Research Article

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Ripening of salted anchovy (*Engraulis anchoita*): development of lipid oxidation, colour and other sensorial characteristics

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Abstract

BACKGROUND: Changes in lipids are in general associated with quality deterioration, though earlier studies in anchovy indicate that the volatile compounds of importance to the characteristic flavour could be generated by lipid oxidation. The aim of the present paper was to study the changes in the fatty acid composition and the lipid oxidation of *Engraulis anchoita* during ripening and their relationship with the development of the typical sensorial characteristics.

RESULTS: As a result of ripening, polyunsaturated fatty acids decrease from 4.27 to 2.42 and 2.00 g kg^{-1} fatty acids in gutted fish and fillet, respectively. The most affected class of fatty acids was the n-3 fatty acids, especially docosahexaenoic acid. Moreover, saturated fatty acids were the most stable to the process. 2-Thiobarbituric acid reactive substances (TBARS) increased throughout ripening, and the use of fillets instead of gutted fish reduced this value. The *a value was the parameter most modified, showing a marked increase which correlates with the acquisition of a uniform pink colour. Sensory analysis did not show development of off-odour or undesirable changes in colour during ripening.**

CONCLUSION: As a result of ripening, the fatty acid profile was modified and an increment of TBARS was observed. However, these changes did not lead to deterioration in the quality of the product.
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Keywords: ripened anchovy; fatty acid profile; lipid oxidation; TBARS; colour; sensorial assessment

INTRODUCTION

Salting is one of the oldest treatments in food preservation, and is still extensively used. In the presence of salt, some pelagic species may suffer physicochemical modifications, resulting in a product called 'ripened' or 'matured'. Some examples of traditional salted–ripened fish are herring (*Clupea harengus*) (typically from Russia, Scotland, Norway, Iceland and Germany), anchovy, produced from *Engraulis encrasicholus* in the southern part of Europe and from *E. anchoita* and *E. ringens* in Latin American countries, and Scandinavian anchovies, commonly produced from sprat (*C. sprattus*) and herring (*C. harengus*).[1]

The conventional process of salting–ripening of anchovy can be divided into two stages. The first stage is salting, characterized by salt penetration into the fish flesh and accompanied by the elimination of water from it. The second stage is ripening, slower than the first, which implies several transformations, including proteolysis, lipolysis and lipid oxidation.[2–5] As a result of these changes the product acquires a firm consistency, reddish colour, juicy texture and characteristic odour and flavour.[6,7]

It is widely accepted that the ripening of salted fish takes place via enzymatic pathways. However, there are three major hypotheses regarding the ripening mechanisms of salted fish: the microbiological theory, which established that the bacterial flora determines the type of changes taking place during the process; the autolytic theory, which assigns a key role to the enzymes of the muscles and the gastrointestinal tract; and finally the enzymic theory, which is actually a combination of the above.[5,8–11]

The digestive enzymes seem to be responsible for the complete ripening and the development of the characteristic taste and odour in salted herring and sprat.[1] Besides, Durand (1981) mentioned that partial evisceration, as practised in the factories, eliminates part of the digestive enzymes; otherwise a bitter taste develops in the fish.[2] On the other hand, studies on salted–ripened *E. anchoita* showed that anchovies which have been filleted ripen slowly and do not acquired the characteristic colour.[12]

During ripening, anchovies are exposed to highly pro-oxidant conditions: high NaCl concentration, low a_w (water activity), and presence of endogenous and bacterial enzymes.[13–17] In addition, the species *E. anchoita* is rich in long-chain polyunsaturated fatty acids (PUFA),[18] which are very susceptible to oxidative processes. Earlier studies showed an increase in free fatty acid content during ripening of *E. anchoita*.[3] Besides, Hernández-Herrero *et al.*[4] reported that 2-thiobarbituric acid reactive substances (TBARS)

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value and peroxide index increase during the ripening of *E. encrasicholus*. Even when these results suggest that the ripening process involves a modification in the lipid fraction, no information is specifically given about changes in the fatty acid composition. In general, changes in lipid during processing, such as lipolysis and lipid oxidation in fish muscle, are associated with quality deterioration.[19] However, Triqui and Reineccius[10] have identified some of the volatiles of importance to ripened *E. encrasicholus* flavour and proposed that these compounds could probably be generated by lipooxigenase activity and autoxidation of PUFA. On the other hand, lipid oxidation has been associated with changes in colour in muscle foods such as fish[20] and cephalopods.[21]

In this context it have been hypothesized that the compounds generated by lipid oxidation contribute to the development of sensorial characteristics of salted–ripened anchovy (*E. anchoita*). Likewise, another hypothesis is that the presence of viscera is essential for the complete ripening and acquisition of the characteristic flavour. Thus the purpose of the present paper was to study the lipid oxidation of different cuts of *E. anchoita* during ripening, measured as TBARS value, and their relationship with the development of the typical sensorial characteristics.

MATERIALS AND METHODS

Raw material

Anchovy (*Engraulis anchoita*) used for the experiments were caught near Mar del Plata, Argentina (38 °S, 57° 33′ W), during November 2007. Following catching, fish were placed in bins with ice and maintained in this condition until they arrived at the laboratory. Fresh fish was classified as 28–34 pieces per kilogram.

Sample preparation and experimental design

Anchovy was arranged into two lots depending on the gutting method applied. One lot (lot W) was processed according to the traditional method: whole fish was immersed in saturated brine for 24 h, with a brine to fish ratio of 1 : 1. Following this, anchovies were beheaded and partially gutted by hand (leaving gonads and pyloric caeca) and placed in barrels, alternating layers of fish and salt, finishing with a layer of salt. A disc of wood was placed on the top layer of salt and weights were put in place to a pressure of 80 g cm^{-2}. The final salt to fish ratio was 1 : 5.

A second lot (lot F) was filleted previous to the brining stage. Then, fillets were immersed in saturated brine for 24 h and placed into barrels, alternating layers of fish and salt, and pressed (identical conditions to those of lot W). Each lot, with different cuts, was carried out in duplicate.

Throughout the ripening process, the barrels were maintained in the ripening chamber of a local factory at room temperature. Samples of ∼1 kg were taken immediately before starting the ripening process (day 0), after 24 h in brine (day 1) and after 10, 28, 73, 100, 127, 158, 255 and 385 days of ripening, respectively. For sample extraction, the two superior layers of fish were discarded.

Proximal composition

Fresh anchovy was analysed to determine the proximal composition. Minced anchovy flesh was analysed for water content by oven drying at 105 ± 1 °C until constant weight, fat by the acid hydrolysis method,[22] proteins by Kjeldhal and ashes by incineration at 500 °C.[23] Sodium chloride was determined by Mohr's method.[24] Analyses were conducted in triplicate for moisture content and in duplicate for protein, fat, ash and sodium chloride content.

Fatty acid composition

Lipids were extracted from 100 g ground samples with a solvent mixture of chloroform–methanol–distilled water (2 : 2 : 1.8, v/v/v) according to the method of Bligh and Dyer.[25] Two independent extracted lipids for each sample were stored under nitrogen in the dark at −20 °C for further analysis.

The fatty acid profile was determined by fatty acid methyl ester (FAME)/gas chromatography (GC) using KOH in methanol as a reagent for transesterification according to the norm ISO 5509.[26] 100 mg of the lipid sample was dissolved in hexane and treated with KOH 1 mol L^{-1} (in methanol). The mixture was heated in a water bath at 70 °C for 10 min. FAMEs were separated and quantified using a gas chromatograph (Shimadzu® GC-17A, Kyoto, Japan) equipped with a 30 m fused silica capillary column (Omegawax 320, Supelco Inc., Bellefonte, PA, USA) (0.32 mm i.d.; 0.25 µm film) and a flame ionization detector. The temperature of the injector port and detector was held at 250 °C. Column oven temperature was programmed from 150 to 225 °C, at a rate of 1.5 °C min^{-1} with an isotherm of 13 min at 225 °C. The injected volume was 1 µL and the carrier gas was nitrogen. Identification of fatty acids was achieved by comparing the relative retention times of FAME peaks from samples with the standard PUFA-1 (Marine Source, Supelco).

Determination of TBARS

TBARS determinations were performed on trichloroacetic acid extracts from 2 g minced muscle according to Tironi *et al*.[27] Two independent extracts were obtained for each sample and subjected to the thiobarbituric acid (TBA) reaction (30 min, 70 °C) in duplicate. The spectrum of the reaction products between TBA and carbonyl compounds shows two main maxima, at 530 nm (pink pigment) and at 450 nm (yellow pigment), corresponding to malondialdehyde (MDA) and to other aldehydes, respectively. Thus absorbance readings were made at 532 nm and at 455 nm. TBARS (mg kg^{-1} muscle) was calculated according to Eqn (1), where Abs is absorbance, PM is molecular weight (g mol^{-1}); V_a is volume of the aliquot (mL); V_e is volume of extract (mL); ε is extinction coefficient (mol L^{-1} cm^{-1}); b is optical pathway (1 cm) and m is weight of the sample (g). At 532 nm an ε of 1.56×10^5 mol L^{-1} cm^{-1} was used, corresponding to MDA, and at 455 nm an ε of 0.457×10^5 mol L^{-1} cm^{-1} was used as an average of the ε obtained for five aldehydes (propanal, butanal, hexanal, heptanal and propanal-dimethylacetal).[28] The results at 455 and 532 nm were expressed as TBARS_{455} and TBARS_{532}, respectively.

$$\mathrm{TBARS} = \frac{\mathrm{Abs} \times \mathrm{PM} \times V_a \times V_e \times 1000}{\varepsilon \times b \times m} \qquad (1)$$

Colour measurement

For colour measurement anchovies were cleaned to remove adhering salt and filleted. The colour measurement was performed on the internal surface of the fillet in both the ventral and the tail area. Five different fillets were measured for each sample. A colorimeter (model NR-3000, Nippon Deshoku, Tokyo, Japan) with illuminant D65, 2° Standard Observer and CIE (1976) colour scale was used. Lightness value, L^*, indicates how dark/light the sample is (varying from 0 (black) to 100 (white)), a^* is a measure of greenness/redness (varying from −60 to +60), and b^* is the grade of blueness/yellowness (also varying from −60 to +60).

Sensory evaluation

Anchovies for sensory analysis were washed to remove adhering salt and blotted with adsorbent paper. The progress of ripening

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was evaluated by six trained panellists, according to the table proposed by Filsinger *et al*.[6] For each sampling time anchovies were selected at random and two specimens were presented to each judge. The sensory characteristics evaluated by the panellists were flavour, odour, flesh colour, flesh consistency and adherence of fillets to backbone, which ranged from 0 for fresh anchovy and 8, corresponding to overripened anchovy. A score of 0 corresponds to characteristic fresh fish flavour, odour and colour, a very elastic and damp texture and fillets very adherent to the backbone. A score of 6 corresponds to a fully ripened product, which presents a uniformly distributed pink tone, a ham-like flavour and a characteristic anchovy odour, with compact fillets which are easily separated from the spine. A score of 8 corresponds to a product with off-flavour and odour, a dark red or black colour with red or black blots and a flimsy flesh consistency that becomes torn in the filleting process.

Statistical analysis

Analysis of variance was carried out to determine the effects of the gutting method and time on the fatty acid profile, TBARS, colour and sensory scores. Difference between means was analysed using Tukey's test for post hoc comparison ($P < 0.01$). Analyses were performed using STATISTICA 6.0 (Statsoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Characterization of raw material

The weight fractions of water, fat, ashes and proteins in raw anchovies are shown in Table 1. Initial salt content is also presented. The proximal composition found in this study is within the range of values reported in the literature for the species.[18,29]

The fatty acid composition of fresh anchovy is presented in Table 2. PUFA) were the most abundant, followed by monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA), respectively. Within PUFA, the n-3 fatty acids predominated; the main fatty acid found in raw material was docosahexaenoic acid (DHA, 22 : 6 n-3) with a concentration of 20.41%, followed by erucic (22 : 1), oleic (18 : 1) and palmitic (16 : 0) acids. Eicosapentaenoic acid (EPA, 20 : 5 n-3) represented 4.47% of the total fatty acid content.

Changes in fatty acid composition

As shown in Table 2, the fatty acid composition present modifications during the ripening of the both lots studied. In general, during the salting–ripening process a decrease in PUFA was observed in comparison with fresh anchovy. This effect was more evident in lot F ($P < 0.01$). MUFA showed a fluctuating behaviour, which exhibited a reduction after 1 month of ripening. During the brining stage, the PUFA decreased sharply in comparison with fresh fish ($P < 0.01$). Within this fraction, the most affected were the n-6 fatty acids, especially the 20 : 2 n-6 and 20 : 4 n-6 fatty acids. During the first 4 months of ripening PUFA continued to decrease gradually, and after 10 months a marked reduction was observed. Within this period, n-3 fatty acids were particularly affected, while n-6 fatty acids remained almost constant. MUFA decreased from 3.68 to 2.91 g kg^{-1} after 1 month of ripening and then increased again to 4.88 g kg^{-1} after 10 months (average values of both lots). This fact coincides with a marked decrease in PUFA between 1 and 10 months of ripening, especially DHA, the content of which content was reduced approximately 26% and 53% in lots W and F, respectively. This behaviour can be attributed to the high susceptibility of PUFA to chemical and enzymatic oxidation compared to MUFA. In this study SFA were the most stable during the process. These results are similar to those obtained by other authors when changes in fatty acid profile during curing ham – a similar process to ripening of anchovy for the type of reactions involved – were studied. In this sense, the dry-curing ham process generally resulted in an increase in SFA and a decrease in PUFA.[30]

Table 1. Proximal composition and NaCl content in fresh anchovy (*E. anchoita*)

	g kg^{-1}
Water	7.57 ± 0.05
Lipid	0.35 ± 0.03
Protein	2.22 ± 0.08
Ashes	0.15 ± 0.001
NaCl	0.03 ± 0.001

Mean ± standard deviation.

Lipid oxidation

TBARS value was used as an index to assess the level of lipid oxidation during the ripening of anchovy. $TBARS_{455}$ showed similar behaviour to $TBARS_{532}$ (Fig. 1) in both lots and the evolution of these indexes can be divided into five periods: brining (day 0–1), ripening for 1–10 days, for 10–28 days, for 28–127 days and for 127–385 days.

$TBARS_{532}$ increased in both lots as a result of brining reaching a higher value in lot W ($P < 0.01$). During this stage the pro-oxidant action of the NaCl and the sharp reduction in a_W can promote lipid oxidation, in accordance with Labuza (1980).[31] Lipid oxidation as a result of salting has been widely studied and the consequent increase in $TBARS_{532}$ was reported in other fish species, such as horse mackerel and salmon.[14] From day 1 to 10 of ripening a decrease in $TBARS_{532}$ was observed ($P < 0.01$). This fact can be explained considering that during this period the lots were heavily pressed (160 g cm^{-2}) and a large amount of water was removed from the fish muscle, dragging lipids and soluble compounds such as proteins, peptides and nitrogen compounds[2,4] and probably also secondary oxidation products. At day 10 some blocks were lifted off and a pressure of 80 g cm^{-2} was maintained during ripening, and the water content in the muscle remained constant (between 5.2 and 5.4 g kg^{-1}). Throughout the ripening process, $TBARS_{532}$ continued increasing in both lots, reaching an average maximum of 12.01 mg kg^{-1} for lot W and 9.23 mg Kg^{-1} for lot F at day 28 ($P < 0.01$). From day 28 to 127 a sharp reduction in $TBARS_{532}$ was observed, more marked in lot W at day 73, which resulted in the absence of differences between the lots towards the end of ripening ($P < 0.01$). The reduction in TBARS can be attributed to decomposition of the secondary products of oxidation into tertiary products. A similar evolution of $TBARS_{532}$ was detected during the ripening of *E. encrasicholus*, with a maximum value of 13.46 mg MDA kg^{-1} after 6 weeks.[4]

During the process, $TBARS_{455}$ was superior in lot W compared to lot F, except at day 73 ($P < 0.01$). While the maximum TBARS value reached corresponded to the pink pigment ($\lambda = 532$ nm), the decay of $TBARS_{455}$ was smooth and the yellow pigment ($\lambda = 455$ nm) predominated after day 127. The development of yellow pigment in the presence of TBA has been associated with saturated aldehydes such as propional and butanal.[32]

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Table 2. Fatty acid composition of anchovy (*E. anchoita*) during ripening (lot W: pre-salting of whole fish, ripening H&G lot F: pre-salting and ripening fillets) (g kg^{-1} lipids)

	Fresh anchovy	Brining		Ripening time (months) 1		4		10	
		Lot W	Lot F	Lot W	Lot F	Lot W	Lot F	Lot W	Lot F
14 : 0	0.25a	0.16a	2.06a	0.58b	0.65b	0.59b	0.60b	0.68b	0.74b
15 : 0	0.02a	0.36ab	0.03 ab	0.04bb	0.05b	0.05b	0.06b	0.02a	0.04a
16 : 0*	1.36a	2.12Abd	1.87Bc	2.20Abd	2.17Abd	2.28Ad	2.27Ad	1.43Aa	1.98Bbc
16 : 1*	0.51a	0.67Abc	0.78Acd	0.57Aab	0.52Aa	0.49Aa	0.49Aa	0.87Ad	0.73Bc
17 : 0*	0.09ad	0.14Abcd	0.14Abcd	0.06Aa	0.06Aad	0.10Aab	0.10Aabd	0.19Ac	0.12Bd
18 : 0	0.09a	0.24b	0.20b	0.24b	0.26b	0.32c	0.32c	0.18b	0.24b
18 : 1*	1.42a	1.68Ab	1.45Ba	1.20Acd	1.16Ad	1.33Aac	1.32Aac	1.29Aacd	1.42Aa
18 : 2 n-6*	0.28a	0.20Abc	0.21Ace	0.47Ad	0.28Bae	0.21Abce	0.21Abc	0.16Abc	0.20Aabc
18 : 3 n-3*	0.13a	0.09Aabd	0.06Bcde	0.06Acde	0.08Abdf	0.08Abe	0.08Abcde	0.04Acf	0.04Ac
18 : 4 n-3*	0.22acd	0.30Ab	0.21Bcde	0.24Aad	0.25Aa	0.17Ae	0.76Ae	0.17Ae	0.16Ae
20 : 1*	0.00a	0.33Ab	0.52Bc	0.35Ab	0.38Ab	0.61Ac	0.63Ac	0.76Ad	0.83Ad
20 : 2 n-6	0.44a	0.02b	0.07b	n.d.[a] b	n.d.b	n.d.b	n.d.b	0.06b	n.d.b
20 : 4 n-3	0.08a	0.06bc	0.06bc	0.06c	0.05c	0.08a	0.08a	0.05b	0.05b
20 : 4 n-6*	0.50a	0.02Ab	0.02Abc	n.d.Ad	n.d.Ad	0.06Aef	0.05Ae	0.04Ac	0.07Bf
20 : 5 n-3*	0.45Aab	0.52Aa	0.71Bcd	0.79Ac	0.77Ac	0.64Ad	0.65Ad	0.38Ab	0.40Ab
22 : 1	1.70a	1.17Ab	1.15Ab	0.76Ac	0.77Ac	0.90Ac	0.91Ac	1.95Ad	1.70Bb
22 : 5 n-6*	0.09a	0.07Abc	0.09Aab	0.04Ad	0.05Be	0.05Acde	0.05Ade	n.d.Af	n.d.Af
22 : 5 n-3*	n.d.a	n.d.Aa	n.d.Aa	0.09Ab	0.11Bc	0.09Ab	0.09Ab	0.05Ad	0.05Ad
22 : 6 n-3*	2.04ab	2.50Aab	2.04Aab	1.91Aa	2.15Bb	1.70Ac	1.69Ac	1.42Ad	1.01Be
Others[b]	0.34	0.13	0.20	0.35	0.22	0.24	0.23	0.21	0.22
Σ SFA*	1.82a	2.69Abcf	2.45Abf	3.12Acdf	3.19Ace	3.35Ade	3.35Ade	2.56Af	3.12Bce
Σ MUFA	3.68a	3.95a	4.06a	2.93b	2.89b	3.32c	3.34c	4.99d	4.76d
Σ PUFA*	4.27a	3.36Ab	3.47Ab	3.81Ac	3.82Ac	3.20Ad	3.19Ad	2.42Ae	2.00Bf
Σ n-3*	2.96ab	3.05Aac	3.08Aa	3.16Aad	3.42Ad	2.88Abc	2.88Abc	2.14Ae	1.72Bf
Σ n-6*	1.31a	0.31Abc	0.39Ab	0.65Ad	0.40Bbc	0.32Abc	0.31Abc	0.27Ac	0.27Ac

[a] n.d., not detected.
[b] Includes fatty acids found in traces.
* Significant differences between lots through ripening ($P < 0.01$). Different capital letters (A, B) in the same row for a given time indicate significant differences between lots.
Different lower-case letters (a, b, c, d, e, f) in the same row indicate significant differences within ripening time ($P < 0.01$).

It is well known that lipid oxidation can be catalyzed by the haem proteins and iron, as well as lipoxygenase. Endogenous lipoxygenase has been found in fish species such as herring[13] and silver carp,[16] among others. On the other hand, enzymatic oxidation of lipids is influenced by NaCl content. Studies on salted herring showed that salt lowered the lipoxygenase activity of the herring muscle, and its inhibiting action was proportional to its concentration. However, after salting (with a salt content of 0.98–1.83 g kg^{-1}) a significant residual lipoxygenase activity was observed.[13] Thus lipoxygenase activity is expected during the ripening of anchovy. The difference in TBARS between lots is probably caused by the effect of bleeding, with the resulting different haem protein content. The study of Richards and Hultin[33] supports this hypothesis, since these authors showed that bleeding significantly reduced rancidity in fish muscle.[33]

Changes in instrumental colour

Figure 2 shows the changes in colour parameters (L^*, a^* and b^*) during salting–ripening. The values corresponding to fresh anchovy were 34.66 ± 2.42; 12.68 ± 1.65 and 8.98 ± 0.55 for L^*, a^* and b^*, respectively. No significant differences were detected between the ventral or the tail area ($P > 0.01$).

Changes of L^*, a^* and b^* during brining

The main parameter modified after brining was a^*. In lot W this value decreased from 12.68 ± 1.65 to 3.38 ± 1.63 in the ventral area, whereas no significant modification was observed in the tail area. In lot F, a^* was reduced in both areas, reaching an average value of 5.51 ± 1.55. The parameter a^* has been related to myoglobin, dependent upon its derivatives and concentration.[34] Therefore, the reduction of a^* during brining is associated with the bleeding that occurs at this stage. In whole fish the ventral area bleeds easily through the gills, whereas in the area of the tail fillets are tightly stuck together, which makes bleeding difficult. When brining fillets the internal surface (muscle) is directly exposed to brine, which makes a uniform bleeding easy; thus no differences were observed between areas. L^* showed a slight increase in lot F, possibly associated with the formation of a thick crust of salt and protein at the surface of the muscle.[35] Finally, b^* was not modified by this operation ($P > 0.01$).

Changes in L^*, a^* and b^* during ripening

The a^* value, which was the most modified, showed a marked increase during ripening in both lots ($P < 0.01$). According to the scheme employed for sensory evaluation, at the beginning of

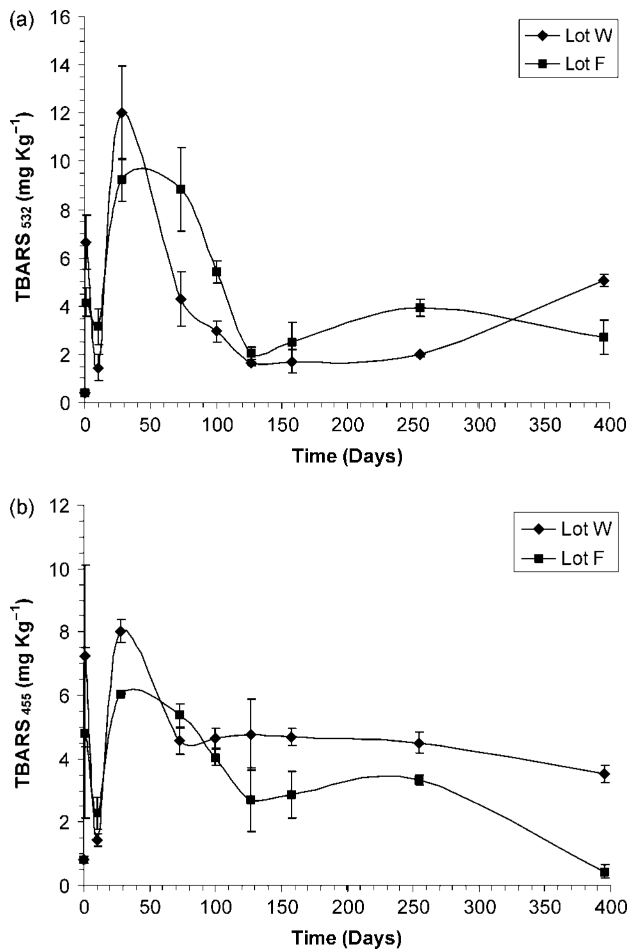

Figure 1. TBARS in salted anchovy during ripening, measured at (a) 532 nm and (b) 455 nm. Lot W, pre-salting of whole fish and ripening beheaded and gutted fish (H&G). Lot F, pre-salting and ripening fillets.

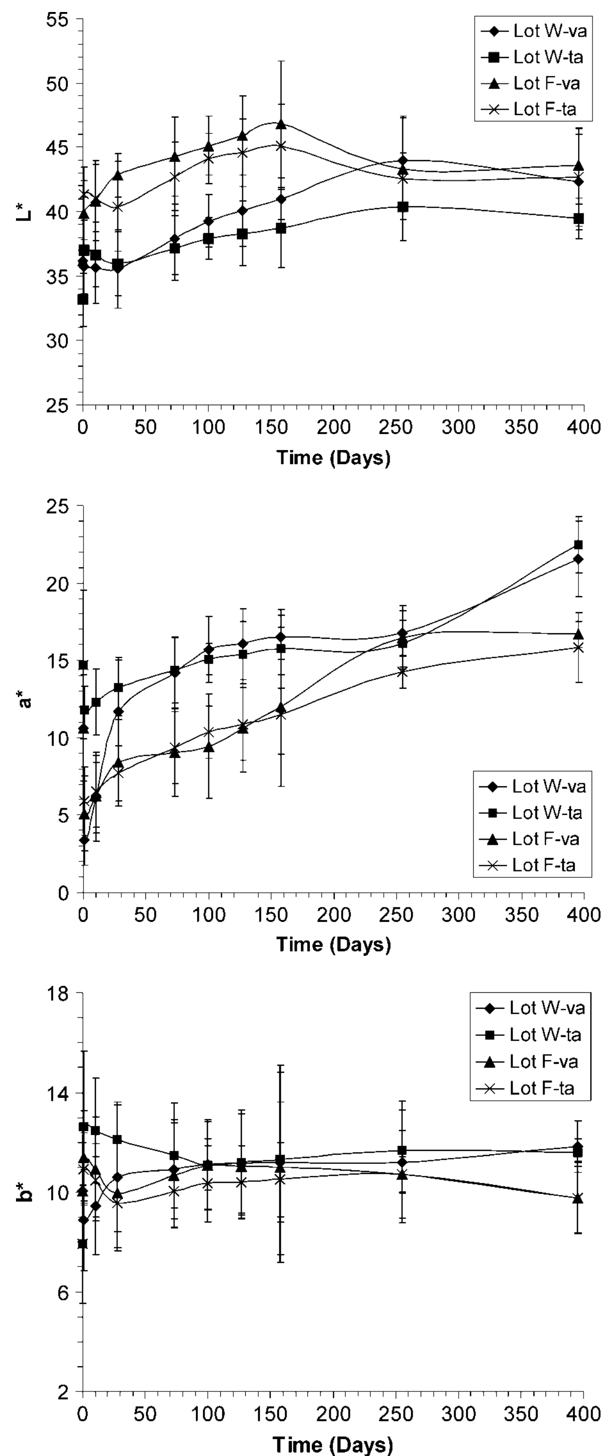

Figure 2. Colour changes during ripening of different cuts of salted anchovy. Lot W, pre-salting of whole fish and ripening beheaded and gutted fish (H&G). Lot F, pre-salting and ripening fillets; va, ventral area; ta, tail area.

ripening anchovy flesh presented a dark-red colour in the middle, corresponding to the zone of the backbone, and a natural light colour in the rest of the muscle. Throughout ripening, the flesh acquired a uniform pink colour.[6,7] The diffusion of colour from the centre to the periphery correlates with the increase in the a^* value. In any of the lots studied significant differences were found in a^* for the ventral and tail areas at the end of ripening ($P < 0.01$). The a^* value for lot F is below that corresponding to lot W ($P < 0.01$), which can be explained by the high bleeding that occurred in the fillets during brining.

The L^* value showed a slight increase in lot W after 127 days of ripening, whereas it remained constant in lot F. The parameter b^* did not show significant modification in this stage ($P > 0.01$).

Sensory evaluation

Figure 3 shows the evolution of sensorial characters, presented in disaggregated form. Usually, the results of sensory evaluation according to similar schemes to that employed in this paper are presented as a mean or the sum of the score obtained on each attribute.[3,9] However, in this paper it was considered that more information is obtained from the analysis of the disaggregated attributes.

In lot W, all the sensorial attributes increased during the first 158 days and then remained constant ($P < 0.01$). The final product obtained presented the typical characteristics desired. It is notable that although there is evidence of lipid oxidation in this lot during ripening, the judges do not find off-odour or undesirable changes in colour. This result is consistent with those corresponding to the studies of Triqui and Reineccius[10] related to the volatiles that contribute to the typical flavour of

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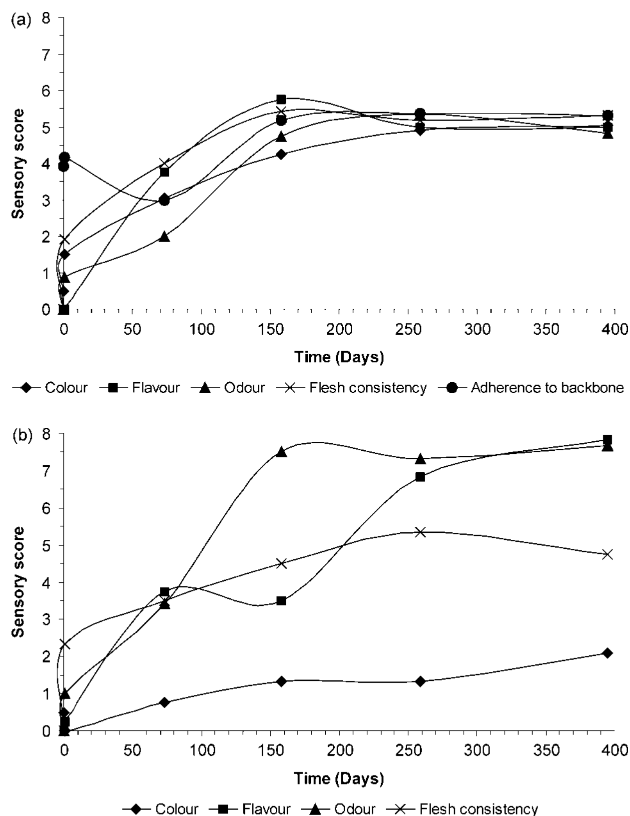

Figure 3. Evolution of sensorial attributes during ripening of different cuts of anchovy: (a) lot W, pre-salting of whole fish, ripening H&G (b) lot F, pre-salting and ripening fillets.

salted–ripened *E. encrasicholus*. These authors identified eight carbon alcohols and ketones in this product that impart plant-like aromas, and (*E*,*Z*)-2,6- nonadienal, which contributes a cucumber-like aromas, suggesting that precursors of these compounds are probably polyunsaturated n-3 fatty acids, considering the existence of 12- and 15-lipoxygenase activities. They also identified various conjugated carbonyl compounds, probably products of autoxidation of n-3 and n-6 PUFA, which impart fatty and fat-like aromas. Another volatile contributing highly to the anchovy flavour found by these authors was 3-methyl-2,4-nonanedione, a product of furanoid fatty acid oxidation, which imparts lard-like, strawy and fruity aromas.[10] Similarly, the studies of Girard and Durance[36] concerning the compounds associated with the characteristic flavour of canned salmon, and of Varlet *et al.*[37] concerning aldehyde participation in the flavour of smoked fish, indicate that volatile compounds derived from lipid oxidation could contribute to the typical aroma of other fish products. Therefore, taking into account the aforementioned authors' considerations, it is possible to consider that the chemical products of lipid oxidation contribute to the characteristic flavour of salted–ripened *E. anchoita*.

In lot F, colour was an attribute that was less developed, showing a slight increase after 158 days of ripening ($P < 0.01$). The final score of colour was 2.1, well bellow that of lot W corresponding to a fully ripened anchovy. These results are in agreement with the evolution of a^*, considering that the major characteristic of the colour in the final product is the acquisition of a pink tone. Flavour and odour increase during the first 255 and 158 days, respectively, and then remain constant. The final score of these attributes corresponds to an overripened product without a sensorial indication of rancidity.

CONCLUSIONS

Salting–ripening of anchovy caused important changes in fatty acid composition simultaneously with an increase in lipid oxidation, measured as TBARS. However, the implementation of a new cut, as the fillet, affected the fatty acid profile though reduced the level of lipid oxidation with respect to traditional processing.

Colour analysis showed that a^* value was the parameter which presented more changes during the ripening process, in correlation with the acquisition of a uniform pink tone.

The chemical products of lipid oxidation might therefore contribute to the typical flavour of salted–ripened *E. anchoita*.

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