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FOOD COMPOSITION AND ANALYSIS

Effect of different preservation processes on chemical composition and fatty acid profile of anchovy (*Engraulis anchoita*)

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Abstract

The effects of salting–ripening, canning and marinating processes on chemical composition and fatty acid profile of anchovy (*Engraulis anchoita*) were evaluated ($p = 0.01$), with emphasis on long-chain polyunsaturated fatty acids. Fresh anchovy showed a high proportion of PUFAs (~45 g/100 g total lipid) with an eicosapentaenoic (EPA) + docosahexaenoic (DHA) content of 27.08 g/100 g total lipid. The salting–ripening process led to the largest changes in the chemical composition and the fatty acid profile, which resulted in a reduction of ~70% on the total EPA and DHA contents (g/100 g edible portion). Contrary, canned and marinated anchovy presented a fatty acid profile similar to that of fresh anchovy. The use of vegetable oil as covering liquid led to final products with increased ω-6 PUFAs content. Despite the modifications observed, the total amount of essential EPA and DHA fatty acids provided by these products remained high compared with values reported in literature for other foods.

Keywords

Canning, DHA, EPA, fish, marinating, salting-ripening

History

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Introduction

Fish has long been recognized as a healthy food with excellent nutritional value, providing high-quality protein, minerals, vitamins, essential fatty acids and trace elements. Fish proteins are easily digestible and contain significant amounts of all the essential amino-acids, principally lysine and the sulphur-containing amino-acids (methionine and cysteine) that are often present in low quantities in vegetables, cereals and legumes. Fish protein can therefore be used to complement the amino acid pattern and the overall protein quality in human diet. Moreover, fatty fish is the richest source of long-chain polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (Huss, 1995; James, 2013; Pozo et al., 1992). This type of fatty acids play an important function in neurodevelopment in unborn babies and infants and have also assumed great nutritional significance owing to their protective role against diseases such as hypertension, inflammation, arrhythmias, psoriasis, cancer and cardiovascular events. In this sense, a number of countries as well as different health organizations recommend a consumption of ~0.5–1.8 g/day of EPA + DHA or two servings of fatty fish per week to reduce the risk of death from coronary heart disease. Taking into account the small contribution of aquatic products to the diet of numerous occidental countries, an increase in the fish and fish-derived products consumption would be recommended (Carrero et al., 2005; FAO, 2011; James, 2013; Kris-Etherton et al., 2002).

The motivation for this research is to investigate how different conservation processes affect the chemical composition and nutritional value of fish. Data on the physicochemical characterization of fish are generally available for raw fish or for fish subjected to a certain type of conservation process (Gladyshev et al., 2007; Jabeen & Chaudhry, 2011; Murillo et al., 2014; Rodríguez et al., 2009; Sirot et al., 2008; Tarley et al., 2004). However, there is limited information about the effect of different processes applied to a fish species and on intermediate products obtained after each processing step.

In this work, products obtained from *Engraulis anchoita* (anchovy) were selected for studying due to the commercial importance (concrete and potential) of this fish species for South American countries and also because it is used as raw material for the obtention of very different products. According to biological data, *E. anchoita* is the most abundant and widely distributed species of the South-western Atlantic Ocean. Since it has been established that the species is underexploited, regional efforts are in course in order to generate new technological alternatives to obtain products with value added. Nowadays, Argentine is the only country that exploits this resource and manufactures products for direct human consumption. *E. anchoita* is used as raw material mainly to obtain salted-ripened anchovy fillets in oil, canned anchovy and marinated anchovy. These products play an important role on the Argentinean exports and internal market. Approximately 95% of the salted–ripened anchovy produced in this country is exported in barrels as intermediate product mainly to Spain, Peru, United States, Italy and Morocco; where it is reprocessed to obtain anchovy fillets in oil (final product). On the

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other hand, salted–ripened anchovy fillets in oil and marinated anchovy (ready-to-eat products) are exported in small volumes as delicatessen with a high market price. Finally, canned anchovy is produced mostly to supply internal market and to export to adjacent countries (Pastous Madureira et al., 2009).

The three mentioned products involve different preserving mechanisms. Salted–ripened and marinated anchovy products can be classified as preserves. In the first one, commercial stability is given by the reduction of a_w and by the high NaCl content, which creates an unfavourable environment for the microbial development. For its part, marinated anchovy is preserved by the simultaneous action of organic acids (acetic acid) and salt which retard the action of enzymes and prevent the growth of pathogenic bacteria and the majority of spoilage bacteria. These two processes result in products with a characteristic flavour and an extended shelf life. On the other hand, canned anchovy is thermally processed at temperatures above 100 °C to achieve the total inactivation of all vegetative bacteria and partial or total inactivation of spores. Thus, the ''commercial sterility'' at ambient temperature for long-term storage is ensured. Since the conservation principles are different, the effects of these processes on proximate chemical composition and fatty acid profile are expected to be different.

Accordingly, the aim of this study was to determine the effect of salting–ripening, marinating and canning processes on proximate chemical composition and fatty acid profile of fishes belonging to the *E. anchoita* species, contributing to knowledge about this important fish resource and covering processes that imply very different conservation principles.

Materials and methods

Raw material

Anchovies (*E. anchoita*) used for the experiences were caught in the coastal region of Argentina, near the Mar del Plata Port (38°S, 57°33′W), during the harvest season. After capture, fish was maintained in ice until arriving to an industrial establishment. There, fish (classified as 33–35 pieces/kg) were processed to obtain salted–ripened anchovy fillets in oil, canned anchovy and marinated anchovy. The processes were effectuated according to the usual industrial procedures.

Processing conditions

Salting–ripening process: obtention of salted–ripened anchovy fillets in oil

Whole anchovies were immersed in saturated brine (brine-to-fish ratio was 1:1) for 24 h. Then, specimens were beheaded and partially gutted by hand, leaving gonads and pyloric caeca. Following this, they were placed in plastic barrels (with a capacity of 200–240 kg) for final salting and ripening. A layer of salt (NaCl) was first put in the bottom of the barrel, then a layer of fish and so on, forming alternate strata with a set amount of salt between each layer. The salt to fish final ratio was 1:5. A plastic lid was put on each barrel and also weights were placed over it to keep fish pressed at 80 g/cm^2. Anchovies were ripened in a closed room for 9 months at an average temperature of 15 °C. The ripening degree was assessed by means of sensorial analysis, according to Filsinger et al. (1982). Once the appropriate degree of maturity was reached, weights and lids were removed and salted–ripened anchovies were taken out of the barrels. Successively washing steps in saturated brine baths at different temperatures were used to remove the remaining salt crystals and to help the anchovies skinning. Finally, anchovies were opened by hand in order to remove the backbone and separate the two fillets. Fillets were packed in glass jars and covered with sunflower oil.

Canning process

Beheaded and gutted fresh anchovies were steam pre-cooked at atmospheric pressure during 15 ± 2 min. Fish was then cooled at room temperature (10–12 °C) under forced convection. The tail was manually removed and specimens were placed in flat rectangular cans (12 cm × 6 cm × 3 cm, net weight ~160 g). A pre-fixed amount of NaCl was added to each container in order to achieve a final salt concentration in fish muscle of ~1.5 g/100 g of edible food. Soybean oil was used as coating liquid. The cans were vacuum-sealed and sterilized at 115 °C for 90 min.

Marinating process

Anchovies used as raw material for marinated products were previously frozen at −20 °C and kept in this condition for 48 h to prevent the risk of the anisakis parasite presence (Reg. EC No. 853/2004). After that, whole anchovies were defrosted, washed and manually headed, gutted and filleted. After immersion in brine (NaCl, 10 g/100 g) for 1 h at a 1:1 (fish:brine) ratio, fillets were placed in closed vessels with a marinating solution containing acetic acid, 3 g/100 g and NaCl, 10 g/100 g. Anchovy fillets were kept in the marinating bath for 9 days at 15 ± 1 °C until the required texture was achieved (Yeannes & Casales, 1995). Then, the fillets were removed, drained to eliminate the excess of marinating solution and packed in glass jars covered with sunflower oil.

Sampling procedure and sample treatment

Proximate chemical composition and fatty acids composition were determined in fresh anchovy and in final products. In addition, samples of intermediate products were taken from the production line, considering the stages in which major changes in composition would be expected, and analysed. Thus, for salting–ripening process and obtention of fillets in oil, samples were taken: (i) after brining (designated ''brined''); (ii) from barrels at the end of ripening (designated ''salted–ripened anchovy''); (iii) from packed anchovy fillets in oil stored for 6 months (designated ''fillets in oil''). For canning process, samples were taken: (i) after steaming (designated ''cooked''); (ii) after sterilization (designated ''canned''). Finally, for marinating process, samples corresponding to: (i) 48 h frozen anchovy (''frozen'') and (ii) marinated anchovy fillets in oil stored for 7 days at refrigeration temperature (5 ± 0.5 °C) (designated ''marinated''), were collected.

In all cases, a stratified random sampling procedure was applied in order to obtain a representative sample. A pool consisting of ~2 kg was analysed in each case.

Prior to analyses, canned, marinated and anchovy fillets in oil were separated from the coating liquid by a 10-min drainage and then blotted with absorbent paper to eliminate the remaining superficial liquid. For the analysis of salted–ripened anchovy, specimens were filleted and remaining scales or viscera were removed. Samples were minced and analysed immediately.

Physicochemical analyses

Samples were analysed for proximate chemical composition, NaCl content, pH and water activity (a_w) of the edible portion (fillets). Acidity was determined in marinated anchovies. Water content was determined by oven drying at 105 ± 1 °C until constant weight (AOAC, 1990, Sec 984.25). Fat content was determined by the acid hydrolysis method (AOAC, 1990,

Sec. 948.15) and ash content by calcination at 550 ± 5 °C (AOAC, 1993, Sec. 945.46). Protein content was measured by Kjeldhal, using the factor 6.25 to convert total nitrogen into protein nitrogen (AOAC, 1993, Sec. 920.152). NaCl content was determined by the Mohr method (Kirk et al., 1996). For pH determination, 10 g of sample was homogenized with 10 ml of distilled water, according to AOAC (1990, Sec 981.12) and measured with a digital pH-meter (Instru RS-232) equipped with a combined glass electrode. Acidity was determined by titration with NaOH 0.1 N (Kirk et al., 1996). Results were expressed as percentage of acetic acid. Water activity was measured by a digital hygrometer Aqualab, model CX-2T (Decagon, Pulman, WA). All analyses were conducted in triplicate.

Analysis of the fatty acid composition

The lipid to be used for fatty acid profile determination was extracted from 100 g of ground samples according to Bligh & Dyer (1959). The extracted lipid was stored under nitrogen in the dark at −20 °C for further analyses. The coating oil medium of salted–ripened fillets, canned and marinated anchovy was also analysed. Fatty acid profile was determined by fatty acid methyl ester (FAMEs)/gas chromatography (GC). For this, 100 mg of the lipid sample was dissolved in hexane and treated with a solution of KOH in methanol according to the norm ISO 5509 (2000). Fatty acids methyl esters were separated and quantified using a gas chromatograph (Shimadzu GC-17A, Japan) equipped with a 30-m fused silica capillary column Omegawax 320 (Supelco Inc., Bellefonte, PA) (0.32 mm i.d.; 0.25 µm film) and a flame ionization detector. Carrier gas was nitrogen. Sample injected volume was 1 µl. Column oven temperature was programmed to begin at 150 °C, increase to 225 °C at 1.5 °C/min and held for 13 min. The temperature of the injector port and the detector was held at 250 °C. Peak retention times and area percentages of total fatty acids were identified by comparison with the standard PUFA-1, Marine Source from Supelco (Bellefonte, PA). The polyene index (PI) was calculated as the following fatty acid ratio: (C20:5ω3 + C22:6ω3)/C16:0 (Lubis & Buckle, 1990).

Statistical analysis

Analysis of variance (ANOVA) was carried out to find the differences in proximate chemical composition and the fatty acid composition due to processing methods evaluated. An independent ANOVA was used for each processing method. Differences between means were analysed using the Tukey's test for *post hoc* comparison ($p < 0.01$). Analyses were performed using STATISTICA 6.0 (Statsoft, Inc., Tulsa, OK).

Results and discussion

Chemical and fatty acid composition of fresh anchovy

The fresh anchovy chemical composition is shown in Table 1. The values determined for the main constituents are in the range of those reported in available literature for this species. As other pelagic species, *E. anchoita* can show important seasonal variations in proximate chemical composition – especially on water and lipid contents – linked to the reproductive cycle. Sex, age and external factors (such as temperature and salinity of the water, composition and availability of food) have also been pointed out to affect proximal chemical composition of fish (Aizpún de Moreno et al., 1979; Huss, 1995; Pozo et al., 1992). Consequently, the values of lipid content found in literature for anchovy captured during the harvest season varies in the range 1.68–6.46 g/100 g of edible food (Aizpún de Moreno et al., 1979; Czerner & Yeannes, 2014; Czerner et al., 2011; Massa et al., 2007, 2012; Yeannes & Casales, 1995).

The fatty acid composition of fresh anchovy is presented in Table 2. It can be seen that PUFAs were the most abundant, followed by monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). DHA (C22:6 ω-3) and EPA (C20:5 ω-3) accounted for >85% of total ω-3 PUFAs. The major MUFAs were cetoleic (22:1 ω-11) and oleic (18:1) acids. Among SFAs, palmitic acid (16:0) was the most concentrated. This fatty acids pattern is typical of fatty fish species and has been previously reported in literature for different species (Huynh & Kitts, 2009; Jabeen & Chaudhry, 2011; Massa et al., 2007, 2012; Sirot et al., 2008; Zlatanos & Laskaridis, 2007). For comparison, Massa et al. (2007, 2012) reported for *E. anchoita* a lipid content of 7.37–10.04 g/100 g of total food, with the following composition given in g per 100 g of total lipids: SFAs, 31.01–31.47; MUFAs, 41.75–35.29; PUFAs, 28.94–27.07 and ω-3, 24.02–25.89. It has to be remarked that these authors informed higher total lipid and SFA contents than those determined in this article, with a lower proportion of PUFAs and ω-3 fatty acids. A direct relationship between total lipid content and SFA content has also been reported for other fish species. It has been pointed out that SFAs are accumulated for energy storage, and therefore its concentration increases during periods of enhanced feeding activity when the lipid content is high. In addition, seasonal variation in the feed composition (plankton) has been identified as another possible factor that could modify fatty acids profile in fish (Murillo et al., 2014; Zlatanos & Laskaridis, 2007).

Table 1. Chemical composition and other physicochemical parameters of fresh and processed anchovy (g per 100 g of edible food).

	Water	Protein	Lipid	Ash	NaCl	pH	a_w
Fresh anchovy	77.65 ± 0.67^{a}	16.24 ± 0.82^{a}	4.25 ± 0.09^{a}	1.16 ± 0.06^{a}	$0.13 \pm 3.5 \times 10^{-5a}$	6.07	0.999 ± 0.001
Salting-ripening							
Brined sample	55.15 ± 0.42^{b}	25.56 ± 0.24^{b}	5.12 ± 0.04^{ab}	17.84 ± 0.05^{b}	17.12 ± 0.04^{b}	5.75	0.812 ± 0.010
Salted-ripened sample	51.32 ± 0.37^{c}	23.65 ± 0.70^{c}	4.58 ± 0.29^{ab}	18.97 ± 0.04^{b}	17.75 ± 0.17^{b}	5.68	0.766 ± 0.004
Fillets in oil	50.21 ± 0.30^{c}	26.70 ± 0.68^{b}	5.69 ± 0.29^{b}	17.41 ± 0.09^{c}	16.52 ± 0.10^{b}	5.65	0.780 ± 0.008
Canning							
Cooked sample	67.5 ± 0.03^{b}	27.53 ± 0.37^{b}	4.05 ± 0.10^{a}	1.15 ± 0.00^{a}	0.16 ± 0.05^{a}	Nd	0.985 ± 0.003
Canned sample	64.82 ± 0.06^{b}	26.74 ± 0.74^{b}	6.23 ± 0.38^{b}	2.21 ± 0.01^{b}	0.29 ± 0.02^{b}	6.12	0.982 ± 0.003
Marinating							
Frozen sample	77.80 ± 0.06^{a}	16.40 ± 0.30^{a}	4.68 ± 0.22^{a}	1.13 ± 0.02^{b}	$0.16 \pm 1 \times 10^{-4a}$	6.41	0.998 ± 0.001
Marinated sample	64.79 ± 0.05^{b}	21.53 ± 0.48^{b}	6.81 ± 0.35^{b}	6.87 ± 0.07^{c}	5.02 ± 0.03^{b}	4.20	0.961 ± 0.001

Mean ± standard deviation. Nd: not determined. An independent ANOVA was carried out for each processing method, always including composition data corresponding to fresh anchovy. Different lower-case letters (a, b, c) in the same column indicate significant differences within stages of the different process ($p < 0.01$).

Table 2. Changes in fatty acids profile (g per 100 g of total lipid) of anchovy during salting–ripening and obtention of fillets in oil.

Fatty acid	Fresh anchovy	Brined sample	Salted-ripened sample	Fillets in oil	Covering liquid
C14:0	2.54 ± 0.15^{a}	1.61 ± 0.16^{a}	6.75 ± 0.05^{b}	1.64 ± 0.08^{a}	0.25 ± 0.13
C16:0 (NS)	13.55 ± 0.76	16.23 ± 0.54	14.84 ± 0.24	13.66 ± 0.07	6.72 ± 0.75
C16:1	5.08 ± 0.91^{a}	6.71 ± 0.23^{b}	8.69 ± 0.15^{b}	4.09 ± 0.24^{a}	Nd
C18:0	0.97 ± 0.33^{a}	2.45 ± 0.12^{b}	1.78 ± 0.23^{ab}	2.54 ± 0.08^{b}	1.29 ± 0.07^{a}
C18:1	14.18 ± 0.15^{a}	16.87 ± 1.32^{a}	12.88 ± 0.15^{a}	24.88 ± 0.18^{b}	25.81 ± 0.18
C18:2 ω-6	2.77 ± 0.08^{a}	2.07 ± 0.04	1.58 ± 0.07^{a}	41.57 ± 0.87^{b}	65.94 ± 1.12
C18:3 ω-3	1.26 ± 0.04^{a}	0.95 ± 0.14^{ab}	0.42 ± 0.04^{b}	3.55 ± 0.07^{c}	Nd
C18:4 ω-3	2.17 ± 0.03^{a}	3.08 ± 0.11^{b}	1.66 ± 0.03^{c}	0.38 ± 0.06^{d}	Nd
C20:1 ω-11	4.42 ± 0.02^{a}	0.23 ± 0.45^{b}	0.55 ± 0.02^{b}	Nd	Nd
C20:4 ω-6	4.99 ± 0.01^{a}	0.28 ± 0.06^{b}	0.38 ± 0.01^{b}	Nd	Nd
C20:5 ω-3	4.47 ± 0.11^{a}	5.21 ± 0.34^{a}	3.83 ± 0.11^{a}	1.47 ± 0.01^{b}	Nd
C22:1 ω-11	17.00 ± 0.48^{a}	11.97 ± 0.08^{b}	19.50 ± 0.54^{a}	Nd	Nd
C22:6 ω-3	22.61 ± 0.81^{a}	25.07 ± 0.23^{a}	14.22 ± 0.33^{b}	4.81 ± 0.34^{c}	Nd
Others	3.86 ± 0.15^{a}	10.95 ± 0.46^{b}	12.56 ± 0.17^{b}	1.58 ± 0.06^{c}	Nd
∑SFAs	18.15 ± 1.31^{a}	26.92 ± 1.32^{b}	25.55 ± 0.59^{ab}	17.84 ± 0.23^{a}	8.25 ± 0.94
∑MUFAs	36.80 ± 1.59^{a}	39.52 ± 1.96^{a}	49.94 ± 0.90^{b}	30.54 ± 0.49^{a}	25.81 ± 0.18
∑PUFAs	44.92 ± 1.13^{a}	33.65 ± 1.42^{b}	24.15 ± 0.65^{b}	51.78 ± 1.34^{a}	65.94 ± 1.12
∑ω-3	31.81 ± 0.98^{a}	30.53 ± 0.83^{a}	21.39 ± 0.55^{b}	10.21 ± 0.47^{c}	Nd
∑ω-6	8.69 ± 0.13^{a}	3.16 ± 0.53^{b}	2.20 ± 0.21^{b}	41.57 ± 0.87^{c}	65.94 ± 1.12
PI	2.00 ± 0.04^{a}	1.87 ± 0.03^{b}	1.22 ± 0.01^{c}	0.46 ± 0.02^{d}	

Mean ± standard deviation. Nd: not detected. Different lower-case letters (a, b, c) in the same raw indicate significant differences within stages of the canning process ($p < 0.01$). NS, not significative differences.

Changes in chemical and fatty acid composition during processing

Salting–ripening process: obtention of salted–ripened anchovy fillets in oil

As a result of the salting–ripening process, the anchovy proximate chemical composition showed important modifications (Table 1). Clearly, the stage of brining led to the largest changes in proximate chemical composition. The constituents most affected were water, NaCl and ashes. The reduction of water content with an increase in ashes and NaCl contents ($p < 0.01$) is expected for this type of process. The higher protein content determined after brining and in the subsequent samples is explained by the changes occurred in the base of calculus (wet basis).

After ripening in barrels, the proximate composition was slightly modified. Water content showed a second reduction that is related to the partial gutting and the press applied in this stage, which promote additional water loss. During ripening in barrels, proteolysis occurs (Hernández-Herrero et al., 1999) and is reflected by a minor reduction in the protein content ($p < 0.01$). The lipid content showed a slight increase in fillets in oil compared with fresh anchovy ($p < 0.01$), possibly due to imbibition of covering liquid.

The pH, a_w and NaCl content determined in both, salted–ripened anchovy and fillets in oil were within the typical values expected for these products and agrees with available literature (Filsinger et al., 1978; Hernández-Herrero et al., 1999).

As shown in Table 2, the fatty acid composition was also modified during this process. Brined and salted–ripened anchovies showed a sharp decrease of PUFAs compared with raw anchovy, ω-6 fatty acids being the most affected while the SFAs fraction was the most stable over the process. The hard reduction in the PI observed during processing indicates an important oxidative rancidity (Lubis & Buckle, 1990). These results can be explained considering that PUFAs are highly susceptible to chemical and enzymatic oxidation, which are enhanced by the extremely pro-oxidant conditions that occur during brining and also in barrels. In first place, the a_w value (0.766) places this product within the zone of maximum lipid oxidation and of important hydrolytic and enzymatic activities (Labuza, 1980). Moreover, the high NaCl concentration and the presence of some remaining blood and bacterial enzymes are factors that could promote lipid oxidation (Ashton et al., 2002; Czerner & Yeannes, 2014). Finally, lipolysis occurring during ripening (Roldán et al., 1985) would favour rancidity, since free fatty acids oxidizes more readily than the esterified lipids (Ashton et al., 2002). It must be considered that previous studies indicate that the compounds generated by lipid oxidation contribute to the development of the typical sensorial characteristics of salted–ripened anchovy (Czerner et al., 2011). Thus, the increased PI does not imply a quality loss in this product.

The fatty acids profile of anchovy fillets in oil was different from that for salted–ripened anchovy, displaying a significant increment of PUFAs specially given by the increased linoleic (C18:2 ω-6) fatty acid content (Table 2). This fatty acid, and also the C18:1, are abundant in the sunflower oil used as covering liquid and could be absorbed into anchovy fillets during storage. As a result of this, the proportion of SFAs, MUFAs and PUFAs in anchovy fillets in oil was similar to that of fresh anchovy. But, conversely to fresh anchovy, ω-6 fatty acids prevail within PUFAs, whereas EPA and DHA fatty acids, which predominated in fresh anchovy, only represented the 61% of the total ω-3 fatty acids in the packed fillets.

Canning process

Variations on anchovy chemical proximate composition as a result of the canning process are shown in Table 1. Water content was reduced after steam-cooking, leading to a simultaneous relative increase in the protein content ($p < 0.01$). This fact has been reported by other researchers (Rodríguez et al., 2009) and can be attributed to protein coagulation by heat and the consequent reduction on the water holding capacity of myofibrillar proteins. For lipid content in muscle, a slight reduction ($p < 0.01$) was found after the steam-cooking step which could be associated to lipid release during this stage. After sterilization, lipid content increased ($p < 0.01$) due to the coating oil absorption into the anchovy muscle. These findings are in agreement with those

Table 3. Changes in fatty acids profile (g per 100 g of total lipid) of anchovy during canning process.

Fatty acid	Fresh anchovy	Cooked sample	Canned sample	Covering liquid
C14:0	2.54 ± 0.15^{a}	4.75 ± 0.31^{b}	2.05 ± 0.13^{a}	0.58 ± 0.01
C16:0	13.55 ± 0.76^{a}	25.75 ± 0.44^{b}	20.29 ± 0.34^{c}	12.78 ± 0.71
C16:1	5.08 ± 0.91^{a}	Nd	Nd	Nd
C18:0 (NS)	0.97 ± 0.33	1.47 ± 0.11	1.89 ± 0.03	2.13 ± 0.08
C18:1	14.18 ± 0.15^{a}	10.91 ± 0.11^{b}	17.70 ± 0.20^{c}	22.12 ± 0.31
C18:2 ω-6	2.77 ± 0.08^{a}	1.49 ± 0.05^{b}	28.45 ± 0.15^{c}	54.23 ± 0.46
C18:3 ω-3	1.26 ± 0.04^{ab}	0.67 ± 0.00^{a}	3.04 ± 0.13^{b}	6.70 ± 1.14
C18:4 ω-3	2.17 ± 0.03^{a}	1.94 ± 0.05^{a}	0.53 ± 0.00^{b}	Nd
C20:1 ω-11	4.42 ± 0.02^{a}	Nd	Nd	Nd
C20:4 ω-6	4.99 ± 0.01^{a}	Nd	Nd	Nd
C20:5 ω-3	4.47 ± 0.11^{a}	5.75 ± 0.18^{b}	4.36 ± 0.15^{a}	Nd
C22:1 ω-11	17.00 ± 0.48^{a}	7.60 ± 0.03^{b}	1.14 ± 1.19^{c}	Nd
C22:6 ω-3	22.61 ± 0.81^{a}	36.06 ± 0.31^{b}	18.85 ± 0.65^{a}	1.16 ± 0.01
Others	3.86 ± 0.15^{a}	3.95 ± 0.24^{a}	1.88 ± 0.05^{b}	Nd
$\sum$SFAs	18.15 ± 1.31^{a}	31.97 ± 0.87^{b}	24.39 ± 0.49^{ab}	15.49 ± 0.81
$\sum$MUFAs	36.80 ± 1.59^{a}	21.83 ± 0.29^{b}	20.56 ± 1.43^{b}	22.12 ± 0.31
$\sum$PUFAs (NS)	44.92 ± 1.13	46.53 ± 0.68	55.22 ± 1.08	62.70 ± 1.61
$\sum$ω-3	31.81 ± 0.98^{a}	44.41 ± 0.53^{b}	26.78 ± 0.93^{a}	8.47 ± 1.15
$\sum$ω-6	8.69 ± 0.13^{a}	2.12 ± 0.14^{b}	28.45 ± 0.15^{c}	54.23 ± 0.46
PI	2.00 ± 0.04^{a}	1.62 ± 0.01^{b}	1.14 ± 0.02^{c}	

Mean ± standard deviation. Nd: not detected. Different lower-case letters (a, b, c) in the same raw indicate significant differences within stages of the canning process ($p < 0.01$). NS, not significative differences.

reported by García-Arias et al. (1994) for canned tuna (*Thunnus alalunga*) and Selmi et al. (2008) for tuna (*Thunnus thynnus*) and sardine (Sardina *pilchardus*), between others. The increment observed in the NaCl content ($p < 0.01$) is partly related to the addition of this salt into the cans before the sterilization.

The FAs profile of samples taken during the canning process and also of the covering liquid taken after thermal treatment is shown in Table 3. Steam-cooking mainly affected the MUFAs and ω-6 fatty acids, whose contents were significantly reduced after this operation ($p < 0.01$). This decrease could be related to leaching loss, as shown the reduction of fat content after this step (Table 1), and also to lipid damage due to high temperatures. Concerning the fatty acids pattern in the sterilized sample, an important effect of this step was observed. As shown in Table 3, canned anchovy exhibited a marked increase of the ω-6 fatty acids, especially in linoleic acid content (C18:2 ω-6), if compared with fresh or cooked anchovy. This fact is consistent with the rise in total fat content observed after thermal treatment (Table 1) and can be attributed to the incorporation of soybean oil, rich in C18:2 ω-6, into the anchovy muscle. A water-fat exchange was observed in other canned fish species, for which the fat composition in muscle after sterilization was similar to that of the oil used as covering liquid (García-Arias et al., 1994; Selmi et al., 2008). The changes observed in the fatty acid pattern are reflected in the decreased PI. The proportion of SFAs, MUFAs and PUFAs was different in canned and fresh anchovy. The total amount of MUFAs decreased due to the process, given specially by the reduction in the cetoleic acid (C22:1 ω-11). The relative content of the essential EPA and DHA fatty acids was comparable in fresh and canned anchovy ($p > 0.01$).

Marinating process

Changes in the proximal chemical composition that take place during the marinating process are shown in Table 1. Freezing did not lead to important changes in the chemical composition. Nevertheless, marinating conduced to a reduction of the water content with an increment on the relative amounts of fat, protein and ashes ($p < 0.01$). The increased ash content is also related to the diffusion of NaCl from the marinating bath into the flesh. Similar findings have been previously reported for marinated anchovy (Cabrer et al., 2002) and also other fish species (Bilgin et al., 2011; Özden, 2005).

As a result of the marinating process, a_w and pH were reduced to 0.961 ± 0.001 and 4.2, respectively, with an acidity value of 1.28 ± 0.05 g of acetic acid per 100 g of edible food. The combined action of low a_w and pH, places this product within the inhibition zone for the growing of pathogenic bacteria and also of many of the spoilage microorganisms (Bilgin et al., 2011).

As shown in Table 4, the fatty acid composition was significantly affected by the marinating process. Freezing step reduced the relative amounts of C18:3 ω-3, C20:1 ω-11, C20:4 ω-6 and C22:1 ω-11 fatty acids ($p < 0.01$). The main change in marinated anchovy was the increase of oleic (C18:1) and linoleic acids (C18:2 ω-6), indicating imbibitions of the covering sunflower oil into the flesh. A remarkable result is that EPA and DHA fatty acids contents were not significantly diminished by the marinating process. Analogous outcomings have been reported for marinated Engraulis *encrasicholus* (Özden, 2005). The proportion of SFA, MUFA, PUFA, total ω-3 and ω-6 fatty acids were not modified as net result of the marinating process compared with fresh anchovy.

Nutritional value of anchovy products from fatty acids signature

In general, fish is believed to be a good source of proteins with high biological value, minerals and vitamins essential for the human health. Moreover, the high PUFAs content makes this food an excellent choice for a healthy human diet. As shown in Tables 1–4, all the products studied in this article meet these characteristics, showing high protein content, a low fat content and a PUFAs content over 2.95 g/100 g of edible food. These values are much higher than those reported for other popular protein sources (meats cuts) in which PUFAs content does not exceed 1.2 g/100 g of edible food (INFOODS, 2014). Within fish species, the protein content rise 22–24 g/100 g of edible food and the fat content is in general <20 g/100 g of edible food. The PUFAs content is variable, but values in the range 0.18–2.13 g/100 g of edible food are commonly reported for fatty fish species with a high proportion of ω-3 and ω-6 fatty acids (Agustinelli & Yeannes, 2015; INFOODS, 2014; Massa et al., 2012; Sirot et al., 2008).

Table 4. Changes in fatty acids profile (g per 100 g of total lipid) of anchovy during marinating process.

Fatty acid	Fresh anchovy	Frozen sample	Marinated sample	Covering liquid
C14:0	2.54 ± 0.15^{a}	6.05 ± 0.65^{b}	4.82 ± 0.26^{b}	Nd
C16:0 (NS)	13.55 ± 0.76	19.87 ± 1.76	16.65 ± 0.20	5.32 ± 0.22
C16:1	5.08 ± 0.91^{a}	7.96 ± 0.91^{b}	5.93 ± 1.20^{ab}	Nd
C18:0 (NS)	0.97 ± 0.33	1.47 ± 0.33	1.74 ± 0.11	3.67 ± 0.45
C18:1	14.18 ± 0.15^{a}	12.87 ± 0.15^{a}	21.41 ± 0.41^{b}	29.05 ± 0.84
C18:2 ω-6	2.77 ± 0.08^{a}	1.68 ± 0.08^{a}	12.73 ± 1.95^{b}	61.39 ± 0.64
C18:3 ω-3	1.26 ± 0.04^{a}	0.82 ± 0.04^{b}	Nd	Nd
C18:4 ω-3	2.17 ± 0.03^{a}	2.78 ± 0.03^{b}	Nd	Nd
C20:1 ω-11	4.42 ± 0.02^{a}	Nd	Nd	Nd
C20:4 ω-6	4.99 ± 0.01^{a}	0.47 ± 0.01^{b}	Nd	Nd
C20:5 ω-3 (NS)	4.47 ± 0.11	5.63 ± 0.11	5.27 ± 0.95	Nd
C22:1 ω-11	17.00 ± 0.48^{a}	8.53 ± 0.48^{b}	Nd	Nd
C22:6 ω-3	22.61 ± 0.81^{a}	25.71 ± 0.81^{ab}	31.45 ± 0.56^{b}	Nd
Others	3.86 ± 0.15^{a}	5.05 ± 0.22^{b}	Nd	0.52 ± 0.10
∑SFAs (NS)	18.15 ± 1.31	27.58 ± 2.78	23.22 ± 0.57	8.99 ± 0.67
∑MUFAs (NS)	36.80 ± 1.59	33.23 ± 1.62	27.34 ± 1.61	29.05 ± 0.84
∑PUFAs (NS)	44.92 ± 1.13	38.08 ± 1.19	49.44 ± 3.46	61.39 ± 0.64
∑ω-3 (NS)	31.81 ± 0.98	35.26 ± 1.03	36.71 ± 1.51	Nd
∑ω-6 (NS)	8.69 ± 0.13	2.61 ± 0.13	12.73 ± 1.95	61.39 ± 0.64
PI	2.00 ± 0.04^{a}	1.58 ± 0.09^{b}	2.20 ± 0.06^{c}	

Mean ± standard deviation. Nd, not detected. Different lower-case letters (a, b) in the same raw indicate significant differences within stages of the marinating process ($p < 0.01$). NS, not significant differences.

Table 5. Amounts of fatty acids from anchovy products¥ (g per 100 g of edible food): effect of salting–ripening, canning and marinating processes.

	∑SFA*	∑MUFA*	∑PUFA	∑ω-3*	∑ω-6*	EPA	DHA*	∑ω-3/∑ω-6
Fresh anchovy	$0.77 \pm 0{,}07^{a}$	1.56 ± 0.10^{a}	1.91 ± 0.09	1.35 ± 0.07^{ab}	0.36 ± 0.01^{a}	0.19 ± 0.01	0.96 ± 0.05^{ab}	3.75
Salting–ripening								
Salted–ripened sample	2.44 ± 0.28^{b}	4.77 ± 0.52^{b}	2.31 ± 0.27	2.05 ± 0.24^{b}	0.10 ± 0.02^{a}	0.37 ± 0.04	1.36 ± 0.15^{abc}	20.5
Fillets in oil	1.01 ± 0.06^{a}	1.74 ± 0.12^{a}	2.95 ± 0.23	0.58 ± 0.06^{a}	2.37 ± 0.17^{c}	0.08 ± 0.00	0.27 ± 0.03	0.25
Canning								
Steamed sample	1.26 ± 0.14^{ab}	0.86 ± 0.09^{a}	1.84 ± 0.19	1.75 ± 0.17^{ab}	0.09 ± 0.01^{a}	0.23 ± 0.03	1.42 ± 0.14^{abc}	19.44
Canned sample	1.52 ± 0.19^{ab}	1.28 ± 0.23^{a}	3.44 ± 0.44	1.67 ± 0.24^{ab}	1.77 ± 0.12^{bc}	0.27 ± 0.04	1.17 ± 0.17^{b}	0.94
Marinating								
Frozen sample	1.29 ± 0.19^{ab}	1.55 ± 0.15^{a}	1.78 ± 0.14	1.65 ± 0.13^{ab}	0.12 ± 0.01^{a}	0.26 ± 0.02	1.20 ± 0.09^{abc}	13.49
Marinated sample	1.58 ± 0.12^{ab}	1.86 ± 0.21^{a}	3.37 ± 0.41	2.50 ± 0.23^{b}	0.87 ± 0.18^{ab}	0.36 ± 0.08	2.14 ± 0.15^{c}	2.88

¥Calculated in relation to the lipid value obtained by the acid hydrolysis method.
*Different lower-case letters (a. b) in the same column indicate significant differences within stages of a given process compared to fresh anchovy ($p < 0.01$).

The ω-3/ω-6 fatty acids ratio is another important guide for comparing the nutritional value of foodstuffs since the consumption of foods containing relatively high levels of ω-3 PUFA and lower amounts of ω-6 PUFA is favourable for human health. As shown in Table 5, fresh anchovy has a high ω-3/ω-6 ratio, comparable to values obtained in fresh fish belonging to other species (Huynh & Kitts, 2009; Murillo et al., 2014). The processing methods considered in this study affected this ratio, which resulted considerably reduced for canned anchovy and salted–ripened anchovy fillets in oil. Taking into account the ω-3/ω-6 values calculated for the intermediate products (cooked and salted–ripened, respectively) this reduction can be associated, at least in part, with the adsorption of covering oil occurring during canning and storage. In this sense, the use of other covering liquid, such as brine, or the vacuum package could benefices the nutritional quality of the final product.

Apart from the ω-3/ω-6 ratio, the total amount of the essential EPA and DHA fatty acids provided by these products is high and covers an important percentage of the daily nutritional requirements. A daily ingestion of 300–400 mg of EPA and DHA has been recommended as reference by different health organizations (FAO, 2011; Kris-Etherton et al., 2002). Thus, a portion of 60 g of canned anchovy (the serving size indicated in nutritional labels) supplies ≈1000 mg of ω-3 fatty acids and duplicates the suggested daily intake of EPA + DHA. Moreover, one portion of marinated anchovy (12 g) contains ∼300 mg of ω-3 fatty acids, covering 75–100% of the EPA + DHA requirements. Finally, the consumption of three fillets of salted–ripened anchovy in oil (12 g) provides ≈70 mg of ω-3 fatty acids and 10–14% of the EPA + DHA recommended. It is important to mention that the last two products are in fact consumed as appetizers or as part of other preparations which could enhance its nutritional value.

Conclusions

The experimental work and analysis performed in this study shows how chemical and fatty acid composition of anchovy (*E. anchoita*) is affected when different processing technologies are used. It is well documented that this species has an intrinsic high nutritional quality, especially from long-chain PUFA signature. However, modifications in composition would be expected when raw fish is processed. In this article, the investigation was focussed on salting–ripening, canning and marinating, which are the processes most commonly applied to anchovy. Our results

indicate that instead the studied processes alter the chemical composition and fatty acids profile; final products are still an important source of proteins and long-chain PUFAs. Salting–ripening was the process that leads to the major changes in chemical and fatty acid composition, followed by canning and marinating. Extremely pro-oxidant conditions throughout ripening results in a significant reduction of ω-3 fatty acids content, especially EPA and DHA. On the other hand, total amounts of ω-3, EPA and DHA in canned and marinated anchovy are comparable to that in fresh samples. Due to their high ω-3 content, the consumption of anchovy-derived products can contribute to balance the human diet, generally rich in ω-6 fatty acids, in order to achieve the recommended ω-3/ω-6 equilibrium. In addition, the evaluated products appear to be valuable for human nutrition taking into account their high content of the essential EPA and DHA fatty acids.

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