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Effect of cooking method on the formation of 7-ketocholesterol in Atlantic hake (*Merluccius hubbsi*) and smooth weakfish (*Cynoscion leiarchus*) fillets

M.T. Freitas [a, c], C.A.A. Amaral [a, c], M.X. Coutrim [b], R.J.C.F. Afonso [b], R.G. Junqueira [c, *]

[a] Departamento de Alimentos, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil
[b] Laboratório de Caracterização Molecular e Espectrometria de Massas, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil
[c] Programa de Pós-Graduação em Ciência de Alimentos, Universidade Federal de Minas Gerais, 31270-010 Belo Horizonte, MG, Brazil

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ABSTRACT

The levels of cholesterol and 7-ketocholesterol were measured in raw Atlantic hake (*Merluccius hubbsi*) and smooth weakfish (*Cynoscion leiarchus*) fillets and in fillets subjected to the following cooking methods: baking in an electric or microwave oven; baking, grilling or stewing in a steam-convection oven; simmering on a stove; electric grilling; and deep frying. The raw samples from both fishes exhibited significantly ($p < 0.05$) higher cholesterol levels (62.71 ± 6.06 mg/100 g–74.16 ± 3.96 mg/100 g) than the processed fillets. In all of the samples, 7-ketocholesterol was detected at significantly ($p < 0.05$) different levels depending on the cooking method and the type of fish. Steam cooking keeping the surface of the product moist produced small decrease in the cholesterol content (26.65%–29.96%) and a low level of 7-ketocholesterol in the samples (6.90 ± 0.21 μg/g–6.47 ± 0.28 μg/g). Baking in electric or steam-convection ovens at high temperatures and long times greatly reduced the cholesterol content (52.77%–65.08%), which was associated with a large increase in 7-ketocholesterol levels (11.54 ± 0.45 μg/g–13.94 ± 1.17 μg/g). These results indicate the necessity of revising the baking procedures for fish to increase the healthiness of food.

1. Introduction

Cholesterol (cholest-5-en-3β-ol) is an essential structural and functional component of cell membranes (Rayner et al., 2010) that is found in significant amounts in fish products and other foods of animal origin (Tai, Chen, & Chen, 1999). Because it is an unsaturated alcohol, cholesterol is readily susceptible to oxidation, forming compounds known as cholesterol oxidation products, oxysterols or cholesterol oxides (Otaegui-Arrazola, Menéndez-Carreño, Ansorena, & Astiasaran, 2010). Several cholesterol oxides have been shown to be cytotoxic (Nury, Samadi, Zarrouk, Riedinger, & Lizard, 2013), mutagenic (Cheng, Kang, Shih, Lo, & Wang, 2005), and carcinogenic (Poli, Biasi, Gargiulo, & Leonarduzzi, 2013) and to have undesirable biological effects, such as interfering with sterol metabolism and changing the properties of the cell membrane (Schroepfer, 2000). There is evidence that dietary cholesterol oxides promote atherogenesis in rats (Soto-Rodríguez et al., 2009) and the development of hypercholesterolemia-induced liver lesions in rabbits (Hur et al., 2014).

In heat-processed foods, the oxidation of cholesterol is initiated mainly through the removal of a hydrogen atom at the C-7 position of the sterol ring followed by the addition of an oxygen molecule, leading to the formation of 7α- and 7β-hydroperoxycholesterol. During the cooking of foods, these hydroperoxides readily decompose and undergo further dehydration to form 7-ketocholesterol (Smith, 1996). Other cholesterol oxidation products are also formed by oxidation at C-5 and C-6, which produces epoxides such as 5α,6α-epoxycholesterol and 5β,6β-epoxycholesterol. Side-chain oxidation promotes the formation of monohydroperoxides, primarily including 20α-, 25-, and 26-hydroxycholesterol, as well as their degradation products (Brown & Jessup, 2009). Selected cholesterol oxides formed during autoxidation in heat-processed foods are shown in Fig. 1. Of the several cholesterol oxidation products that have been documented to occur in foods, 7-ketocholesterol is a useful marker of cholesterol oxidation in processed foods because it is produced in

* Corresponding author. Tel.: +55 031 3409 6913; fax: +55 031 3409 6989.
E-mail address: rjunqueira@ufmg.br (R.G. Junqueira).

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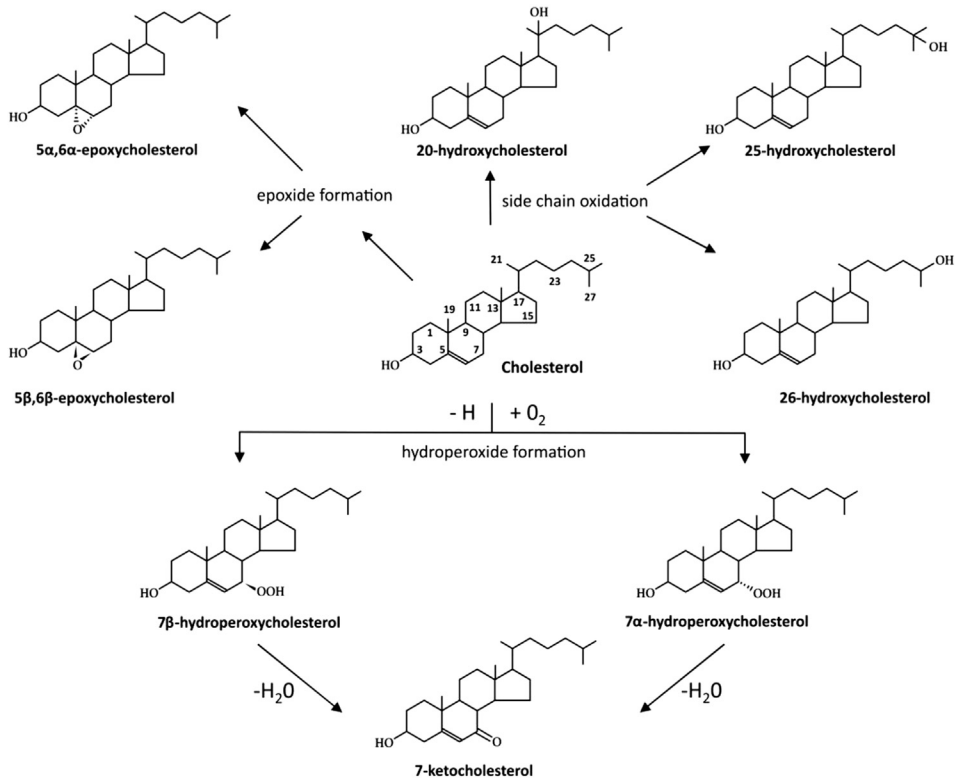

Fig. 1. Selected cholesterol oxides formed during autoxidation in heat-processed foods.

large quantities during the early stages of the oxidation process, particularly in fish and other seafood (Rodriguez-Estrada, Garcia-Llatas, & Lagarda, 2014).

Fish is considered one of the foods most susceptible to oxidation due to its cholesterol and polyunsaturated fatty acid content (Hur, Park, & Joo, 2007). The presence of polyunsaturated fatty acids markedly favors the formation of cholesterol oxides during processing and storage (Xu, Sun, Liang, Yang, & Chen, 2011). Fish is sometimes eaten raw but is generally subjected to culinary processes that involve heating, favoring lipid oxidation and leading to a loss of nutritional value (Saldanha, Benassi, & Bragagnolo, 2008).

The formation of cholesterol oxides in processed fish has been previously reported (Al-Saghir et al., 2004; Saldanha et al., 2008). However, there are few studies examining Atlantic hake (*Merluccius hubbsi*) and smooth weakfish (*Cynoscion leiarchus*) that have been subjected to many different culinary processes. These fishes are often used by Brazilian food services, but limited knowledge is available on the effect of commonly used cooking methods on the formation of cholesterol oxidation products during cooking. Thus, the aim of this study was to investigate cholesterol oxidation by assessing 7-ketocholesterol formation in Atlantic hake and smooth weakfish fillets prepared using common cooking methods, including simmering, grilling, frying, and baking in electric, microwave, and steam-convection ovens.

2. Materials and methods

2.1. Samples

Atlantic hake (*M. hubbsi*) and smooth weakfish (*C. leiarchus*) fillets (30 kg) were purchased frozen from Campesca (Belo Horizonte, Brazil) and maintained at −18 °C in a freezer (BD 400, Termisa, Maracanaú, Brazil) until all of the experimental processing was complete, which occurred in 30 days or less. After thawing under refrigeration (4 °C), approximately 500 g of each fillet was cut into standardized pieces 15-cm long, 6-cm wide, and 2-cm thick. The fillets (27 samples) were randomly distributed into an uncooked control group (RAW) and eight other groups, each one of which was subjected to a different cooking method, in three replicates. After cooking, the raw and processed samples were ground using a food blender (Hamilton Beach Brands, Glen Allen, VA, USA), vacuum-packed and stored at −18 °C for no longer than four weeks until the analysis was performed. The moisture content of the samples was determined gravimetrically at 105 °C with three replicates.

2.2. Cooking methods

The samples were cooked using the following methods: baking in an electric oven (BEO) at 200 °C for 30 min using a semi-industrial electric oven (ITC Eletro, Guaramirim, Brazil); simmering on a stove (SOS) for 18 min using a domestic stove (Maxim's Giromagic, Continental 2001, São Paulo, Brazil) and a stainless steel pan (N° 22, Rochedo, São Paulo, Brazil), slowly adding 100 mL of boiling water; deep frying (FRY) by immersing the fillets in preheated fresh soybean oil at 180 °C for 3 min in a deep fryer (Tedesco, Caxias do Sul, Brazil); heating in a microwave oven (MIW) using a domestic microwave oven (Maxi Gratine Inox, Brastemp, São Paulo, Brazil) at 820 W and 60 Hz for 15 min; grilling on an electric grill (GRI) in a Multi-Grill Express Baby (Multi-Grill Express, Londrina, Brazil) by preheating the equipment surface to 180 °C for 10 min, then heating the fillets for 4 min. Three other cooking methods were performed using a steam-convection (combined) oven (EC3-Gourmet Pratica Technicook, Pouso Alegre, Brazil), as follows: baking in the combination oven (BCO) at 180 °C for 25 min, 90% relative humidity, and 0.1 MPa; stewing in the

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combination oven (SCO) at 150 °C for 30 min, 90% relative humidity, and 0.1 MPa; and grilling in the combination oven (GCO) at 220 °C for 8 min using the convection mode. All of the cooking times and temperatures used for each process are normally used in Brazilian food services or are recommended by the equipment manufacturers. At the end of each cooking process, the internal temperature of the fillets was monitored by contact using a calibrated thermometer (Equitherm, Gravataí, Brazil). The conditions of time and temperature used for the different cooking methods and the internal temperature achieved by the final products are shown in Table 1.

2.3. Extraction procedures

Lipid extraction was carried out by the procedure described by Folch, Lees, and Stanley (1957). Briefly, homogenized samples (10.0 ± 0.5 g) were extracted twice with 300 mL of chloroform:methanol (2:1, v/v). The suspension was vortexed at room temperature for 3 min and filtered to a separating funnel using Whatman No. 1 filter-paper containing 5 g of anhydrous sodium sulfate. After addition of 0.1 mol/L KCl (66 mL) the mixture was allowed to stand for 3 min and the two phases were collected separately. The chloroform lower layer was collected and the lipid fraction was recovered after evaporating the solvent under vacuum in a rotary evaporator. The saponification procedure was performed according Chen and Chen (1994), by taking 0.1 g of the lipid fraction and adding 20 mL of 1 mol/L KOH in methanol and leaving the mixture in the dark for 18 h at 25–30 °C with mechanical agitation. Then, 10 mL of distilled water were added to the mixture and the unsaponified fraction was extracted with 10 mL of hexane three times in a separating funnel. The hexane layers were collected, combined and washed with 10 mL of 1 mol/L KOH in methanol, and with 10 mL of distilled water, three times. The resulting extract was filtered using Whatman No. 1 filter-paper containing 5 g anhydrous sodium sulfate, and the material retained after filtration was washed with 20 mL of hexane. The combined filtrate was evaporated to dryness under vacuum at 40 °C and the residue was dissolved in 1 mL of pyridine and transferred to vials for chromatographic analysis. 5 α-Cholestane (10 μg/mL) purchased from Sigma–Aldrich (Milford, MA, USA) was used as the internal standard, and three replicates of the extraction procedure were performed per sample.

Table 1
Time and temperature settings used for the different cooking methods and the final internal temperatures achieved in the processed Atlantic hake (*Merluccius hubbsi*) and smooth weakfish (*Cynoscion leiarchus*) fillets.

Cooking method	Temperature setting (°C)	Time (min)	Final internal temperature (°C)	
			Atlantic hake	Smooth weakfish
BEO	200	30	89 ± 2^{abA}	87 ± 4^{abA}
BCO	180	25	84 ± 2^{bcA}	82 ± 3^{bcA}
GCO	220	8	87 ± 1^{abA}	84 ± 2^{bcB}
GRI	180	4	80 ± 2^{cdA}	77 ± 2^{deA}
MIW	–*	15	82 ± 1^{cA}	81 ± 2^{cdA}
SOS	96	18	92 ± 1^{aA}	90 ± 1^{aA}
SCO	150	30	77 ± 1^{dA}	74 ± 2^{eA}
FRY	180	3	89 ± 1^{abA}	91 ± 2^{aA}

*820 W and 60 Hz; BEO: baking in an electric oven; BCO: baking in a combination oven; GCO: grilling in a combination oven; GRI: grilling on an electric grill; MIW: heating in a microwave oven; SOS: simmering on a stove; SCO: stewing in a combination oven; FRY: deep frying. The final internal temperatures are the mean values ± standard deviation of three results. Different superscripted lowercase letters (a-e) indicate significant differences ($p < 0.05$) between the mean internal temperatures reached using the different cooking methods for the same fish, as determined using the Tukey test. Different superscripted capital letters (A–B) indicate significant differences ($p < 0.05$) between the mean internal temperatures of the two fishes attained using the same cooking method, as determined using the Tukey test.

2.4. Chromatographic analysis

The samples were analyzed using on-line derivatization gas chromatography tandem mass spectrometry with a chromatograph GCMS-QP2010 Plus Shimadzu (Tokyo, Japan) equipped with a 30 m × 0.25 mm i.d., 0.10 μm film thickness Zebron ZB-XLB-HT INFERNO capillary column (Phenomenex, Torrance, CA, USA). Helium was used as the carrier gas at a constant flow rate of 1.46 mL/min. The injections were performed in splitless for 1 min in order to concentrate the analytes on the column and then in split mode with a flow rate of 1/50. The volume of each injected sample was 2 μL. The initial temperature of the column was set and maintained at 100 °C for 1 min. The temperature was then increased by 10 °C/min to 200 °C, increased again by 15 °C/min to 250 °C, then finally increased by 3 °C/min to 280 °C and was maintained at this temperature for 5 min. The injector and detector temperatures were set to 280 °C. The samples were analyzed using online derivatization with N,O-bistrimethylsilyltrifluoroacetamide/trimethylchlorosilane at 99:1 (Sigma–Aldrich), as previously described by Wu, Hu, Yue, Yang, and Zhang (2009). The mass detector was operated in selected ion monitoring (SIM) mode, in which the ions of particular m/z values for 5α-cholestane (217; 218; 357; 372), cholesterol (129; 329; 368; 458) and 7-ketocholesterol (367; 382; 457; 472) were selected as the most characteristic for quantification and confirmation purposes. The calibration curves were prepared using sunflower oil at six concentrations in three independent trials. The concentrations of the standard solutions employed to produce the calibration curves ranged from 10 to 450 μg/mL for cholesterol and from 0.1 to 15 μg/mL for 7-ketocholesterol. Each calibration solution was prepared to contain a fixed concentration (10 μg/mL) of 5α-cholestane. The limits of quantification were estimated using software Shimadzu GC–MS Solutions (Tokyo, Japan) based on a signal/noise ratio of 10:1 (Miller & Miller, 2010, chap. 5). Typical chromatograms of the 5α-cholestane, cholesterol, and 7-ketocholesterol in Atlantic hake fillets grilled in a combination oven is shown in Fig. 2.

2.5. Statistical analysis

All of the samples were processed in triplicate, and each processed fillet was analyzed in three replicates ($n = 3$). The calibration curves were generated using the ordinary least squares method and were tested for lack-of-fit and homoscedasticity based on the procedure described by Souza and Junqueira (2005). The data were evaluated using ANOVA with a 2 × 9 (fish × cooking method) factorial design. The differences between the values according to the type of fish and the cooking methods were considered significant when p was < 0.05 based on the Tukey test (Montgomery, 2013, chap. 5). Pearson product–moment correlation coefficients (r) were calculated for the relationship between the cholesterol and 7-ketocholesterol content in the fish fillets using a significance level of $p < 0.05$, $p < 0.01$, and $p < 0.001$ (Miller & Miller, 2010, chap. 5). Statistical analyses were performed with IBM SPSS Statistics package version 19.0 (IBM Corporation, Somers, NY, USA).

3. Results and discussion

3.1. Raw fillets

Although the lipid and cholesterol content of fish tissues differ between species, gender, geographical origin, and season and can be affected by other environmental factors (Özogul, Özogul, & Alagoz,

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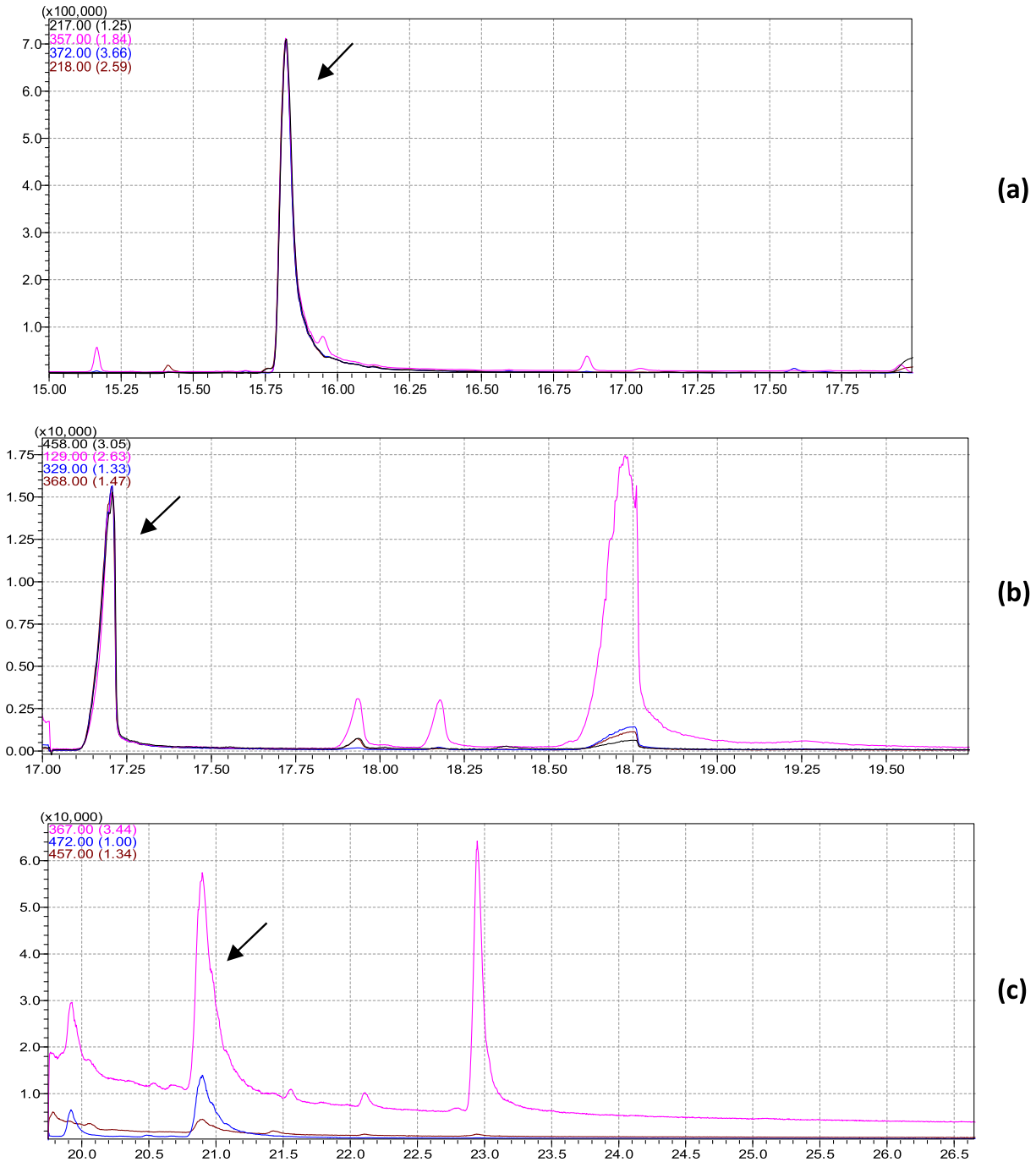

Fig. 2. Typical GCMS chromatograms of the 5α-cholestane (a), cholesterol (b), and 7-ketocholesterol (c) in Atlantic hake fillets grilled in a combination oven. 5α-cholestane: t_r 15.915 min, m/z 217, 218, 357, 372; cholesterol: t_r 17.177 min, m/z 129, 329, 368, 458; 7- ketocholesterol: t_r 20.900 min, m/z 367, 382, 457, 472. Conditions: on-line derivatization gas chromatography tandem mass spectrometry with a chromatograph GCMS-QP2010 Plus Shimadzu with a 30 m × 0.25 mm i.d., 0.10 μm film thickness Zebron ZB-XLB-HT INFERNO capillary column. Helium at 1.46 mL/min flow rate was used as the carrier gas.

2007), as well as by, the analytical method used (Georgiou, Constantinou, & Kapnissi-Christodoulou, 2014), the amounts of lipids and cholesterol observed in the raw Atlantic hake and smooth weakfish fillets (Table 2) were in the range reported by other researchers for both species (Saldanha & Bragagnolo, 2008, 2010; Sancho, Lima, Costa, Mariutti, & Bragagnolo, 2011). Comparing the fishes, smooth weakfish exhibited significantly ($p < 0.05$) higher fat and cholesterol levels and less moisture than Atlantic hake. These results are consistent with those reported by Rebah, Abdelmouleh, Kammoun, and Yezza (2010), who observed that the lipid content in fish is inversely proportional to the water content.

It has been previously demonstrated that 7-ketocholesterol is absent or present at very low levels in raw samples (Lugasi et al., 2007). As expected, small amounts of 7-ketocholesterol were observed in the raw fillets from both types of fish, and no significant ($p > 0.05$) differences were detected between them (Table 2).

3.2. 7-Ketocholesterol formation

The relationship between cholesterol and 7-ketocholesterol content in raw and heat-processed fillets on a dry-weight basis are depicted for Atlantic hake (Fig. 3a) and smooth weakfish

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Table 2
Moisture, total lipids, cholesterol and 7-ketocholesterol (wet basis) content of raw Atlantic hake (*Merluccius hubbsi*) and smooth weakfish (*Cynoscion leiarchus*) fillets.

Analyte*	Atlantic hake	Smooth weakfish
Moisture (g/100 g)	81.16 ± 0.95[a]	78.25 ± 0.34[b]
Total lipids (g/100 g)	1.99 ± 0.12[b]	2.92 ± 0.18[a]
Cholesterol (mg/100 g)	62.71 ± 6.06[b]	74.16 ± 3.96[a]
7-ketocholesterol (μg/g)	0.045 ± 0.009[a]	0.050 ± 0.001[a]

*The values are the mean values ± standard deviation of the nine trials (three batches analyzed in three replicates). Different superscripted lowercase letters (a, b) indicate significant differences ($p < 0.05$) between the mean values for the fishes, as determined using the Tukey test. The limits of quantification of cholesterol and 7-ketocholesterol were 1.41 mg/100 g and 0.043 μg/g, respectively.

(Fig. 3b). The level of cholesterol in the processed samples was significantly ($p < 0.05$) lower than in the raw fillets, which was accompanied by a significant ($p < 0.05$) increase in 7-ketocholesterol levels in samples from both fishes. These findings are consistent with the results of other studies reported for fish that were processed using different methods (Al-Saghir et al., 2004; Saldanha et al. 2008; Stephen, Shakila, Jeyasekaran, & Sukumar, 2010). Atlantic hake exhibited lower levels of total lipids and cholesterol than smooth weakfish as raw fillets (Table 2) but higher levels of 7-ketocholesterol in most of the heated samples (Fig. 3). Cholesterol oxidation occurs through a process that is affected by several factors including characteristics of the food itself, such as water content, pH, the abundance of various forms of cholesterol, the types of fatty acids present, and antioxidant and pro-oxidant levels, as well as the interactions between these components and degradation products that form during processing and storage (Cardenia, Rodriguez-Estrada, Boselli, & Lercker, 2013; Ohshima, Shozen, Ushio, & Koizumi, 1996). Therefore, differences between the tissues of the two species could explain the different behavior for the oxidation of cholesterol.

Negative and significant ($p < 0.001$) Pearson product–moment correlation coefficients between the cholesterol content and the 7-ketocholesterol content were observed for both fishes (Fig. 3). Excluding the FRY samples, for which other variables might affect cholesterol levels, these data demonstrated that the reduction in cholesterol content was directly related to its oxidation, as indicated by the linear increase in 7-ketocholesterol levels concomitant with the decrease in cholesterol levels in the processed samples. Saldanha et al. (2008) also found a negative correlation ($r \leq -0.84$) between the amounts of cholesterol and cholesterol oxides in sardines that were grilled or stored for 120 days. Correlations between the cholesterol content and the cholesterol oxide content formed during the grilling and storage of Atlantic hake fillets have been observed by Saldanha and Bragagnolo (2008). However, 7-ketocholesterol was not detected in raw, grilled or stored samples; instead, 19-hydroxycholesterol and 25-hydroxycholesterol were the prominent oxides assessed in all of the samples. In the present study, we confirmed the role of 7-ketocholesterol as a marker of cholesterol oxidation in heated samples (Rodriguez-Estrada et al., 2014) because this oxide was quantifiable in all of the cooked fillets and showed a significant ($p < 0.001$) trend to increase as the cholesterol content decreases.

3.3. Fried samples

The FRY fillets contained a smaller quantity of 7-ketocholesterol (4.89 ± 0.15 μg/g in Atlantic hake and 5.32 ± 0.19 μg/g in smooth weakfish) than was expected based on the reduction in the cholesterol content (54.56% in Atlantic hake and 39.69% in smooth weakfish), which was significantly different ($p < 0.05$) from the other cooking treatments. When the FRY samples were included

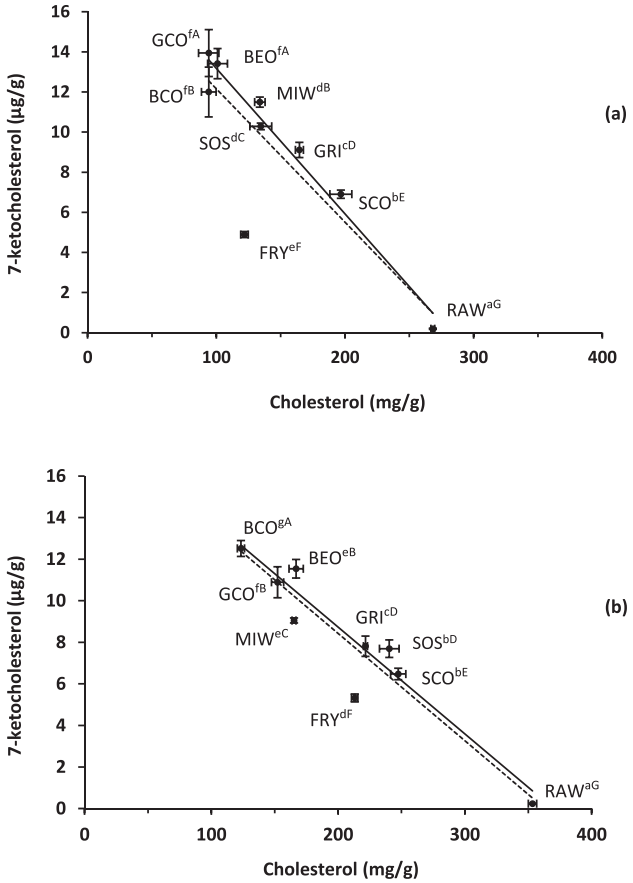

Fig. 3. Relationship between the cholesterol and 7-ketocholesterol levels for Atlantic hake (a) and smooth weakfish (b) fillets in the raw state and after using eight different cooking methods. RAW: uncooked control group; SOS: simmering on a stove; BEO: baking in an electric oven; GRI: grilling on an electric grill; GCO: grilling in a combination oven; SCO: stewing in a combination oven; BCO: baking in a combination oven; MIW: heating in a microwave oven; FRY: deep frying. The mean values ± standard deviation ($n = 3$) were compared using the Tukey test with a significance level of 0.05. The superscripted lowercase letters (a–g) differentiate the means of the treatments in the same fish with respect to the cholesterol content. Different superscripted capital letters (A–G) indicate significant differences between the mean 7-ketocholesterol content of the treatments of the same fish; ———— correlation analysis excluding the FRY samples $r = -0.9816$ ($p < 0.001$) for Atlantic hake and $r = -0.9757$ ($p < 0.001$) for smooth weakfish; - - - - correlation analysis including the FRY samples $r = -0.8510$ ($p < 0.001$) for Atlantic hake and $r = -0.9467$ ($p < 0.001$) for smooth weakfish; r: Pearson product–moment correlation coefficient.

when computing the Pearson correlation coefficients, the r value changed from $r = -0.9816$ to $r = -0.8510$ for Atlantic hake and from $r = -0.9757$ to $r = -0.9467$ for smooth weakfish (Fig. 3). These results might be explained by concomitant factors. First, the short period of heat exposure during frying did not increase the internal temperature sufficiently (Table 1). The oil penetrates the sponge-like network that forms after the partial evaporation of water at the end of the frying procedure and acts upon the food for a very short period (Stephen et al., 2010). Other contributing factors are related to the exchange that occurs between the fats in the fish and the frying oil, which significantly change the cholesterol content. During frying, cholesterol is released by the frying medium (Wu & Lillard, 1998). Furthermore, fried samples normally absorb oil at the end of the frying period, leading to dilution of the cholesterol content (Sánchez-Muniz, Viejo, & Medina, 1992). Our results agree with those of Al-Shagir et al. (2004), who observed high levels of cholesterol oxides in farmed salmon fillets that were fried. Likewise, a decrease in the cholesterol content was observed by Mai,

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Shimp, Weihrauch, and Kinsella (1978) after pan frying or deep frying white sucker and bluegill lake trout fillets, which was attributed to the elution of cholesterol by the frying oil.

3.4. Baked samples

Using the BEO, BCO, and GCO methods significantly ($p < 0.05$) reduced the cholesterol content (52.77%–65.08%) and increased the 7-ketocholesterol content of the fillets compared to the RAW samples (Fig. 3). However, differences between the baked treatments were observed according to the type of fish, being found the highest levels of 7-ketocholesterol for treatment BEO (13.41 ± 0.75 μg/g in Atlantic hake and 11.54 ± 0.45 μg/g in smooth weakfish), BCO (12.00 ± 1.24 μg/g in Atlantic hake and 12.51 ± 0.38 μg/g in smooth weakfish), and GCO (13.94 ± 1.17 μg/g in Atlantic hake and 10.89 ± 0.75 μg/g in smooth weakfish). The conditions used for baking were harsher than those used for the other cooking methods (Table 1), which might explain why the highest level of 7-ketocholesterol and the lowest level of cholesterol were found in this group of treatments. The effect of conventional oven baking on the cholesterol content of catfish fillets was examined by Wu and Lillard (1998), but these authors found that the cholesterol concentration increased when the fillets were baked at 204 °C, possibly due to water loss. Our results are similar to those of Mai et al. (1978), who observed a significant decrease in the cholesterol content baking three types of fish at 190 °C.

3.5. Grilled samples

Although the GRI samples were processed at temperatures similar to those used for baking the fillets, the processing time used for grilling was much shorter than for baking. This factor resulted in products with lower final internal temperatures (Table 1), lower reduction of the cholesterol content (38.65% in Atlantic hake and 37.28% in smooth weakfish), and lower 7-ketocholesterol levels (9.11 ± 0.38 μg/g in Atlantic hake and 7.81 ± 0.49 μg/g in smooth weakfish) than those observed for the baked samples. Previous studies examined the formation of cholesterol oxidation products during grilling by determining the 7-ketocholesterol content (Saldanha et al., 2008; Saldanha & Bragagnolo, 2010; Shozen, Ohshima, Ushio, & Koizumi, 1995). Ohshima et al. (1996) suggested that the oxidation of cholesterol from grilling fish products occurs through the peroxidation of lipids, particularly the polyunsaturated fatty acids present in triacylglycerides.

3.6. Simmered samples

After cooking, the SOS samples exhibited high final internal temperatures (Table 1). However, a relatively small reduction in cholesterol (49.84% in Atlantic hake and 31.98% in smooth weakfish) and low levels of 7-ketocholesterol were observed in these samples (10.28 ± 0.16 μg/g in Atlantic hake and 7.69 ± 0.42 μg/g in smooth weakfish). Sancho et al. (2011) observed that after cooking white hake meatballs in boiling water (95 ± 1 °C) for 30 min, the cholesterol and cholesterol oxide contents of samples were unchanged, indicating that this treatment does not cause cholesterol oxidation. Water was added at the same time the SOS samples were cooking to keep the surface of the product moist. Therefore, although the samples reached a high internal temperature, the added water might have generated a protective effect by hindering the oxidation of cholesterol in these samples, possibly by reducing the availability of oxygen.

3.7. Microwaved samples

The quantity of 7-ketocholesterol in the MIW samples for smooth weakfish fillet was lower than expected based on the observed reduction of the cholesterol levels (Fig. 3b), as noted for Atlantic hake (Fig. 3a). Although the reduction in cholesterol content was comparable in both fish samples (to approximately 2-fold less than that of the raw samples), the amount of 7-ketocholesterol in the fillets was quite different after MIW treatment. The 7-ketocholesterol content increased 60-fold in Atlantic hake fillets (11.50 ± 0.25 μg/g), whereas a 38-fold increase was observed in smooth weakfish fillets (9.05 ± 0.12 μg/g). This result could be attributed to the degradation of 7-ketocholesterol or the formation of other species-dependent compounds. It is known that cholesterol oxidation products other than 7-ketocholesterol are present in food products (Ohshima et al., 1996; Tai et al., 1999). Moreover, the thermal degradation of cholesterol leads not only to oxide formation but also to the formation of other high- and low-molecular-mass products (Chien, Wang, & Chen, 1998). During microwave heating, the heating performance is affected by dielectric properties. The cooking time decreases with an increase in the lipid content because the dielectric constant and the loss factor decrease with increasing lipid content (Chandrasekaran, Ramanathan, & Basak, 2013). Accordingly, the observed differences between the fish during microwave processing could be explained based on species-specific differences in the total lipids and moisture content of the raw fillets (Table 2). However, the reduction of cholesterol and the formation of 7-ketocholesterol occurred to a lesser extent during MIW processing of the fillets compared to baking them in conventional or steam-convection ovens (Fig. 3).

3.8. Steam-convection cooked samples

As described above, the conditions used for the BCO and GCO methods resulted in high internal temperatures for the final products (Table 1) and had comparably strong effects on cholesterol oxidation as assessed by the 7-ketocholesterol content of the samples (Fig. 3), despite the use of steam for BCO cooking and the short duration of GCO cooking. The temperature setting used for GCO cooking was much higher than that used for BCO cooking; however, hot steam causes the release of a large quantity of latent heat when it condenses on the surface of the samples (Vittadini, Rinaldi, Chiavaro, Barbanti, & Massini, 2005). This phenomenon increases the amount of heat available in the oven and can result in a rapid temperature increase in the products (Murphy et al., 2001). On the other hand, the conditions used for the SCO cooking method simulated stewing in that the surface of the fillets were kept moist, which hampered surface-crust development. Moreover, the lowest temperature setting was used for SCO cooking, which resulted in average internal temperatures for the final products that were approximately 10 °C lower than the average internal temperatures of the baking group (Table 1). The different temperature gradients in the samples might have a strong effect on the rate and extent of chemical changes (Vittadini et al., 2005). The outcomes might explain the low level of cholesterol oxidation in the SCO samples. Indeed, the SCO treatment was the cooking method that produced the smallest decrease in the level of cholesterol (26.65% in Atlantic hake and 29.96% in smooth weakfish) and the lowest content of the oxide 7-ketocholesterol in the processed fillets (6.90 ± 0.21 μg/g in Atlantic hake and 6.47 ± 0.28 μg/g in smooth weakfish).

4. Conclusions

In this study, it was found that the levels of cholesterol for cooked samples were markedly lower than those for raw fillets.

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This decrease was associated with an increase in the 7-ketocholesterol content in the cooked samples from both of the studied fishes. Cooked Atlantic hake fillets had higher levels of 7-ketocholesterol than cooked smooth weakfish fillets, although raw Atlantic hake fillets had a lower cholesterol content. Steam cooking under mild conditions and simultaneously keeping the surface of the product moist produced a relatively low internal temperature, a small decrease in the cholesterol content (26.65%–29.96%), and a low level of 7-ketocholesterol in the samples (6.90 ± 0.21 μg/g–6.47 ± 0.28 μg/g). Thus, based on the lowest level of 7-ketocholesterol on fillets, the SCO method could be considered the best cooking method. However, baking in electric or steam-convection ovens at high temperatures greatly reduced the cholesterol content (52.77%–65.08%), which was associated with a large increase in 7-ketocholesterol levels (11.54 ± 0.45 μg/g–12.51 ± 0.38 μg/g). Considering the toxicity of cholesterol oxidation products, these results highlighted the necessity of revising the baking procedures for fish to increase the healthiness of food.

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