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Acid and salt uptake during the marinating process of *Engraulis anchoita* fillets influence of the solution:fish ratio and agitation

Absorção de ácido e sal durante marinado de Engraulis anchoita: influência da agitação e da proporção solução:pescado

María Eugenia CAPACCIONI¹, María Rosa CASALES^{2,3}, María Isabel YEANNES^{2,3*}

Abstract

The aims of this research were to determine the effect of different conditions of the marination stage on the salt and acid uptake, immersion time, and sensorial characteristics during the marinating process of anchovy (*Engraulis anchoita*). Different solution:fish ratios and the agitation effect during this stage were analyzed. The ratios used were: 0.77:1, 3:1 and 10:1 (with and without agitation). An increase of marinating solution:fish ratio causes a higher speed of acid and salt penetration. The product obtained with the 10:1 ratio had a dry and fibrous texture and a slightly salty taste. Salt concentration was statistically significantly lower ($p < 0.01$) in the samples with agitation. Agitation did not influence the acid uptake, and the salt penetration speed decreased, but rancidity was detected in this product. The ratio 3:1 decreases the marinating time without damaging sensory attributes and can be used in the fish marinating process.

Keywords: anchovy; marination stage; marinating solution:fish ratio; agitation.

Resumo

Os objetivos desta pesquisa foram determinar o efeito de diferentes condições na etapa de marinado para absorção de ácido e sal, tempo e características sensoriais da anchoita (*Engraulis anchoita*) marinada. As proporções utilizadas de solução:pescado foram: 0,77:1, 3:1 e 10:1 (com e sem agitação). O aumento da proporção permitiu uma velocidade maior de penetração do ácido e sal. O produto obtido na proporção 10:1 apresentou textura seca e fibrosa e leve gosto salgado. A concentração de sal foi significativamente menor ($p < 0,01$) nas amostras com agitação. A absorção do ácido não foi influenciada pela agitação; foi detectado ranço nos produtos produzidos com agitação. A proporção 3:1 diminui o tempo de marinação sem danificar os atributos sensoriais e pode ser usada para processar os marinados.

Palavras-chave: anchoita; marinado; proporção solução:pescado na marinação; agitação.

1 Introduction

Marinades are solutions, that can include sugar, spices, oil, acids (from vinegar, fruit juice, wine), and they are used to improve tenderness, juiciness, flavor, and aroma and to extend shelf life of meat, poultry, seafood, and vegetables (CADUN; CAKLI; KISLA, 2005). Marination preserves meat, poultry, or fish through the simultaneous action of salt and organic acids. It involves an increase in ionic strength and a decrease in pH (POLIGNE; COLLIGNAN, 2000).

Marinated fish are semi-preserved fish products, ready-to-eat with no heat treatment and are a high-value delicatessen (FUENTES et al., 2010). Acetic acid and salt are added to fish to retard the action of bacteria and enzymes; this process results in a product with a characteristic flavor and an extended but limited shelf life (MEYER, 1965; MC LAY, 1972). The objective of fish marinating is not only to prevent spoilage from microorganisms and enzymes, but also to increase the value of the fish (POLIGNE; COLLIGNAN, 2000; BISPO et al., 2004).

In addition to herring (traditionally used species), eels, hake, cod, mackerel, sea salmon, dogfish and shrimps are also processed as marinades in Europe (SHENDERYUK; BYKOWSKI, 1994). In Spain, fresh anchovy, sprat, mussel, and cockle are usually used for marinades (LÓPEZ BENITO; SAMPEDRO, 1974; LÓPEZ BENITO; SAMPEDRO, 1975). In Great Britain, herring and usually three species of shellfish (cockles, mussels, and whelks) are processed as marinades (MC LAY, 1972).

There are several methods to elaborate fish marinades. On the basis of the preparative procedures, cold, fried, cooked and sometimes pasteurized marinades may be distinguished (MEYER, 1965; YEANNES, 1991). In cold marinades, fish is treated in a marinating bath with a relatively high acetic acid and salt contents. The immersion time depends on the species characteristics (lipid content, texture, and size among others) (MEYER, 1965; BERTULLO, 1975; YEANNES; CASALES, 1995; CABRER; CASALES; YEANNES, 2002; KILINC; CAKLI, 2005; DUYAR; EKE, 2009; FUENTES et al., 2010).

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In Argentina, marinades can be elaborated using anchovy (*Engraulis anchoita*) (BERTULLO, 1975; YEANNES; CASALES, 1995; MADUREIRA et al., 2009). There are some previous studies on marinated *Engraulis anchoita* such as the development of product (YEANNES; CASALES, 1995), physical and chemical changes during the marinating process (CABRER; CASALES; YEANNES, 2002; YEANNES; CASALES, 2008), and also microbiological studies on each stage of the process (FUSELLI et al., 1994) and isolation and identification of the typical flora (FUSELLI et al., 1998). Several of these studies also take into account different pretreatments of raw materials (fresh, pre-salted-on-board, and frozen-on-board) concluding that a very good product may be obtained under any of these conditions (FUSELLI et al., 1994; YEANNES; CASALES, 1995).

The composition of the marinating bath, the ratio of fish to liquid, and the exact way of treating the product are of decisive importance for a successful final quality (MEYER, 1965). According to the process conditions proposed by Yeannes and Casales (1995), the marinating time of *Engraulis anchoita* is around 7-9 days to obtain a pleasant acid and salty taste and an agreeable fish pickled odor with a firm and consistent texture. These sensorial characteristics arise from the treatment of fish with acetic acid or vinegar and salt. At industrial level, it is important to reduce the marinating time to obtain the same final characteristics of pH, a_w , and sensorial attributes. This can be done by increasing either the salt and acid contents of the marinating solution or the marinating solution:fish ratio, or by agitation during the marinating process.

In previous experiences with marinated fish elaborated using marinating solution with salt content greater than 14% and acid content greater than 7%, the immersion time decreased but the fillet had a high salt and acid contents with negative changes in the sensorial characteristics. Some authors found that an increase in the vinegar content of the marinating solution to achieve a longer shelflife may cause defects in the taste and odor of the final product (YEANNES; CASALES, 1995; POLIGNE; COLLIGNAN, 2000; SEN; TEMELLI, 2003; KILINC; CAKLI, 2004; SALLAM et al., 2007). According to Rodger et al. (1984) as acid and salt levels were increased in the marinating solution, the water content in the fish decreased, and the texture and water content of the fillet were dependent on the levels of salt and acid that the fish tissue takes.

The aims of this research were to determine the effect of different conditions of the marination stage on the salt and acid uptake, immersion time, and sensorial characteristics during the marinating process of anchovy (*Engraulis anchoita*). Different solution:fish ratios and the agitation effect during this stage were analyzed.

2 Materials and methods

2.1 Raw materials

Anchovy (*Engraulis anchoita*) were caught in September, frozen on-board, and stored during two months at $-30\text{ }^{\circ}\text{C}$ and used to make marinades.

2.2 Process

The marinating process was performed according to Yeannes and Casales (1995) and consisted of the following stages: thawing, washing, heading and gutting, cutting off the tail, filleting, washing, brining (in a bath with 10% w/v of sodium chloride -food grade- for 1 hour at room temperature ($15\text{ }^{\circ}\text{C}$) with a fish:solution ratio of 1:1), marinating with different solution:fish ratios (in a solution composed of 3 % v/v of acetic acid - Merk pro analysis- and 10 % w/v of sodium chloride -food grade-), packing in glass containers with corn oil, and storing (at $8 \pm 2\text{ }^{\circ}\text{C}$). The solution:fish ratios were: 0.77:1, 3:1 and 10:1; two batches (10 kg each) were produced for each ratio. The ratio 0.77:1 was previously used for this species by Yeannes and Casales (1995) and Cabrer, Casales and Yeannes (2002). The marinating using the ratio 10:1 was performed with and without agitation on an orbital shaker at 50 rpm.

Marinating was carried out in 10 l capacity metal closed recipients at $20 \pm 1\text{ }^{\circ}\text{C}$ until the end point of texture was sensorially assessed according to the table of Yeannes and Casales (1995). The end point of texture was previously determined for the marinating solution:fish of 0.77, and it is considered as reference for experiences with different ratios.

2.3 Sampling

Ten fillets of each recipient were removed from the marinating bath at different times and left to drain for 30 minute, and then they were analyzed for water content, sodium chloride, and acetic acid.

Determinations of water, sodium chloride and acetic acid contents, and sensorial analyses of texture were carried out for all the marinating solution:fish ratios at: 0; 0.5; 2; 4.5; 7; 10; 14; and 24 hours.

The pH and a_w values were determined after washing, at the end of the brining stage, and during 24 hours of marinating for all experimental conditions assayed.

2.4 Physical and chemical analyses

Water content was determined at $105\text{ }^{\circ}\text{C}$ until a constant weight was obtained (ASSOCIATION..., 1993, Sec. 984.25). Ashes were determined by ignition of the dry sample in an oven at $500 \pm 10\text{ }^{\circ}\text{C}$ until white ashes were obtained (ASSOCIATION..., 1993, Sec. 945.46). Sodium chloride was determined by the Mohr method (KIRK; SAWYER; EGAN, 1996), and the acidity determination was made by titration with sodium hydroxide (KIRK; SAWYER; EGAN, 1996). Lipids were determined according to Association... (1993, Sec 922.06). Proteins were determined by Kjeldhal (ASSOCIATION..., 1993, Sec. 920.152). Rancidity was determined by the TBA technique (VYNCKE, 1975). The value of pH was obtained using a pHmeter with glass electrode (Instrumental Parsec, Vega VI) in a fish:distillated water ratio 1:1 (ASSOCIATION..., 1993, Sec. 981.12).

All determinations were carried out in triplicate, and water content for sextuple.

The water activity of the samples was measured using a digital hygrometer Aqualab, model CX-2T (decagon, Pullman, USA). All determinations were carried out in quadruplicate.

2.5 Sensorial analysis

Sensorial tests were carried out by six trained sensory panelists experienced in fishing products. The samples were always presented in the same way. This uniformity included portion quantity, temperature (21 ± 1 °C.), recipients, and utensils. A table of aleatory numbers was used to codify the samples (INTERNATIONAL..., 1981).

During marinating, changes in texture, color, and acid, salty, and rancid tastes were analyzed twice a day for the ratios 0.77:1, 3:1, and 10:1 (with and without agitation). Four samples were assessed in each session.

Texture and salty and acid tastes were analyzed for all ratios considering that full marinating was reached when the marinated fillet obtained the sensorial characteristics of processed marinade using our reference solution:fish ratio, 0.77:1.

A scale based on textural changes (YEANNES; CASALES, 1995; CABRER; CASALES; YEANNES, 2002) was applied to analyze the behavior of these attributes in the different solution:fish ratios. The scale ranged from 1 to 4: 1 for "inadequate texture for either deficient or excessive marinating" (typical of raw fish without marinating or excessively soft, respectively); 2 corresponds to both dry and hard texture, resistant to cut with a table fork (due to deficient marinating), or soft and watery (due to excess of marinating); 3 corresponds to less hard, dry, and fibrous texture (due to deficient marinating), or less firm texture (due to excess of marinating), and 4 for "the best degree of marinating with a firm and consistent texture" (fillets are cut easily with a table fork). This scale was developed in six sessions carried out by eight trained judges assessing the changes in texture during the immersion of anchovy fillets in marinating bath by means of common utensils.

Color changes were analyzed by a structured scale developed for this product. Point 0 refers to the typical coloration of the marinated fillet (white-grayish color) and point 4 corresponds to a dark yellow, brownish fillet; intermediate points are

determined as follows: 1: yellowish toward the edges of the fillet; 2: stronger yellowish toward the edges, yellowish diffused toward the center; 3: yellowish uniform color. The scale was developed in six sessions carried out by eight trained judges, who assessed the color changes of a recently marinated anchovy fillet until dark yellow, brownish color appeared.

Rancid taste was analyzed by a structured scale developed for this product. The scale was developed in six sessions carried out by eight trained judges who assessed the taste of a recently marinated anchovy fillet (with 1.65 ± 0.31 mg malonaldehyde/kg values). The taste changes were evaluated until it became rancid (5.17 ± 0.23 mg malonaldehyde/kg values). Score ranges from 0 to 3. Point 0 corresponds to the typical taste of the marinated fillet; point 1, to lightly rancid taste; point 2, to rancid taste and point 3, to extremely rancid taste.

The acid and salty tastes were individually evaluated using structured scales according to Pedrero and Pangborn (1989). The scale for acid/salty ranges from 0 "not acid/salty" to 10 "extremely acid/salty"; the intermediate points are as follows: 2: scarcely perceptible; 4: lightly acid/salty; 6: moderately acid/salty; and 8: very acid/salty.

3 Results and discussion

Salt and acid penetration for 0.77:1, 3:1 and 10:1 ratios were analyzed to determine the influence of marinating solution:fish ratio in the salt and acid uptake and its influence in the sensorial characteristics. The results of chemical analyses, Figure 1, shows that there was a higher concentration of salt and acid in the fillet during marinating with the increase in the ratio. It was also observed that as the solution:fish ratio increases, a higher expulsion of water was produced.

Table 1 shows the percentage of uptake of sodium chloride and acetic acid at different solution:fish ratios. It can be observed that the acetic acid penetrated the anchovy flesh faster than salt for the analyzed ratios. The uptake of both sodium chloride and acid was higher with the increase in the ratio.

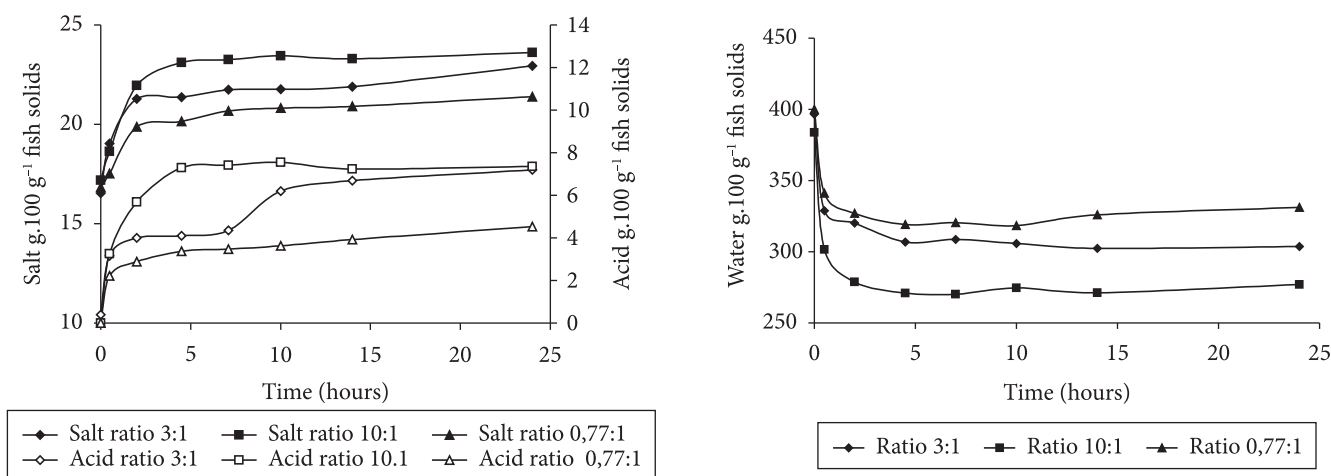


Figure 1. Behavior of salt, acid, and water at different marinating solution:fish ratios.

Table 1. Salt and acid uptake during marinating.

Time (hours)	% Salt uptake				% Acid uptake			
	Solution:fish ratio							
	0.77:1	3:1	10:1	10:1 with agitation	0.77:1	3:1	10:1	10:1 with agitation
0	0	0	0	0	0	0	0	0
0.5	4.0	10.0	10.7	11.2	16.0	23.3	26.9	30.8
2.0	10.2	15.6	21.7	18.8	21.3	30.0	48.9	51.7
4.5	11.5	17.2	25.7	21.3	25.3	31.7	63.6	64.0
7.0	12.5	17.8	26.1	21.6	26.0	33.3	64.7	65.4
10.0	13.0	18.0	25.9	21.6	27.3	47.7	65.0	66.1
14.0	12.4	18.6	26.1	21.7	29.0	51.7	62.9	67.1
24.0	12.8	20.7	26.0	22.0	33.0	55.2	62.9	66.4
48.0	17.4	20.0	26.0	21.6	31.5	52.0	65.0	65.0

Table 2. Values of pH and a_w in the anchovy fillet at different stages of marinating process.

Sample	Acetic acid (g.100 g ⁻¹)	pH	Sodium chloride (g.100 g ⁻¹)	a_w
Anchovy fillet	0 ^a 0 ^b	6.53	0.12 ^a 0.57 ^b	0.99
Brined fillet	0 ^a 0 ^b	6.52	3.35 ^a 16.85 ^b	0.97
Marinated fillet (ratio 0.77:1) (24 hours)	0.99 ^a 4.52 ^b	4.40	4.68 ^a 21.39 ^b	0.96
Marinated fillet (ratio 3:1) (24 hours)	1.66 ^a 7.20 ^b	4.22	5.29 ^a 22.95 ^b	0.95
Marinated fillet (ratio 10:1; without agitation) (24 hours)	1.8 ^a 7.34 ^b	4.14	5.79 ^a 23.62 ^b	0.94
Marinated fillet (ratio 10:1; with agitation) (24 hours)	1.9 ^a 7.12 ^b	4.12	5.43 ^a 20.22 ^b	0.94

References: compound sample (n = 20 fillets). a: g.100 g⁻¹ wet sample; and b: g.100 g⁻¹ of fish solids.

Rodger et al. (1984) obtained uptake percentages of 31.5 and 54.5 for sodium chloride and of 50.05 and 83% for acetic acid at 4 and 48 hours, respectively for marinated herring at 20 °C with a marinating solution containing 14% of salt and 7% of acid in a ratio 1:1. The values obtained for the aforementioned authors were higher than those obtained in the present study for all the ratios at the same times, this behavior can be explained considering the differences in the respective driving force. According to Rodger et al. (1984), these results could explain the importance of the solution:fish ratio in salt and acid levels in marinated products.

Values of pH and a_w for the anchovy, brined, and marinated fillet at 24 hours of marinating for the different ratios of solution: fish are shown in Table 2. In the brining and marinating stage,

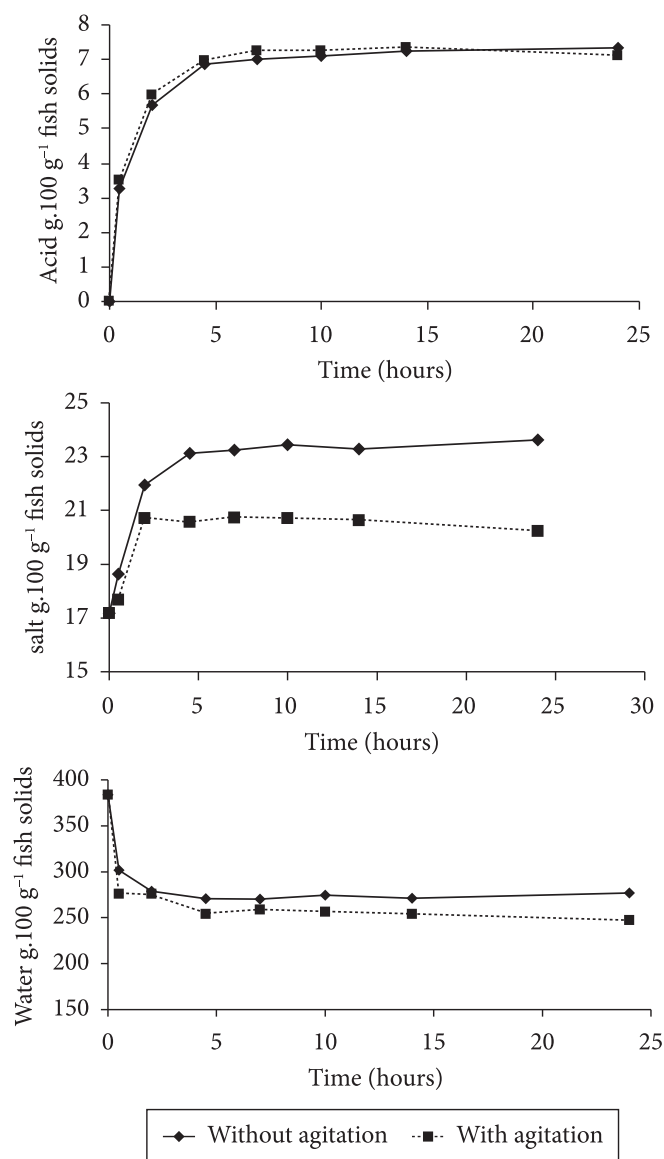
**Figure 2.** Behavior of water, salt, and acid contents during marinating with and without agitation.

Table 3. Effect of marinating conditions on sensorial attributes.

Marination time (days)	Sensorial score					
	Marinating solution:fish ratio	Texture	Acid taste	Salty taste	Rancid taste	Color
1	0.77:1	2	2.50	1.50	0.20	0
	3:1	2	2.30	1.50	0.20	0
	10:1	2.25	4.50	1.50	0.25	0
	10.1 (1)	2	4	1.50	1.50	0.75
2	0.77:1	3.16	4.16	3.33	0.16	0.50
	3:1	2.50	3.90	3.33	0.20	0.45
	10:1	2.66	5.16	3.33	0.50	0.16
	10.1 (1)	2.50	4.66	2.66	2.50	2.50
3	0.77:1	3	3.83	1.33	1	0.33
	3:1	3.10	3.80	1.33	0.90	0.32
	10:1	2.50	6.16	1.33	0.33	0.14
	10.1 (1)	2.33	6.83	1	2.66	3
5	0.77:1	3.50	4	1.67	1	0.53
	3:1	3.50	4	1.82	1.10	0.28
	10:1	2.67	6.50	2.33	0.50	0.20
	10.1 (1)	2.83	6.70	3	2.83	3.70
6	0.77:1	3.45	3.83	1.69	0.63	0.38
	3:1	3.70	3.90	2	0.70	0.25
	10:1	3	6	4	0.50	0.31
	10.1 (1)	3	6	4	3	4
8	0.77:1	3.77	4	1.84	0.80	0.30
	3:1	4	4	2	0.50	0.33
9	0.77:1	4	4	2	0.80	0.35

References: (1) with agitation.

there is a significant decrease in the a_w . The lowest pH and a_w values were obtained for the ratio 10:1 (with and without agitation), according to the highest contents of acetic acid and sodium chloride.

The behavior of water, salt, and acid contents in the fillet for the 10:1 solution:fish ratio with and without agitation during 24 hours of marinating are shown in Figure 2. It can be seen that the agitation did not influence the uptake of acetic acid in the fillet ($p > 0.01$). Salt concentration was significantly lower ($p < 0.01$) in the sample with agitation than in the sample without agitation. The results show that agitation promote the water expulsion ($p < 0.01$). According with Collignan et al. (2001), the binary solution of salt and water did not need to be stirred since natural convection is enough to renew the barrier layer at the product/solution interface.

To determine the effect of the different marinating conditions on the characteristics of product, the sensorial attributes were analyzed and compared considering 0.77:1 as reference (Table 3).

The texture of the fillet at the end of marinating stage (9-day immersion) with a 0.77:1 ratio was given a score 4 (good point of marinating), while for the 3:1 ratio the same score was obtained after 8 days of immersion. For the ratio 10:1 (with and without agitation), 3 was the highest score given (dry and fibrous) for 6 days of immersion. The fillets were kept in the marinating bath without obtaining sensory improvements. This nonwished

behavior in the textural characteristic can be explained by the greater impelling force that causes a major uptake of salt in less time and an irreversible modification in the texture.

The acid taste at the end of marinating process was given score 4 (lightly acid) for the ratios 0.77:1 and 3:1, and score 6 (moderately acid) for the ratio 10:1 (with and without agitation). For the salty taste (scarcely perceptible), score 2 corresponded to the ratios 0.77:1 and 3:1, and 4 (lightly salty) to the ratio 10:1 (with and without agitation).

In the products marinated with agitation, a marked rancid taste and a yellow color in the fillets were observed. This is due to the agitation effect that causes an airing during marinating (by the air in the headspace of the recipient) favoring oxidative rancidity.

In order to confirm the effect of agitation on rancidity of fillets during the marinating process, another test was performed. At 24 hours, the sensorial analysis of color and rancid taste showed that for the ratio 10:1 with agitation, the fillets were scored 0.75 (yellowish in the borders) and 1.5 (lightly rancid taste), respectively. These scores increased markedly as the marinating time progressed. For the solution:fish ratios 0.77:1 and 10:1 without agitation, the scores of both color and rancid taste remained unchanged.

The TBA value for thawed raw materials was 0.61 mg malonaldehyde/kg, slightly higher than the value determined

by Del Valle et al. (1984) of 0.22 for fresh anchovy. Kilinc and Cakli (2005) found TBA values of 1.03 mg malonaldehyde/kg in raw sardine (*Sardine pilchardus*). In 0.77:1 ratio marinated anchovy 2.6 mg of malonaldehyde/kg was obtained; in the 10:1 ratio without agitation, the value was 2.45 mg malonaldehyde/kg; for the same ratio with agitation, the value was 3.75 mg malonaldehyde/kg. These results agree with those obtained by other authors for marinated fish. Kilinc and Cakli (2005), studying marinated sardine (*Sardine pilchardus*), found 4.47 mg malonaldehyde/kg values, Duyar and Eke (2009) found 4.27 and 2.18 mg malonaldehyde/kg values at the end of the marinating process of bonito (*Sarda sarda*, Bloch 1793) and anchovy (*Engraulis encrasicolus*, Linnaeus 1758), respectively.

The results of TBA obtained with agitation in the marinating stage showed the adverse effect of stirring on the product.

4 Conclusions

An increase of marinating solution:fish ratio causes a higher acid and salt penetration speed. However, the product obtained with the 10:1 ratio had a dry and fibrous texture and a lightly salty taste. Therefore, in order to define the adequate ratio, the sensorial characteristics must be considered.

Agitation did not influence the acid uptake, and the salt penetration speed decreased. The sensorial analysis, as well as the TBA value, showed a decrease in quality by the oxidative rancidity development and an excessive acidity and hardness in the final product. This indicates that incorporating agitation in the marinating stage was not appropriated.

From the results of this research, it may be concluded that besides the original ratio of 0.77:1, the ratio 3:1 can also be used to elaborate marinades of this species. This ratio decreases the immersion marinating time without damaging the sensorial attributes and maintaining the stability of the marinade.

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