Changes in Biogenic Amines during the Storage of Mediterranean Anchovies Immersed in Oil

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Anchovies are a semipreserved fish product obtained without heating, and for this reason contents of biogenic amines could be modified during its storage. Changes of 10 biogenic amines during the storage of oil-packed anchovies from three different commercial brands at 8−10 °C or at 20 ± 1 °C were studied. Initial contents of spermidine and spermine were very similar in all samples studied, and no changes were observed throughout storage. Putrescine and cadaverine also remained constant during storage, but differences between commercial brands were observed. Agmatine remained constant at refrigeration temperature but decreased at 20 ± 1 °C in samples of two commercial brands. Histamine, tyramine, tryptamine, and β-phenylethylamine increased during storage at both temperatures. Storage under refrigeration, as producers recommend, reduces but does not prevent amine formation.

Keywords: *Biogenic amines; histamine; fish spoilage; anchovy shelf life*

INTRODUCTION

Biogenic amines may be indicators of fish spoilage (Hui and Taylor, 1983), and their appearance is related to microbial decarboxylation of their precursor amino acids. Several biogenic amines (serotonin, histamine, and tyramine) are essential for many physiological functions, but consumption of foods containing high amounts of these amines can also have toxic effects (Brink et al., 1990). In addition, certain drugs [monoamine oxidase inhibitors (MAOI)] decrease the efficiency of the biogenic amine detoxification systems. Moreover, the toxic effects of biogenic amines are strongly dependent on the efficiency of the detoxification mechanisms of different individuals.

High levels of tyramine are usually associated with cheese, but high contents of this amine have also been reported in other foods such as beer (Izquierdo-Pulido et al., 1995), ripened meat products (Brink et al., 1990, Maijala et al., 1995), and semipreserved anchovies (Murray et al., 1982; Veciana-Nogués et al., 1989; Yen and Hsieh, 1991). Histamine is the biogenic amine most frequently related with toxicological effects after fish consumption, but other biogenic amines such as putrescine and cadaverine increase histamine toxicity (Chu and Bjeldanes, 1981). Outbreaks of histamine intoxication are often related with fresh or canned fish consumption (Taylor, 1985; Morrow et al., 1991; Valentini et al., 1991; Hwang et al., 1995). Although high contents of histamine were reported for some semipreserved fish products, only Murray et al. (1982) described histamine intoxication following consumption of anchovies.

Legal limits have been established only for histamine in fish and fish products. European Community and Spanish regulations have fixed a maximum average value of 100 mg/kg in a group of nine samples of fresh or canned fish and lower than twice this value for ripened fish products (Directiva, 1991; Real Decreto, 1993). Recently, the Food and Drug Administration has revised the histamine defect action level to lower it from 100 to 50 mg/kg (FDA, 1995).

"Anxoves de l'Escala" are genuine anchovies prepared exclusively from fresh *Engraulis encrasicholus* by a process of salting and ripening for at least 2 months (Departament d'Agricultura Ramaderia i Pesca, 1987). Spanish regulations established that fish used as raw material to obtain the ripened anchovies called "anxoves de l'Escala" should be classed as excellent quality (grade A). When ripening ends, as judged by appearance and flavor, anchovies were traditionally packed in brine in small glass jars. These ripened anchovies need to be desalted and filleted before consumption. Nowadays, consumers generally prefer to purchase the product desalted, immersed in oil ready to eat and packed in glass jars.

Anchovies, in brine or in oil, are semipreserved, obtained without any heating process to stabilize the end product. Changes during storage can occur, and even at low temperatures, enzymatic processes may lead to a product showing a high degree of proteolysis. Moreover, desalting and filleting increase the risk of secondary bacterial contamination in the end product packed immersed in oil (Cheftel, 1965). Previous studies showed that histamine levels increased during storage of semipreserved anchovies when they were not kept at refrigeration temperature (Karnop, 1988; Veciana-Nogués et al., 1989). Other biogenic amines such as tyramine, β-phenylethylamine, and tryptamine also seem to increase during storage (Veciana-Nogués et al., 1996), but little information is available. Here we studied the evolution of the contents of 10 biogenic amines throughout the shelf life of ripened anchovies from the north Mediterranean Spanish coast, called "anxoves de l'Escala". Changes in biogenic amines during storage of anchovies packed immersed in oil and ready to eat from three different manufacturers were studied to determine whether biogenic amines were produced during 9 months of storage of the end products. The assigned shelf-life period for these anchovies is 6 months. Total mesophilic, psychrotrophic, and enterobacterial counts were also determined to check

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Table 1. Biogenic Amines (mg/kg) in Anchovies Immersed in Oil (before Storage) from Three Different Commercial Brands

	A	B	C
spermine	7.9[a]	7.7	7.5
spermidine	2.3	2.1	2.2
putrescine	7.6	2.3	5.2
cadaverine	38.3	23.1	27.6
agmatine	45.3	24.1	34.2
serotonin	nd[b]	nd	nd
histamine	12.6	3.9	3.10
tyramine	21.6	1.9	24.6
β-phenylethylamine	2.0	0.2	1.7
tryptamine	1.1	nd	0.8

[a] Average value of amine determination in two samples. [b] Not detected.

their possible relationship with biogenic amine production linked to the handling process needed before the final packaging of anchovies immersed in oil.

MATERIALS AND METHODS

Biogenic Amine Determination. Ten biogenic amines, histamine (HI), tyramine (TY), serotonin (SE), β-phenylethylamine (PHE), tryptamine (TR), putrescine (PU), cadaverine (CA), agmatine (AG), spermine (SM), and spermidine (SD), were determined by HPLC (Veciana-Nogués et al., 1995). The method is based on an ion-pair chromatographic procedure on a C_{18} reversed-phase column involving a postcolumn reaction with *o*-phthalaldehyde (OPT) to form fluorescent derivatives with amines.

Microbiological Counts. Mesophilic and psychrotrophic microorganisms and *Enterobacteriaceae* counts were determined at the Food Hygiene Unit of the Faculty of Veterinary Medicine of Universitat Autònoma de Barcelona according to procedures described by Rodriguez-Jerez et al. (1994).

Sampling. Samples of ripened anchovies packed in oil were obtained from three different factories (A, B, and C). The *E. enchrasicholus* used as raw material was caught in the same geographical area (Golfo de Rosas) the first week of July and was processed in the three factories using similar techniques (head removing, gutting, ripening in brine solution, desalting, filleting, immersion in oil, and packing in glass jars of 500 mL). Thirty-two samples of each brand (packed and ready for their merchandising distribution) were split into two batches and stored in their original packaging at 20 ± 1 °C in a climatic test chamber, HC 0020 (Heraeus Vötsch, Frommern, Germany), and at 8−10 °C in a refrigerator, 2F 235 (Zanussi, Zaragoza, Spain). Samples for analysis were taken in duplicate to determine biogenic amines and microbiological counts at zero time (after desalting, filleting, and immersion in oil) and after 1, 2, 3, 4, 5, 6, and 9 months of storage.

Statistical Analysis. All statistical tests were performed by the statistical package SYSTAT (Systat, Inc., Evanston, IL). Comparisons of biogenic amine contents and microbial counts among samples from different commercial brands or stored at different temperatures were made by using ANOVA analysis, after verifying the normality and variance homogeneity, or by using the nonparametrical Kruskall−Wallis test.

RESULTS AND DISCUSSION

Table 1 shows biogenic amine contents in samples from zero time. SE was not detected, and PHE and TR were below 2 mg/kg. Contents of SM and SD were similar in the three commercial brands studied whereas differences were observed in the contents of PU, CA, TY, AG, PHE, and HI, which are related with fish spoilage (Veciana-Nogués et al., 1996). Sample A showed, in general, the highest contents of biogenic amines, and all were higher than in fresh fish (Pechaneck et al., 1980; Ingles et al., 1985), although they were similar to those reported by other authors for anchovy samples (Yen and Hsien, 1991; Soares and Gloria, 1996; Yeanes and Casales, 1995). Levels of biogenic amines were often higher in ripened anchovies than in nonprocessed anchovies and have been linked to the use of poor quality raw material and to the formation of biogenic amines in the first period of the ripening process (Veciana-Nogués, 1996).

Evolution of biogenic amine contents during storage at 8−10 °C or at 20 ± 1 °C depended on the amine considered. SE, which was not detected in samples from zero time, was found in 31% of anchovies only from brand C and always below 0.5 mg/kg. The polyamines SM and SD and the diamines PU and CA were not modified by storage time, whereas changes of AG, TY, HI, PHE, and TR were observed. Similar average contents of SD, SM, PU, and CA were observed in samples stored at 8−10 °C, as producers recommended, and in samples stored at 20 ± 1 °C (Table 2). No differences in SD and SM contents ($p > 0.05$) were found among samples from different commercial brands. Both polyamines are ubiquitous in fish samples (Yamanaka et al., 1989) and are usually present in fish and in other foods, since they are necessary for cellular growth (Pollack et al., 1992). Contents of SM were always higher ($p < 0.05$) than contents of SD, as other authors reported for food from animal origin (Bardocz, 1995). Contents of PU and CA, which have been related with fresh fish spoilage, remained constant throughout storage at both temperatures. By Kruskall−Wallis test, differences ($p < 0.05$) were observed in average contents of PU and CA between samples from different producers.

No changes in AG content were observed in any of the samples kept at 8−10 °C and in samples from brand A kept at 20 ± 1 °C throughout the 9 months of storage (Figure 1). In contrast, a decrease of ca. 95% of initial AG contents was observed in samples from brands B and C stored at 20 ± 1 °C, dropping to 0.8 mg/kg and 2.65 mg/kg, respectively. The AG decrease in stored anchovies agrees with the evolution of AG during fresh anchovy spoilage, since, although AG contents increased in the few hours of nonprocessed anchovy storage, it decreased soon after, while contents of other biogenic amines such as PU, CA, HI, or TY increased (Veciana-Nogués et al., 1996). Yamanaka and Matsumoto (1989) also reported a decrease in AG contents during storage of fresh mackerel and saury pike at low temperatures.

Changes in TY, HI, TR, and PHE contents during storage of the three commercial brands studied are

Table 2. Average Contents (mg/kg) of Spermidine, Spermine, Putrescine, and Cadaverine in Anchovies Immersed in Oil Stored at 8−10 °C and at 20 ± 1 °C for 9 Months

	8−10 °C			20 ± 1 °C		
	A	B	C	A	B	C
spermine	9.5 ± 1.7[a]	9.6 ± 0.9	8.7 ± 0.2	1.8 ± 2.2	11.1 ± 2.9	9.2 ± 0.6
spermidine	1.5 ± 0.4	1.2 ± 0.2	1.1 ± 0.7	1.8 ± 0.3	2.1 ± 2.1	2.6 ± 1.1
putrescine	8.6 ± 2.6	3.8 ± 1.5	6.88 ± 0.9	8.7 ± 1.6	5.4 ± 3.1	7.4 ± 4.6
cadaverine	34.2 ± 3.1	22.6 ± 2.7	25.2 ± 2.5	35.0 ± 5.9	24.7 ± 5.9	31.8 ± 4.0

[a] Average value ± standard deviation ($n = 16$).

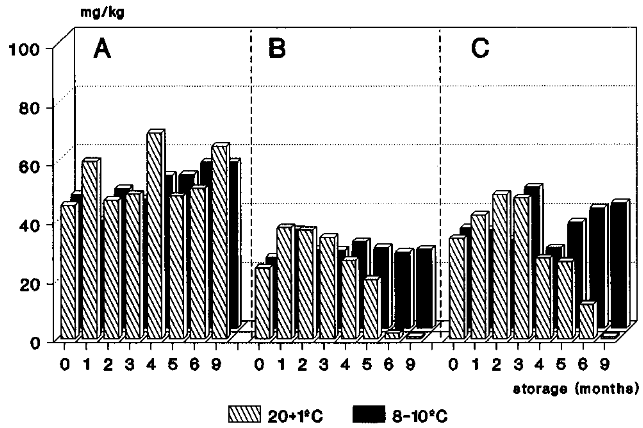

Figure 1. Changes in agmatine contents throughout storage of anchovies immersed in oil from three commercial brands (A, B, C) stored at 8−10 °C or at 20 ± 1 °C.

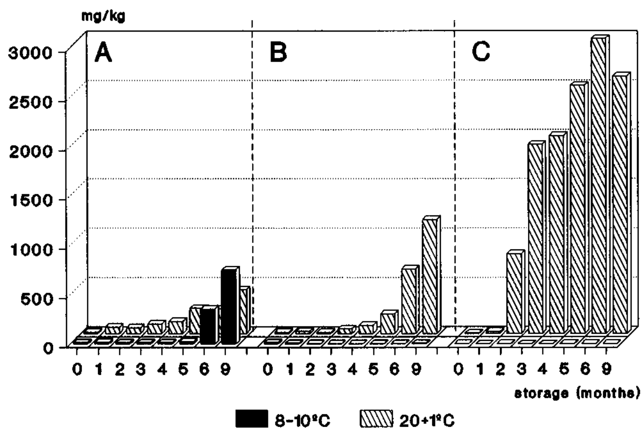

Figure 2. Changes in histamine contents throughout storage of anchovies immersed in oil from three commercial brands (A, B, C) stored at 8−10 °C or at 20 ± 1 °C.

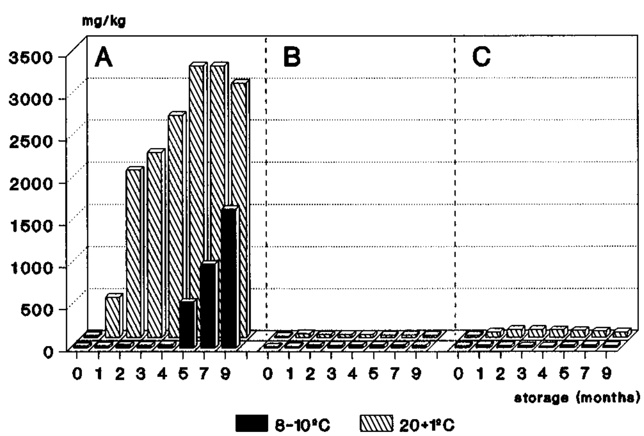

Figure 3. Changes in tyramine contents throughout storage of anchovies immersed in oil from three commercial brands (A, B, C) stored at 8−10 °C or at 20 ± 1 °C.

showed in Figures 2−5. Amine increases were, in general, lower in samples kept at 8−10 °C than in samples kept at 20 ± 1 °C. Therefore, we would point out that storing semipreserved anchovies under refrigeration, as producers recommended, reduces but does not prevent amine formation.

All anchovies stored at 20 ± 1 °C showed increases in HI content whereas in samples kept at 8−10 °C the increase was observed only for samples from A. The highest and earliest formation of HI was observed in samples from C (Figure 2). HI contents at 6 months (shelf-life deadline) of storage at 20 ± 1 °C surpassed,

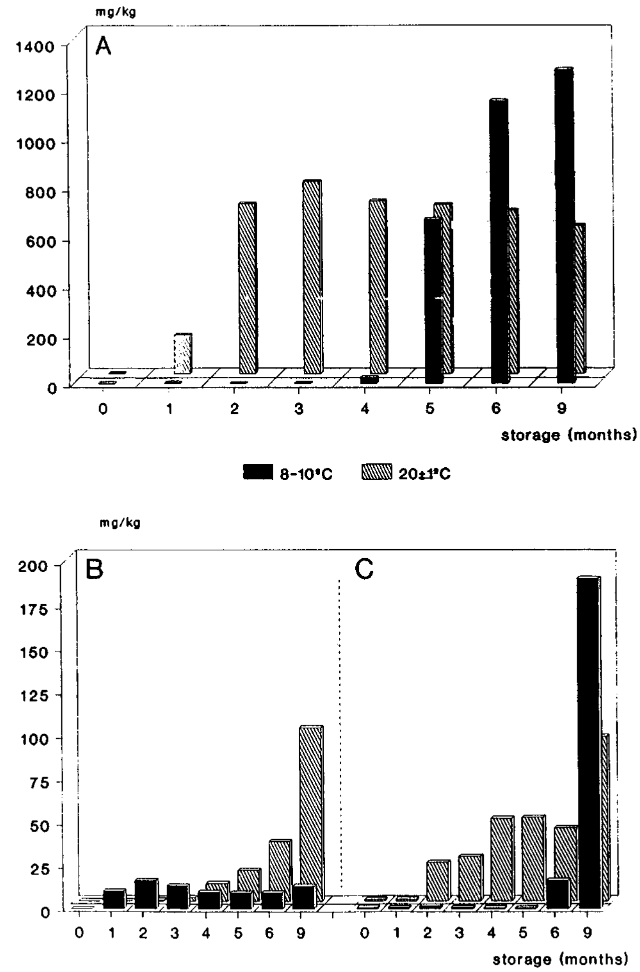

Figure 4. Changes in tryptamine contents throughout storage of anchovies immersed in oil from three commercial brands (A, B, C) stored at 8−10 °C or at 20 ± 1 °C.

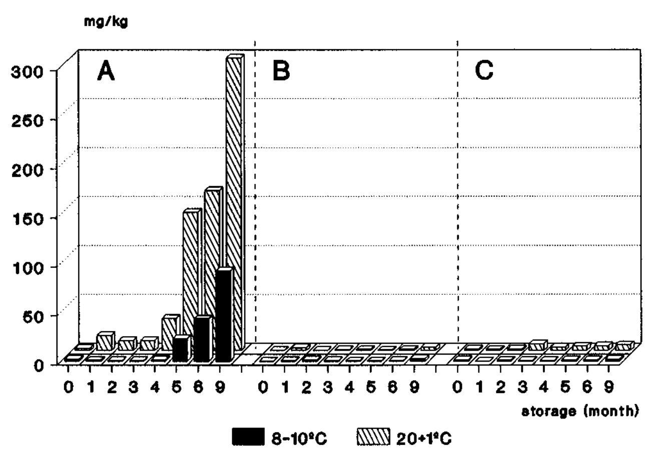

Figure 5. Changes in β-phenylethylamine contents throughout storage of anchovies immersed in oil from three commercial brands (A, B, C) stored at 8−10 °C or at 20 ± 1 °C.

in all A, B, and C samples, the maximum average value (200 mg/kg) permitted for ripened fish products according to Spanish and European regulations. This legal limit was surpassed in C samples after only 2 months of storage at 20 ± 1 °C. HI levels remained constant in B and C samples stored at 8−10 °C, but HI formation was also observed in brand A, surpassing the legal limit after 6 months of storage. Karnop (1988) also reported high HI increases in oil-packed anchovies after 1 year of storage at 20 °C and after 18 months at 5 °C.

Contents of TY remained constant throughout the period studied at both temperatures in B and C samples (Figure 3). In contrast, great increases in TY were observed in A samples stored at 20 ± 1 °C. At the end of the shelf life, TY contents reached ca. 1000 mg/kg in A samples stored at 8−10 °C and ca. 3000 mg/kg in those stored at 20 ± 1 °C.

Increases in TR content were observed in samples from all three commercial brands at both temperatures (Figure 4). The highest formation of TR was also observed in a sample from A. Formation of TR in A anc C samples was earlier at 20 ± 1 °C than at 8−10 °C, but unexpectedly the amount found after 9 months of storage was higher in samples kept under refrigeration. Contents of TR in samples stored under refrigeration were, at the end of shelf life, ca. 100 times higher in A samples than in B or C samples. However, in samples stored at 20 ± 1 °C, TR content was only 20 times higher in A samples than in B or C samples.

PHE contents did not increase during storage of B or C samples, whereas A samples showed progressive increases throughout storage. However, maximum levels reached for PHE were much lower than those reached for TR, TY, or HI. Previous work performed in our laboratory showed that both TR and PHE increased only when spoilage of fish was advanced and when levels of other biogenic amines such as HI, TY, PU, and CA were high. On the other hand, a slight formation of TR, PHE, HI, TY, PU, and CA has been reported during anchovy manufacture (Veciana-Nogués et al., 1996). High contents of TR and PHE in anchovies immersed in oil have been reported previously (Yen and Hsiend, 1991), but no data are available on the evolution of these amines during ripening and storage, except the previous work performed in our laboratory mentioned above.

In samples from zero time, counts of colony forming unit (CFU) mesophilic bacteria ranged from 2.2 to 4.8 log CFU, counts of psychrotrophic bacteria ranged from 2.5 to 3.4, and enterobacteria were not found. Similar counts have been reported previously for other Spanish anchovies immersed in oil (Vieites et al., 1995). In samples from the same commercial brand, no differences were observed in average bacterial counts among samples of different time and temperature storage. In B and C samples, mesophilic and psychrotrophic bacterial counts were, throughout storage, always lower than 3 log CFU/g and enterobacteria were not found. In A samples kept at 20 ± 1 °C, ANOVA analysis showed that average mesophilic (4.36 ± 0.48 log CFU/g), psychrotrophic (4.51 ± 0.48 log CFU/g), and enterobacterial (0.33 ± 0.93) counts were significantly higher ($p > 0.05$) than counts in B or C samples. Similar results were found in samples from A kept at 8−10 °C. Samples from brand A showed higher bacterial counts and also higher increases of TY, TR, and PHE during storage than samples from B and C. However, the increase of HI was lower in samples from A than in those from B and C.

Karnop (1988) reported that increases in HI could be expected only when the total bacterial count of anchovy samples increased more than 5 log CFU/g during storage. In contrast, results presented here show that formation of HI, TY, TR, and PHE could be expected although no increases occurred in bacterial counts throughout storage. Microorganisms commonly related with HI formation in fish belong to the Enterobacteriaceae family (Yoshinaga and Frank, 1982; Leitao et al., 1983). However, a relationship between growth of Enterobacteriaceae and amine formation could not be established because those counts were very low and remained constant during storage. Histamine formation can also be related with the growth of other bacteria as lactobacilli (Karnop, 1988), and also with remaining enzyme activity from halophilic microorganisms, even when low bacterial counts are found (Karnop, 1988; Fujii et al., 1995).

High contents of some biogenic amines in anchovy, even before the end of shelf life, could be related with certain toxic effects. The intake of 10 mg of HI was linked to symptoms of histamine poisoning (Karnop, 1988). Migraine headaches can be provoked by 125 mg of TY and/or 5 mg of PHE (Lüthy and Schlatter, 1983), and 6 mg is the maximum tolerable intake of TY for patients under classic monoamine oxidase inhibitor (MAOI) therapy (Tailor et al., 1994).

Anchovies are usually consumed at low amounts as a snack or as an ingredient of several dishes, and it can be difficult to surpass the levels mentioned above. However, by considering 20 g of anchovies as a usual intake, the contents of HI, TY, and PHE found in all samples stored at 8−10 °C for 6 months are insufficient to provoke either histamine intoxication or migraine headaches. After 9 months of storage, only consumption of anchovies from brand A could provide more than the 10 mg of HI related with histamine intoxication. A and B anchovies stored at 20 ± 1 °C contributed more than 10 mg of HI at the end of shelf life, but the toxic level for HI was surpassed in C anchovies after only 3 months of storage.

Regarding migraine headaches, with an intake of 20 g of anchovies, only samples from brand A stored at 20 ± 1 °C reached PHE contents higher than levels associated with migraine attack. In addition, TY contents in A samples after 1 month at 20 ± 1 °C and 6 months at 8−10 °C could interact with classic nonreversible MAOI drug therapy. TR, which also increases during anchovy shelf life, can also interact with this therapy (Joosten, 1988; Unzeta et al., 1989); nevertheless, no data about maximum tolerable intake of TR have been reported.

Interaction with MAOI drugs is the most serious problem linked to the toxicological effects of biogenic amines in foods. Fortunately, the interaction risk decreases when reversible MAOI drugs are used, since a very high intake of TY (150 mg) is required to produce it (Tailor et al., 1994). Regarding samples of this work, unusually high ingestion of anchovies should be needed to surpass the maximum amount allowed, even in samples from brand A, which showed the highest formation of TY.

Results of this study indicate that anchovies, which showed low or moderate contents of biogenic amines inmediately after packaging, were easily transformed into hazardous products with high contents of HI and also TY, TR, and PHE, especially when they were kept at room temperature. Storage of anchovies immersed in oil at refrigeration temperatures, as manufacturers recommend on labels, delays but does not prevent amine formation. Therefore, more information about the possible biogenic amine formation during the storage of oil-packed anchovies ready to eat should be given to consumers, especially to subjects under MAOI drug therapy. No relationships between mesophilic, psychrotrophic and Enterobacteriaceae microorganisms and biogenic amine formation has been established during storage of anchovies immersed in oil.

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