0.88 ± 0.02E	1.15 ± 0.02cd
∑ ω6	4.19 ± 0.08A	3.42 ± 0.06a	3.61 ± 0.03B	2.89 ± 0.03b	3.13 ± 0.1C	2.09 ± 0.06c	2.62 ± 0.08D	1.70 ± 0.03d	2.21 ± 0.06E	1.28 ± 0.01e
∑ ω3	25.5 ± 0.3A	21.0 ± 0.3a	21.3 ± 0.4B	16.2 ± 0.2b	18.0 ± 0.07C	13.3 ± 0.1c	14.6 ± 0.2D	11.6 ± 0.1d	12.6 ± 0.1E	8.23 ± 0.07e
ω3/ω6	5.00 ± 0.2D	6.12 ± 0.2d	5.90 ± 0.2A	5.61 ± 0.1e	5.76 ± 0.08B	6.36 ± 0.08c	5.56 ± 0.1C	6.81 ± 0.06a	5.70 ± 0.08B	6.43 ± 0.08b

Values are means ± standard deviation of the six analysis (two lots analysed in triplicates). All the raw and grilled samples are significantly different ($p < 0.02$). Values bearing and different letters (capital letters) are significantly differences ($p < 0.02$) at storage time in the raw samples. Values bearing and different letters (small letters) are significantly differences ($p < 0.02$) at storage time in the grilled samples.

contents. However, a significant increase was observed after grilling for all the evaluated samples. Candela et al. (1997) observed that the *trans* fatty acids were not uniformly affected by cooking in fish, however, sole showed the highest level of brassidic *trans* acid (22:1ω9) which increased with cooking. In other studies a significant decrease in levels of the *trans* fatty acids (C16:1, 9*t*; C18:1, 9*t*) during frying and warm holding were found in

salmon, mackerel and sardines samples, and only a significant increase of the linolelaidic acid was observed in fried mackerel (Candela et al., 1998).

Considering the initial time, the ratio of ω3/ω6 in the thawed hake were similar in brand A (5.27 ± 0.2) and brand B (5.00 ± 0.2). In the tissues of marine fish the ratio, on average varied from 5 to 10 (Ahlgren, Blomqvist, Boberg, & Gustafsson, 1994), however, Gladyshev, Sushchik, Gubanenko, Demirchieva, and Kalachova (2006a) observed the ratio to be about 16 in unfrozen humpback salmon. Frozen storage combined with grilling significantly affected the ratio of ω3/ω6, even so these ratios were not uniformly modified in both brands. In brand A the ratio remained constant during storage and after grilling, with significant increase only after 120 days in grilled samples as compared to the thawed samples at zero time. In brand B the ratio of ω3/ω6 was increased during storage and after grilling, except in grilled samples at 30 days. Other authors reported a decrease of ω3/ω6 during a different heat treatment of fish samples (Candela et al., 1998; Gladyshev et al., 2006a, Gladyshev, Sushchik, Gubanenko, Demirchieva, & Kalachova, 2006b), however Al-Saghir et al. (2004) observed that the ω3/ω6 ratio remained constant after frying salmon fillets in olive oil.

3.3. Cholesterol oxides contents

The cholesterol oxide contents, calculated on a dry weight basis are shown on Table 6. In this study twelve cholesterol oxides (Fig. 1) were evaluated, however, only nine products were determined in hake fillets samples. The oxides 19-OH, 22(R)-OH, 24(S)-OH, 22(S)-OH, 25-OH and 5,6β-Ep were found in both samples; 25(R)-OH and 5,6α-Ep in the brand A samples, and 20α-OH in the brand B samples. Many previous studies (Ohshima, 2002; Ohshima et al., 1993; Shozen et al., 1995) showed that 7-keto, 7β-OH, 7α-OH and the epimeric epoxides were the main oxides found in fish foods. In the present study, a significant number of the oxides originated from the lateral chain and only 19-OH, 5,6α-Ep and 5,6β-Ep originated from the main chain. It is important to point out the absence of 7-keto, the most common product found in food samples and considered as a marker of cholesterol autoxidation. The products 19-OH and 25-OH were prominent oxides in all the studied samples. 25-OH is recognised together with triol and epoxides as the most toxic agents. The tertiary carbons (e.g., at position 25) would tend to form relatively stable radicals, thereby favouring formation of peroxides at this position (Finocchiaro & Richardson, 1983). The oxidation at position 25 probably occurs by a superposition of the bilayers of cholesterol making this position at the same time more reactive and more exposed to the attack of reagents than the other atoms (Korahani, Bascoul, & de Paulet, 1982). On the other hand, it must be considered the metabolic origin. In relation to 19-OH, neither autoxidation nor metabolism origin for this sterol has been elucidated, although some studies have found this product in mutton meat (Kowale et al., 1996), buffalo meat (Rao et al., 1996) and fish samples (Saldanha et al., 2006). Probably, the 19-OH had a metabolic origin in fish or the proximity of position 19 with the double bond between C_5 and C_6 made the C_{19} more susceptible to attack from

Table 6
Cholesterol oxides levels (μg/100g, dry basis) in low-oxygen (brand A) and high-oxygen (brand B) permeability packing hake fillets

Cholesterol oxides	Zero time		30 days		60 days		90 days		120 days	
	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled	Raw	Grilled
Brand A										
19-OH	5.6 ± 0.2^E	8.7 ± 0.4^e	8.2 ± 0.1^D	12.3 ± 0.5^d	11.6 ± 0.1^C	18.8 ± 0.8^c	15.6 ± 0.3^B	27.8 ± 0.2^b	18.4 ± 0.5^A	35.4 ± 0.3^a
22(R)-OH	1.9 ± 0.1^E	7.5 ± 0.4^e	3.7 ± 0.2^D	10.3 ± 0.3^d	5.8 ± 0.3^C	12.4 ± 0.2^c	8.3 ± 0.1^C	17.8 ± 0.3^b	12.4 ± 0.3^A	22.7 ± 0.2^a
24(S)-OH	0.6 ± 0.05^E	1.2 ± 0.1^e	2.3 ± 0.2^D	4.5 ± 0.2^d	4.2 ± 0.1^C	8.9 ± 0.2^c	6.9 ± 0.2^B	11.4 ± 0.3^b	13.8 ± 0.1^A	27.5 ± 0.3^a
22(S)-OH	0.7 ± 0.02^E	2.5 ± 0.2^e	1.7 ± 0.1^D	3.8 ± 0.4^d	3.3 ± 0.2^C	5.1 ± 0.2^c	5.4 ± 0.3^B	7.8 ± 0.3^b	8.7 ± 0.1^A	14.4 ± 0.2^a
25-OH	4.6 ± 0.3^E	10.3 ± 0.1^e	8.9 ± 0.1^D	17.3 ± 0.2^d	12.8 ± 0.2^C	31.6 ± 0.2^c	28.5 ± 0.6^B	42.2 ± 0.9^b	51.3 ± 1.0^A	94.2 ± 1.5^a
25(R)-OH	–	–	1.3 ± 0.1^D	4.5 ± 0.1^d	5.7 ± 0.2^C	9.9 ± 0.3^c	8.8 ± 0.1^B	16.60 ± 1^b	12.5 ± 0.6^A	36.6 ± 1.2^a
5,6α-Ep	0.4 ± 0.1^E	2.5 ± 0.2^e	1.5 ± 0.2^D	3.5 ± 0.3^d	4.4 ± 0.1^C	7.6 ± 0.3^c	7.1 ± 0.1^B	8.4 ± 0.3^b	9.5 ± 0.2^A	12.9 ± 0.2^a
5,6β-Ep	2.7 ± 0.2^E	$5.2 \pm 0.1e$	7.8 ± 0.3^D	11.6 ± 0.3^d	10.9 ± 0.5^C	15.4 ± 0.8^c	13.6 ± 0.7^B	20.8 ± 1.0^b	22.6 ± 1.0^A	35.6 ± 1.5^a
Total	16.5 ± 0.1^E	37.9 ± 0.2^e	35.4 ± 0.1^D	67.8 ± 0.3^d	57.7 ± 0.2^C	$1110 \pm 0.4c$	94.2 ± 0.3^B	139 ± 0.5^b	149 ± 0.5^A	279 ± 0.7^a
Brand B										
19-OH	4.8 ± 0.2^E	11.4 ± 0.3^e	8.6 ± 0.6^D	19.2 ± 1.0^d	11.7 ± 0.8^C	32.6 ± 1.0^c	17.5 ± 0.6^B	48.9 ± 2.0^b	25.9 ± 1.0^A	59.9 ± 1.0^a
20α-OH	1.5 ± 0.1^E	4.9 ± 0.2^e	3.6 ± 0.2^D	7.1 ± 0.3^d	4.9 ± 0.2^C	9.5 ± 0.6^c	8.2 ± 0.3^B	16.7 ± 1^b	15.8 ± 0.7^A	29.6 ± 0.5^a
22(R)-OH	1.6 ± 0.1^E	4.3 ± 0.1^e	3.2 ± 0.3^D	5.9 ± 0.2^d	5.8 ± 0.2^C	7.5 ± 0.4^c	7.6 ± 0.2^B	11.4 ± 0.6^b	10.7 ± 0.5^A	18.2 ± 0.4^a
24(S)-OH	0.8 ± 0.0^E	1.4 ± 0.1^e	1.8 ± 0.1^D	3.9 ± 0.1^d	3.8 ± 0.1^C	5.9 ± 0.3^c	5.2 ± 0.1^B	7.5 ± 0.3^b	7.5 ± 0.2^A	10.2 ± 0.2^a
22(S)-OH	0.6 ± 0.0^E	1.4 ± 0.2^e	2.1 ± 0.1^D	3.8 ± 0.1^d	4.4 ± 0.3^C	6.7 ± 0.2^c	7.7 ± 0.2^B	10.9 ± 0.2^b	14.9 ± 0.4^A	19.4 ± 0.6^a
25-OH	2.9 ± 0.1^E	7.8 ± 0.3^e	6.1 ± 0.2^D	14.6 ± 0.8^d	17.8 ± 0.6^C	37.5 ± 1.0^c	28.3 ± 0.5^B	45.7 ± 0.9^b	40.1 ± 1.3^A	$61.9 \pm 2{,}0^a$
5,6β-Ep	0.1 ± 0.0^E	1.7 ± 0.1^e	2.4 ± 0.2^D	6.0 ± 0.3^d	6.9 ± 0.1^C	9.5 ± 0.3^c	14.6 ± 0.3^B	20.3 ± 0.7^b	46.8 ± 1.6^A	65.6 ± 1.8^a
Total	12.3 ± 0.6^E	32.9 ± 0.2^e	27.8 ± 0.2^D	60.5 ± 0.3^d	55.3 ± 0.3^C	109 ± 0.5^c	81.1 ± 0.3^B	161 ± 0.8^b	162 ± 0.7^A	265 ± 0.7^a

Values are means ± standard deviation of the six samples (two lots analysed in triplicates). Values bearing and different letters (small letters) are significantly differences ($p < 0.02$) at storage time in the raw samples. Values bearing and different letters (capital letters) are significantly differences ($p < 0.02$) at storage time in the grilled samples.

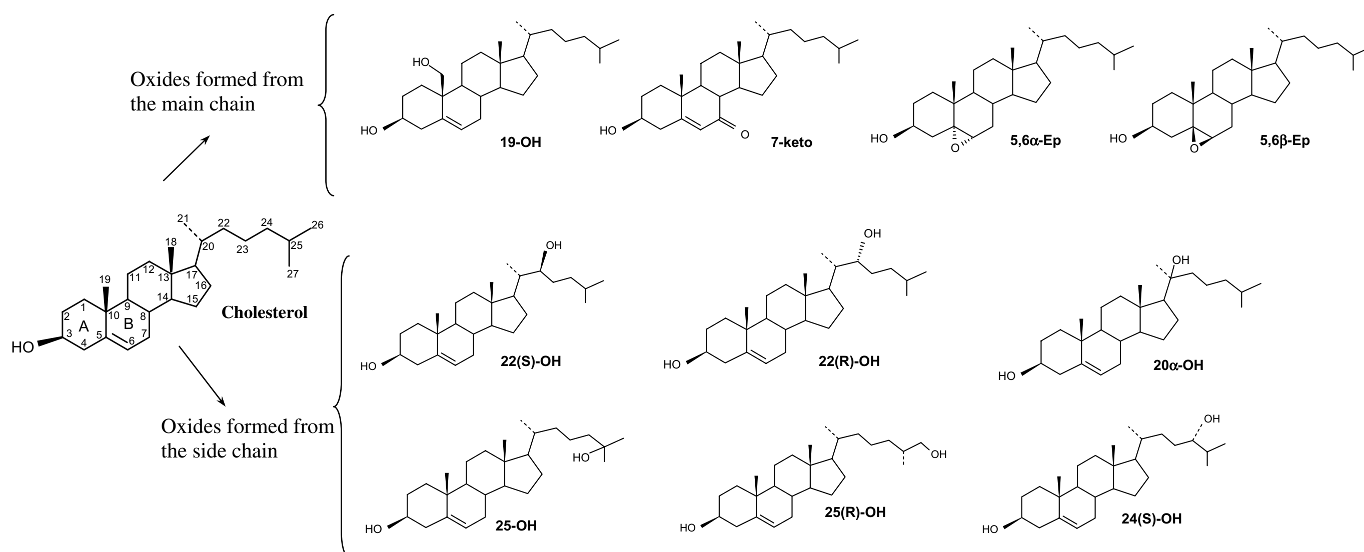

Fig. 1. Chemical structures of cholesterol and cholesterol oxides found in hake fillets.

peroxy radicals, and a higher amount of this oxide was produced during the experiment.

Hake fillets showed relatively high levels of total cholesterol oxides in the zero time. The presence of a great number of the oxides at zero time suggests that lipid oxidation had already occurred producing free radicals, which can act as accelerators of cholesterol oxidation (Smith, 1987) or these products are metabolites, originated enzymatically in fish metabolism. During frozen storage the level of the oxides exhibited a gradual and significant increase ($p < 0.02$) in all the samples evaluated. Of the COP, the production of 24(S)-OH and 5,6α-Ep were highest in brand A samples and 5,6β-Ep, followed by 22(S)-OH in brand B samples. After 120 days of storage, the total increase in oxides in the raw hake was 804% for brand A and 1214% for brand B, demonstrating that the freezing of commercial fish products significantly increases the susceptibility toward oxidation. The oxidation ratio was less accentuated in the brand A samples, leading to the conclusion that low-oxygen permeability packing does not hinder the oxide formation. Although it was more effective than high-oxygen permeability packing. The use of low storage temperatures or vacuum packing for frozen fish can slow the monomolecular reactions of oxidation but it does not stop the problems completely (Khalil & Mansour, 1998).

The levels of the COP increased significantly ($p < 0.02$) after grilling for all the evaluated samples. The increases were from 47% to 130% for brand A, and from 64% to 167% for brand B in total, of oxides, during 120 days of storage. The oxides more affected by the heat treatment were the 5,6α-Ep and 22(R)-OH at zero time in brand A samples; and 5,6β-Ep at zero time in brand B samples. Heat oxidation in cholesterol rich food systems is a dynamic reaction (Kim & Nawar, 1991; Navar, Kim, Li, & Vajdi, 1991), depending on the amount of cholesterol present, the type and severity of the heat treatment and the presence of free radicals in the sample.

4. Conclusion

A continuous increase in the amount of COP was accompanied by a concurrent decrease in the levels of the fatty acids during frozen storage and subsequent grilling, the most common methods used to conserve and prepare fish products. Thus, it can be concluded that commercial frozen storage followed by grilling are important factors in the alterations of the lipid profile in the samples studied, mainly in cholesterol oxidation. In relation to the two types of package studied, it was observed that low-oxygen permeability packing was more effective in retarding lipid oxidation than the high-oxygen permeability packing, although it does not hinder the development of oxidation. Lipid oxidation and the safety question for human health should be discussed at greater length, since the relationship between the long-term consumption of cholesterol oxidation products and human health is still not clear.

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