Fatty acids profile in canned tuna and sardine after retort sterilization and high pressure thermal sterilization treatment

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Summary

Fatty acid composition was asssessed for yellowfin tuna (*Thunnus albacares*) and sardine (*Sardina pilchardus*) canned in three different ways (brine, sunflower oil, olive oil) and subjected to two different sterilization treatments: retorting (as conventional treatment) and high pressure thermal sterilization (HPTS) (as alternative treatment). Impact of treatments on the fatty acid profile of the whole product was evaluated, particularly on the polyunsaturated fatty acids (PUFA) fraction since it is more susceptible to oxidation. Treatments were applied at pilot scale and the threshold for the sterilization factor was 7 min. HPTS treatment did not significantly affect the fatty acid profile of both canned tuna in brine and tuna in sunflower oil as compared with retorting. However, significant differences ($p < 0.05$) were observed in sardine in olive oil, where total PUFA content and the sum of eicosapentanoic acid and docosahexaenoic acid were nearly half in samples treated by HPTS. This work provides novel information on the impact of the combination of thermal treatment and high pressure as compared with classical retorting. Nevertheless, further investigation focused on the role of the prooxidants and antioxidants balance is necessary to confirm the oxidative effect of HPTS on PUFA content in oily fish species.

Keywords

tuna (*Thunnus albacares*); sardine (*Sardina pilchardus*); fatty acid; high pressure thermal sterilization; retort sterilization; canning

Fish is an important source of amino acids and proteins for a large part of the world's population and its consumption reaches 117 million tonnes, the global seafood consumption topping at 17.2 kg per capita in 2009 [1]. In addition, marine fish provide important constituents for the human diet, such as lipid-soluble vitamins, microelements and polyunsaturated fatty acids (PUFA). In general, marine fish contain small amounts of linoleic acid (C18:2, *n*-6) and linolenic acid (C18:3, *n*-3), and large amounts of eicosapentanoic acid (EPA, C20:5, *n*-3) and docosahexaenoic acid (DHA, C22:6, *n*-3). Omega-3-PUFA are essential for normal human growth and development, and may play an important role in the prevention and treatment of cardiovascular and cerebrovascular diseases, hypertension, arthritis as well as other inflammatory and autoimmune disorders, and cancer [2]. It is known that EPA protects cardiovascular health by regulating activities involved in the metabolism of plasma lipids, the aggregation of platelets and the process of blood coagulation [3]. On the other hand, DHA plays an important role in neural function and high intakes were found to inversely correlate with the relative risk of Alzheimer's disease [4]. According to the Food and Nutrition Board, the consumption of omega-3 fatty acids in amounts up to (but not exceeding) 3 g per day is beneficial [5].

Fatty acid composition of fish depends on the

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species, individual, the catching season and the fishing ground, being influenced by environmental conditions and geographical effects [6, 7]. In addition, fatty acid composition may be conditioned by processing, which specifically regards PUFA content, since PUFA are especially susceptible to deterioration by oxidation and hydrolysis because of their high degree of unsaturation [8].

Tuna and sardine represent a large percentage of the total amount of marine fish captured. An important part of their capture is destined for making preserves, being canned in oils, in brine and in various sauces. Canning in particular is the most important way to preserve fish since this packing allows pasteurization and sterilization to ensure the stability of the product by devitalizing microorganisms. In the food industry, the common process used to achieve a sterile product is retort sterilization. This conventional treatment is associated with the application of high temperature for an extended period of time, thus guaranteeing appropriate devitalization of microorganisms but with the possibility of inducing deterioration of product quality. Furthermore, the food can be adversely affected in other ways resulting in undesirable sensorial and nutritional changes and specifically loss of heat-sensitive nutrients [9]. Particularly, PUFA are prone to oxidation under high temperatures, which may decrease the final quality of the product.

Over the past few years, some technologies have emerged, which have a potential to improve traditional thermal processing characteristics. Among them, high pressure thermal sterilization (HPTS) may be an alternative to retort processing. In HPTS, a combination of pressures equal or higher than 600 MPa and temperatures approx. 90–121 °C is applied. These conditions facilitate the implementation of rapid, mainly uniform heating allowing sterilization of the food product but reducing the negative effects associated with the traditional thermal sterilization [10]. Several studies conducted on seafood using high pressure processing have concluded that this technology may be applied to these products to extend their shelf life [11]. However, it has also been found that the application of high pressure to certain foods such as meat and meat-like systems may lead to increased rates of lipid oxidation during subsequent aerobic storage of the food product [12]. As mentioned above, due to the high content of PUFA, lipids in fish are more susceptible to oxidation and, for this reason, changes induced by pressure may be significant. According to Angsupanich and Ledward [13], pressures above 400 MPa can accelerate lipid oxidation due to the release of free metal ions, while pressures of 600 MPa and 800 MPa markedly enhanced lipid oxidation. This subject has been recently revised by Medina-Meza et al. [14].

The aim of the present study was to investigate the impact of retort sterilization (conventional treatment) and high pressure + high temperature (alternative treatment) on the fatty acid composition of two species of marine fish (tuna and sardine) canned in three different ways (in brine, in sunflower oil and in olive oil).

Tab. 1. Recipes for the different canned fish samples.

Samples	Ingredients	Weight [g]	Percentage [%]
Tuna in brine (TB)	Raw tuna (*Thunnus albacares*)	300	75
	Water	97	24.2
	Salt	3	0.7
	Total	400	100
Tuna in sunflower oil (TSO)	Pre-cooked tuna (*Thunnus albacares*)	280	70
	Sunflower oil	120	30
	Total	400	100
Sardines in olive oil (SOO)	Sardines (*Sardina pilchardus*)	280	70
	Olive oil	120	30
	Salt	Traces	Traces
	Total	400	100

Quantities are given for a 425 ml metal can.

MATERIAL AND METHODS

Sample preparation

Yellowfin tuna (*Thunnus albacares*) from Pacific Ocean, and sardine (*Sardina pilchardus*) from Mediterranean Sea were used for this study. Loins of tuna were frozen and stored at –18 °C for 10–12 weeks before processing. Fresh small whole sardines were rapidly frozen and stored at –18 °C for 10–12 weeks before processing.

Samples were defrosted before processing and canned in 425 ml circular tin can coated with lacquers containing phenolic epoxy, epoxy and organosol (100 mm diameter). Recipes for the different canned fish are shown in Tab. 1. Tuna in brine was composed of raw pieces of yellowfin tuna put in brine. Tuna in sunflower oil was composed of pre-cooked pieces of yellowfin tuna put in sunflower oil. Sardine in olive oil was composed of headed/gutted small sardines put in olive oil. Manufacturing processes for the production of

tuna in brine and tuna in sunflower oil were similar, but that for tuna in oil included two additional steps, namely, cooking and cooling. Cooking was necessary to eliminate the water present in the flesh and to avoid formation of water-oil mixture in the final product. The cans were vacuum-sealed and subjected to retort sterilization and high pressure thermal sterilisation.

Retort sterilization treatment

Canned fish samples were sterilized (F_0 = 7 min) in a retort at 116 °C for 60 min. Three separate batches of tuna and sardine were processed as collected in different seasons in order to estimate the variability in the process and to take into account possible variations of the composition of the fish associated with the seasonality of the raw material collection. Batch 1 was collected in November 2011, batch 2 in November 2012 and batch 3 in January 2013.

High pressure thermal sterilization treatment

HPTS treatment for canned fish samples was done as previously described by SEVENICH et al. [15]. Briefly, a high pressure unit U111 (Unipress, Warsaw, Poland), a laboratory-scale system, was used for the treatment of the samples. The temperature and time conditions selected for the treatment were 115 °C and 28 min of dwell time at 600 MPa, which represented an F_0 value equivalent to conventional retorting of fish cans in the fish industry (7 min). Samples for high pressure treatment (Batch 4) were collected in December 2013.

Extraction and determination of fatty acids profile

Processed samples were lyophilized before lipid extraction. Brine was removed before lyophilization, but the total content of tuna in sunflower oil and tuna in olive oil, including both fish and oil, was jointly lyophilized and analysed. Following grinding, lipid fraction of samples was extracted with chloroform/methanol [16]. Free fatty acid content in lipid extracts was determined by the Lowry and Tinsley method [17]. The determination of fatty acids profile in the extracted lipids was conducted by analysis of fatty acid methyl esters (FAME). FAME were prepared by base-catalysed transmethylation of the extracted lipids using 2 mol·l^{-1} KOH in methanol as described by IUPAC [18, 19].

Gas chromatography

FAME were analysed on a HP-6890 gas chromatograph (Hewlett Packard, Avondale, Pennsylvania, USA) equipped with a flame ionization detector (FID). FAME were separated using HP Innowax capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The temperature programme used was: 180 °C for 2 min, followed by 3 °C·min^{-1} to an upper temperature of 230 °C and held there for 20 min. The temperatures of the injector and detector were held at 250 °C. Hydrogen was the carrier gas at a flow rate of 1 ml·min^{-1} with a split ratio of 1 : 40. Individual fatty acids were identified on the basis of their retention times as compared to appropriate standards.

Polyene index assessment

The polyene index (*PI*) was calculated as the following fatty acid ratio [20]:

$$PI = (C_{C20:5} + C_{C22:6})/C_{C16:0} \quad (1)$$

where $C_{C20:5}$ is the content of C20:5, $C_{C22:6}$ is the content of C22:6 and $C_{C16:0}$ is the content of C16:0.

Statistical analysis

Statistical analyses were performed using Statgraphics Centurion XV (Herndon, Virginia, USA). Data were expressed as mean ± standard deviation. Values were calculated for each variable measured. Statistical confidence was given by Student's *t*-test at a 95% confidence level, therefore differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

In the present study, three canned fish products were selected for evaluating their fatty acids profile: tuna in brine, tuna in sunflower oil and sardine in olive oil. These products were selected because they are representative of the European market. Nutrient content per 100 grams of each food model is presented in Tab. 2. Samples were subjected to classical retorting and HPTS as a novel technique. The fatty acid composition in each sample after these two processes was compared. Due to this selection of food models, it was possible to observe different compositions and variations because of species (tuna and sardine in vegetable oils) and toppings (tuna in brine/tuna in oil).

The major difficulty encountered in canned fish production is the seasonality of the raw material. This is due to the variation of the composition of the fish throughout the year. This phenomenon is observed in all species but it is more significant for fat fish during the spawning period or migra-

Tab. 2. Nutrient content per 100 grams of each food model.

Nutrients	TB	TSO	SOO
Energy [kcal]	121	180	190
Energy [kJ]	506	752	794
Protein [g]	26.1	25	21.7
Lipid [g]	1.9	10.8	12.9
Carbohydrates [g]	0	0	0
Sodium [mg]	400	290	300
Vitamin C [mg]	0	0	0

TB – tuna in brine, TSO – tuna in sunflower oil, SOO – sardines in olive oil.

tions. Variations of composition mainly affect water and fat fractions, since these components may represent approx. 80% of the composition of the flesh. Water and fat compensate for each other and involve variation of the composition and technological properties of the food product. To take into account possible variations related to seasonality, different batches of both tuna and sardine collected in different seasons were produced and an average of the results observed was calculated in this study. Following the extraction of lipids in canned fish samples, content of free fatty acids was determined and values as low as 0.4–0.6% were found.

Tab. 3 shows the fatty acid methyl ester profiles found, obtained after transmethylation of the extracted lipids using 2 mol·l^{-1} KOH in methanol, the values being expressed as percentages of total fatty acid methyl esters. In the samples of canned tuna in brine, seventeen fatty acid methyl esters were identified. The global profile was similar to the levels given for fresh tuna by Tables of Food composition [21].

The major fatty acids were C16:0; C18:0; C18:1, *n*-9; EPA (C20:5, *n*-3) and DHA (C22:6, *n*-3). These fatty acids accounted for approx. 80% of total fatty acids. Similar profiles were reported in studies on other tuna species [22, 23] or bonito [7]. The major saturated fatty acid was palmitic acid (C16:0), content of which varied from 15.9% to 16.9% after high pressure and retort sterilization, respectively. These values were in line with those found by Moreira et al. [24] and Maia et al. [25] in several species of farm and freshwater fishes. Content of stearic acid (C18:0) ranged from 5.9% (high pressure) to 8.5% (retorting). These values

Tab. 3. Fatty acid methyl ester composition of lipids extracted from canned fish samples subjected to retorting and high pressure thermal sterilization treatment.

FAME [%]	Tuna in brine		Tuna in sunflower oil		Sardines in olive oil	
	Retort	HPTS	Retort	HPTS	Retort	HPTS
C14:0	1.4 ± 0.4	1.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	1.5 ± 0.2	1.4 ± 0.0
C15:0	0.7 ± 0.2	0.5 ± 0.0	0.0 ±0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C16:0	16.9 ± 2.9	15.9 ± 0.2	4.8 ± 0.2	5.1 ± 0.4	13.4 ± 0.3	13.2 ± 0.0
C16:1, *n*-7	3.2 ± 1.1	2.9 ± 0.5	0.1 ± 0.0	0.2 ± 0.1	2.3 ± 0.4	1.9 ± 0.0
C17:0	1.1 ± 0.2	1.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
C17:1, *n*-9	0.7 ± 0.2	0.7 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
C18:0	8.5 ± 2.3	5.9 ± 0.1	3.7 ± 0.0	2.1 ± 2.3	3.5 ± 0.2	3.4 ± 0.0
C18:1, *n*-9	23.9 ± 6.1	28.9 ± 0.2	55.9 ± 2.0	59.9 ± 2.5	60.6 ± 1.4^{a}	65.8 ± 0.1^{b}
C18:2, *n*-6	4.1 ± 4.0	4.4 ± 0.3	33.8 ± 1.8	30.9 ± 1.3	4.8 ± 0.8	5.6 ± 0.0
C18:3, *n*-3	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.7 ± 0.2	0.8 ± 0.0
C18:4, *n*-3	0.5 ± 0.0	nd	–	–	–	–
C20:1, *n*-9	1.3 ± 1.1	2.8 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	1.5 ± 0.1	1.4 ± 0.0
C22:1, *n*-11	0.7 ± 0.0	nd	nd	0.1 ± 0.1	nd	1.5 ± 0.0
C20:4, *n*-6	2.3 ± 0.3	nd	–	–	0.2 ± 0.0	nd
C20:5, *n*-3	3.7 ± 1.6	4.5 ± 0.1	0.6 ± 0.3	0.1 ± 0.1	2.6 ± 0.5	1.7 ± 0.0
C22:5, *n*-3	1.4 ± 0.4	nd	0.3 ± 0.1	nd	0.4 ± 0.2	nd
C22:6, *n*-3	22.8 ± 7.3	21.7 ± 0.1	0.2 ± 0.0	0.5 ± 0.1	3.8 ± 0.6^{a}	1.6 ± 0.1^{b}

Results are mean ± standard deviation. For retorted samples, values are the mean of the analysis of three different batches. For samples treated by high pressure, values are means of three different analyses of the same batch.
Different letters in the same canned fish sample mean significant differences among the treatments ($p < 0.05$)
FAME – fatty acid methyl esters (percentage of total FAME is given), HPTS – high pressure thermal sterilization, nd – not detected.

Tab. 4. Summary of the fatty acid methyl ester (FAME) composition and polyene index of lipids extracted from canned fish samples subjected to retorting and high pressure thermal sterilization (HPTS) treatment.

FAME [%]	Tuna in brine		Tuna in sunflower oil		Sardines in olive oil	
	Retort	HPTS	Retort	HPTS	Retort	HPTS
SFA	26.4 ± 7.7	25.2 ± 0.2	8.6 ± 0.2 [a]	7.3 ± 1.8 [b]	18.8 ± 0.4	18.3 ± 0.0
MUFA	29.4 ± 5.6	35.3 ± 0.3	56.3 ± 2.0	60.6 ± 2.8	64.6 ± 1.3 [a]	70.8 ± 0.1 [b]
PUFA	34.9 ± 6.8	31.0 ± 0.2	35.0 ± 2.0	31.6 ± 1.0	12.6 ± 1.2 [a]	9.7 ± 0.0 [b]
EPA + DHA	26.5 ± 8.8	26.3 ± 0.1	0.5 ± 0.4	0.6 ± 0.2	6.5 ± 0.6 [a]	3.3 ± 0.1 [b]
Polyene index	1.6 ± 0.6	1.6 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.0 [a]	0.2 ± 0.0 [b]

Results are mean ± standard deviation. For retorted samples, values are the mean of the analysis of three different batches. For samples treated by high pressure, values are means of three different analyses of the same batch.
Different letters in the same canned fish sample mean significant differences among the treatments ($p < 0.05$).
FAME – fatty acid methyl esters (percentage of total FAME is given), HPTS – high pressure thermal sterilization, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, EPA – eicosapentanoic acid, DHA – docosahexaenoic acid.

were similar to those described for marine species and Brazilian fishes [24, 26], and higher than 2.1% reported by MENDEZ et al. [27] for fishes from Río de la Plata. The rest of the saturated fatty acids (C14:0, C17:0 and C15:0) were present in minor quantities. Among the monounsaturated fatty acids (MUFA), oleic acid (C18:1) was found to be the major constituent (23.9% and 28.9% in samples treated by retort and high pressure, respectively) in a similar way to the results presented by several authors for different freshwater fishes [23, 24, 26, 27]. Concerning PUFA, the principal components were EPA (3.7–4.5%) and DHA (21.7–2.8%), in a higher proportion when compared with other marine species [28]. Similar results were observed in farmed and wild specimens of tuna, where the sum of these fatty acids reached approx. 19% and 22%, respectively [23]. According to RAVICHANDRAN et al. [29], lipids of marine fishes are characterized by higher levels of DHA in comparison with EPA, which agrees with the results observed in the samples of canned tuna in brine.

In Tab. 4, fatty acid composition is summarized, according to the degree of unsaturation, in groups of saturated, monounsaturated and polyunsaturated fatty acids, and specifically *n*-3 PUFA, i.e., EPA and DHA. The application of HPTS, when compared with conventional heating, did not significantly affect the lipid profile of canned tuna in brine. The minor differences observed between treatments could be attributed to variability among batches, which might have included different species and seasonality. Thus, samples subjected to retorting presented higher variability since they were obtained from three different batches. Data expressed as polyene index did not show significant differences in these samples.

In the samples of canned tuna in sunflower oil, fifteen fatty acids were identified (Tab. 3). As expected, the profile of fatty acids was conditioned by the composition of oil since the content of the can was taken as a whole. It is known that the major fatty acid in sunflower oil is linoleic acid (C18:2) (~62%), followed by oleic acid (C18:1) (~25%) [21]. The highest contents were observed for C18:1 (55.9% and 59.9% in samples treated by retorting and high pressure, respectively), which reflected the contribution of both the sunflower oil and tuna lipids. Linoleic acid was the second major fatty acid, ranging from 30.9% to 33.8%, whereas the rest of fatty acids, with the exception of C16:0 and C18:0, were found in very small proportions. Due to the high contents of C18:1 and C18:2 in these samples, the proportion of EPA and DHA (approx. 1%) was lower than that found in tuna in brine. Again, no significant differences were found between fatty acids in the samples subjected to the different treatments and hence the sterilization method apparently did not affect the profile of fatty acids in tuna in sunflower oil. Polyene index, therefore, did not show significant differences (Tab. 4).

The composition of fatty acids of sardine in olive oil was also conditioned by the fatty acid composition of the vegetable oil. In these samples, fifteen fatty acids were identified (Tab. 3), the profile of fatty acids being similar to that previously shown for sardines canned in olive oil [30]. Olive oil is characterized by a high content of oleic acid (C18:1) (~69%), followed by important proportions of C16:0 (~12%) and C18:2 (~10%) [21]. These proportions were very close to those found in samples of sardine in olive oil. Oleic acid represented ~60% of total fatty acids in the retorted batches, whereas high pressure treated samples

showed a significantly higher content (65.8%). Regarding the proportions of C16:0 and C18:2, the percentages ranged between 13.2% and 13.4%, and between 4.8% and 5.6%, respectively. Values for EPA and DHA, characteristic of marine fish, varied from 3.3% to 6.5%. Small differences were observed among the different batches in retorted samples, which agreed with the observations of BADOLATO et al. [31], who indicated that, although lipid levels in sardines can vary with seasonality, the fatty acids profile did not significantly change. Significant differences were observed in the total PUFA content and also in the sum of EPA and DHA when sardine subjected to the different sterilization treatments were compared. In this regard, samples treated by HPTS showed the lowest content and, consequently, the MUFA proportion in this latter group of samples was significantly higher when compared with retorted sardine. Polyene index also showed significant differences between treatments ($p < 0.05$), being the high pressure + high temperature treated samples, which denotes the higher lipid alteration (Tab. 4).

The literature data regarding the effect of high pressure treatment on lipid oxidation are controversial [14] and especially the potential consequences of the novel combination of high pressure + heat treatment are not evaluated in a way as they are in the present study. ANGSUPANICH and LEDWARD [13] observed changes in lipid oxidation in cod muscle treated by pressure above 400 MPa, whereas no effects were observed at 200 MPa. Conversely, CHEVALIER et al. [32] reported that the oxidative stability of lipids in turbot (*Scophthalmus maximus*) muscle was particularly affected beyond 180 MPa. Pronounced effects were observed in mackerel muscle lipids after high pressure treatments, attributed to intrinsic components of muscle that can enhance lipid oxidation [33]. In fact, high pressure may induce denaturation of heme proteins in muscle, which facilitates a higher exposure to the catalytical heme group [34] and to the iron ions released to the medium [35]. It is well known that certain metal ions may play an important role in promoting autoxidation of lipids in pressurized fish meat [36].

As mentioned above, lower proportions of PUFA were also observed in samples of tuna in brine and tuna in sunflower oil treated by high pressure as compared with their counterpart retorted samples, although such differences were only significant in sardine canned in olive oil. Mineral composition was previously found higher in canned sardine than in canned tuna, including iron content [21], which might have promoted lipid oxidation in high pressure-treated sardine and justify why these significant effects were not observed in the tuna samples. Anyway, many other factors may have contributed to the results obtained, such as the variability among the batches and possible differences in the content of prooxidants and antioxidants.

CONCLUSIONS

In this paper, the fatty acid composition of yellowfin tuna (*Thunnus albacares*) and sardine (*Sardina pilchardus*) canned in three different ways (in brine, in sunflower oil and in olive oil) and subjected to two different sterilization treatments (retort heating, as conventional treatment, and high pressure thermal sterilization, as alternative treatment) was investigated. Tuna subjected to retort sterilization and HPTS presented similar fatty acid profiles, and no significant differences were observed in EPA and DHA contents, which are the fatty acids most susceptible to deterioration by oxidation due to their high degree of unsaturation. However, significant differences were found in sardine canned in olive oil, where total PUFA content and the sum of EPA and DHA were lower in samples treated by HPTS ($p < 0.05$). These results suggest that combination of high pressure + high temperature could promote lipid oxidation. This fact may be explained by the higher mineral content of canned sardine in comparison with canned tuna, which during HPTS treatment may promote lipid degradation. Nevertheless, further investigation with other oily fish species canned with refined vegetable oils is required to confirm the potential oxidative effect of HPTS on EPA + DHA contents.

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