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Influence of the Freshness Grade of Raw Fish on the Formation of Volatile and Biogenic Amines during the Manufacture and Storage of Vinegar-Marinated Anchovies

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Volatile and biogenic amines from three batches of anchovies, marinated in vinegar, were studied. The anchovies had been vacuum-packed and kept in refrigerated storage for 3 months. Trimethylamine and total volatile basic nitrogen levels were very low and constant throughout marinating and storage process: less than 1 and 10 mg/100 g, respectively. Certain amine levels, mainly those of tyramine and serotonin, increased slightly, particularly during storage. However, even the highest recorded levels were much lower than those considered to be hazardous for human consumption. To study the influence of raw material freshness in the amine profile, two laboratory trials using fresh and spoiled anchovies and simulating the industry standard marinating process were carried out. Levels for both volatile and biogenic amines were dependent on raw material quality, proving consistently higher in those deriving from nonfresh fish. Vinegar marinating leads to a decrease in the accumulation of amines in anchovy while their concentration in the vinegar solution increases due to the vinegar effect as solvent extractor.

KEYWORDS: Anchovy; semipreserves; trimethylamine; total basic volatile nitrogen; biogenic amines; histamine

INTRODUCTION

Anchovy (*Engraulis encrasicholus*) is a pelagic fish belonging to the Engraulidae family. Anchovies caught off the Bay of Biscay and Mediterranean coasts are commonly consumed in Spain as fresh fish between May and October. However, up to 70% of capture is destined to the manufacture of semipreserves, mainly salted but also marinated in vinegar. Marinades are made by treating fresh or frozen fish, or portions of them, with edible acids (usually acetic acid) and salt, before the fish are placed in brine, sauces, creams, or oils (*1*). Marinating increases ionic strength and decreases pH, not only preventing microorganism growth but also involving changes in taste and in the textural and structural properties of fish (*2*).

Although, in general, the quality of raw materials affects the quality of fish derivatives, few data on marinated fish are currently available. Trimethylamine (TMA-N) and total volatile basic nitrogen (TVB-N) have been proposed in several studies (*3*−*5*) as objective indices to evaluate the freshness of marine fish. TMA-N is a volatile basic compound that accumulates during fish spoilage from the reduction of trimethylamine oxide (TMA-O) by bacterial enzymes (*6*). Although TMA has been traditionally recognized as the compound mainly responsible for the characteristic fishy odor (*7*), it seems that other compounds may be even more important (*8*). TMA-N, ammonia, and other basic compounds constitute TVB-N.

Biogenic amines, which are basic organic compounds of low molecular weight, have also been proposed as indices of freshness in fish (*9*−*11*). Biogenic amines stem from decarboxylation of precursor amino acids by bacterial enzymes (*12*, *13*). In fish marinades, acetic acid reduces bacterial growth and enzyme action, resulting in products with extended but limited shelf life. Some biogenic amine accumulation in marinades could be expected, as they are semipreserves wherein certain microorganisms or enzymes may remain active. In addition, acidic conditions make the tissue cathepsins more active, yielding higher amino acid release from proteins (*14*).

Besides their use as freshness indices, biogenic amine levels can also warn consumers to potential health hazards related to fish consumption. Migraine headaches and histamine intoxication (HI) are directly produced by the intake of biogenic amines (*15*−*17*). Moreover, there are also indirect toxic effects related to biogenic amines, mainly tyramine, intake such as their interaction with monoamine oxidase inhibitor (MAOI) drugs (*18*). MAOI drugs block the oxidative deamination pathway of biogenic amines, giving rise to their accumulation and causing hypertensive crises, which can be mortal (*19*).

Histamine, the most widely studied biogenic amine in fish, is the only amine for which legal limits have been established. The U.S. Food and Drug Administration (FDA) recommends

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determining other biogenic amines together with histamine (*20*) because they are known to aggravate histamine toxicity (*21*).

Fish marinades incorporating vinegar have been popular in Mediterranean countries since antiquity, but their consumption has historically been limited to the fishing season. Today, however, anchovies marinated in vinegar are also manufactured in factories, making them available to consumers year round. Although several studies address biogenic amine content and evolution in salt-ripened anchovies or marinades from other fish species (*14*, *22*−*25*), none examine anchovies marinated in vinegar.

The aim of the present work was to study the usefulness of volatile and biogenic amines as quality indices of raw materials in anchovies marinated in vinegar. Samples of raw materials and their corresponding end products were analyzed. As end products are not thermally treated, possible changes stemming from the storage process were also examined. In addition, a laboratory trial simulating the industrial marinating process was carried out to investigate the effects of raw material quality on the amine profile.

MATERIALS AND METHODS

Factory Samples. Samples were provided by an artisan producer who elaborates anchovies marinated in vinegar as follows: Immediately upon arrival, the fresh fish (*E. encrasicholus*) is cleaned, beheaded, gutted manually, and then immersed in a vinegar solution (20% acetic acid v/v). A maceration period of 2 weeks is maintained, during which fish remain under refrigeration. Anchovies are then manually filleted, immersed in oil, and vacuum-packed. Three different batches were studied, including samples of fish and vinegar of both raw materials and end products. To determine whether changes in biogenic amines occurred during storage, end product samples were divided into two groups: samples of the first group were analyzed upon arrival at the laboratory, and samples of the second group were analyzed after a refrigerated storage period of 3 months, which is the manufacturer's recommended shelf life.

Laboratory Trial Samples. Anchovies (2−3 kg) caught off the Mediterranean coast were obtained from a fish market in Barcelona and marinated at the laboratory following the same procedure used by the artisan producer. Individual fish weighed 11−16 g and were 10−15 cm in length. Upon arrival at the laboratory, the freshness state of anchovies was assessed using the European Union (EU) grading scheme for fatty fish (*26*) and resulted in a grade of Extra (E). Whole fish were divided in two batches to proceed with marination.

(a) Batch F (fresh) anchovies were immediately gutted, beheaded, immersed in a 40:60 mixture of wine vinegar/water, and kept refrigerated for 2 weeks.

(b) Batch S (spoiled) anchovies were kept at room temperature until they met C grade of the EU criteria (*26*), which means spoiled fish, unacceptable for human consumption. Then fish were gutted, beheaded, and marinated in an identical way to those of batch F.

Fish and vinegar samples were taken at time 0 (nonmarinated fish and newly acquired vinegar) and after 3, 8, and 14 days of marinating. At each sampling time, 10−12 anchovies, selected at random, were collected and their flesh was homogenized for determination of TMA-N, TVB-N, and biogenic amine levels. This laboratory trial was performed in duplicate.

Chemical Analysis. TMA-N and TVB-N levels were measured by flow injection−gas diffusion following extraction from fish with 7.5% trichloroacetic acid (*27*). Afer alkalinization with NaOH, volatilized amines induced a change in the color of the blue bromothymol solution proportional to the amount of TMA-N or TVB-N, which was detected at λ 635 nm. Quantification was carried out on the basis of a calibration curve calculated from the response given by standard solutions of TMA (for TMA-N) and ammonium chloride (for TVB-N). Standards were obtained from Panreac Quimica SA (Barcelona, Spain).

Biogenic amine levels were analyzed by high-performance liquid chromatography (HPLC) from a 0.6 N perchloric acid extract according to the method of Veciana-Nogués et al. (*28*). Briefly, the method involves the separation of ion pairs formed between biogenic amines and octanosulfonic acid present in the mobile phase on a reverse phase column (Nova Pack C_{18}, Waters Cromatografía, S.A.). A postcolumn derivatization employing *o*-phthalaldehyde (OPA) is followed by an spectrofluorometric detection (*28*). The elution gradient allowed the same-run separation of 12 biogenic amines, the identification of which was done according to the retention times obtained for the standards (**Figure 1**). Quantification of each amine was performed from the corresponding calibration curve calculated after the injection of solutions at different concentration (from 0.25 to 10 mg/L) of amine standards (Sigma, St. Louis, MO), with a satisfactory precision (relative standard deviation below 5%) and sensitivity (limit of quantification below 0.5 mg/kg for all amines).

All chemical analyses were carried out in duplicate.

Statistical Analysis. Statistical analysis of data was conducted using SPSS 11.0 for Windows software (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Table 1 shows the TMA-N, TVB-N, and biogenic amine levels for factory samples both before and following marinating, as well as after 3 months of storage. As expected, TMA-N and TVB-N were not found in fresh vinegar. In fresh and just-packed marinated anchovies, TMA-N content was <1 mg/100 g, the maximum allowance for fish graded as excellent (*29*). Likewise, TVB-N levels were relatively low, similar in both fresh and marinated anchovies, and far from the 25−35 mg of TVB-N/100 g that the EU has set as the maximum level permissible in fish of the Merlucciidae and Gadidae families, as well as the *Salmo salar* species (*30*). For the Engraulidae family, no legal maximum admissible levels have been set. TMA-N levels in marinated anchovies refrigerated for 3 months were similar to those found in just-packed samples, whereas TVB-N levels were slightly higher. Other authors reported changes in both TMA-N and TBV-N in sardines marinated in vinegar and finally packed immersed in oil, but stored for a longer period of time and elaborated using vinegars of low acid acetic concentrations (*25*).

In general, biogenic amines were very low or not detected in raw material (fish and vinegar). Octopamine, tyramine, serotonin, histamine, β-phenylethylamine, and tryptamine were not found in fresh fish. Putrescine and cadaverine and the natural polyamines spermidine and spermine were all low (<3 mg/kg), whereas agmatine exhibited the highest content, being ∼10 mg/kg. This biogenic amine profile is consistent with that reported for fresh anchovies (*22*). In vinegar, very low levels of tyramine, putrescine, cadaverine, and histamine were found. The presence of biogenic amines in vinegar has been previously reported (*31*, *32*).

The increased levels of certain biogenic amines (octopamine, tyramine, serotonin, and histamine) in anchovies during marinating suggest that even with immersion in vinegar, some enzymatic processes can occur in fish with subsequent proteolysis and biogenic amine formation. Biogenic amine accumulation in anchovy samples marinated in vinegar, however, was weak. Indeed, higher biogenic amine levels have been reported for sardines marinated in vinegar, over shorter periods of time (24 h) (*14*), as well as for salt-ripened anchovies (*22*). Although histamine is the most studied biogenic amine in fish and fish products, tyramine concentration was higher than histamine in anchovies marinated in vinegar, which is also the case for salt-ripened anchovies (*22*).

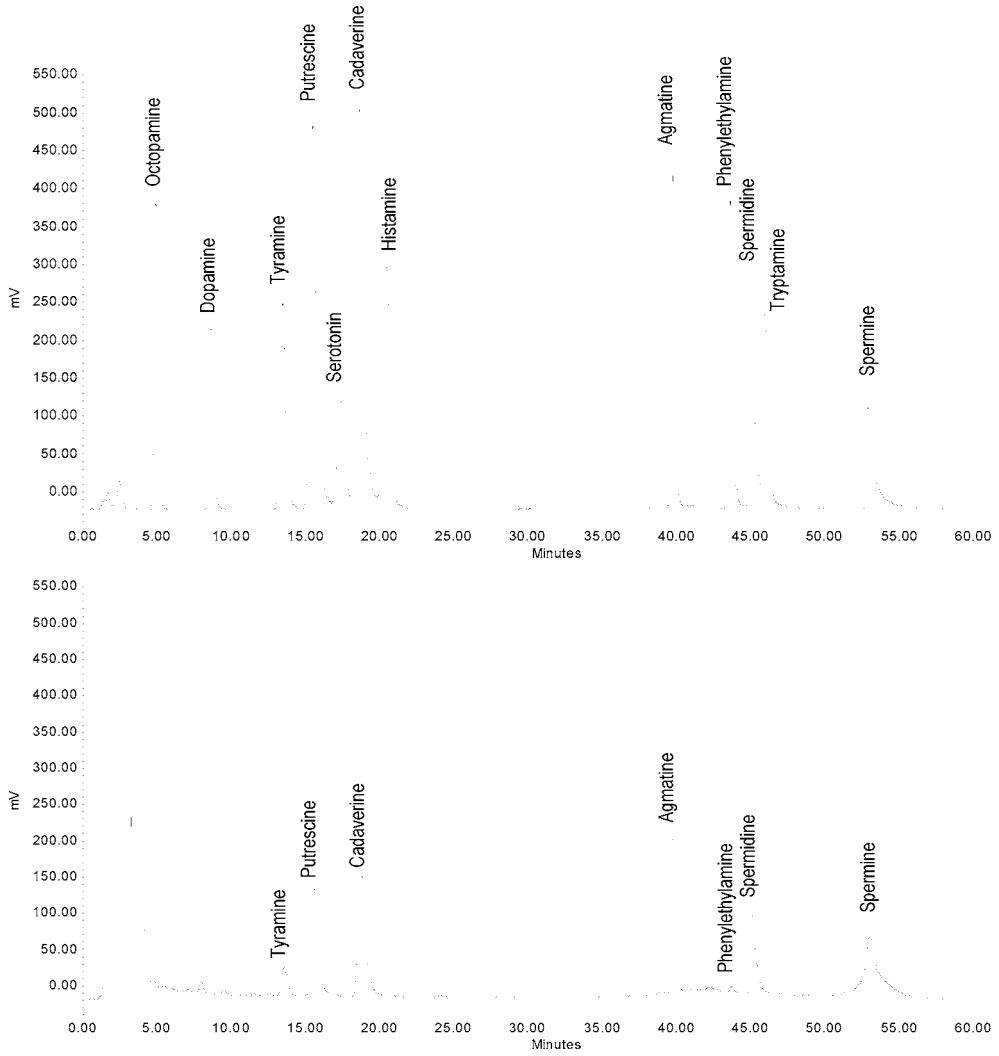

Figure 1. Chromatograms of an 8 mg/L biogenic amine standard solution (top) and an anchovy perchloric acid extract (bottom).

Table 1. Volatile (Milligrams per 100 g) and Nonvolatile (Milligrams per Kilogram) Amines in Raw Materials and in Marinated Product Just Packed and Stored for 3 Months[a]

		raw material		just packed		end date
		vinegar	fish	vinegar	fish	fish
volatile amines	TMA	ND[b]	0.33 (0.17)	ND	0.29 (0.24)	0.41 (0.30)
	TVB-N	ND	7.32 (0.81)	ND	6.04 (1.05)	9.43 (0.55)
nonvolatile amines	OC	ND	ND	2.05 (0.01)	1.95 (0.26)	5.17 (0.46)
	TY	2.23 (0.02)	ND	5.08 (0.18)	5.61 (0.29)	18.86 (0.63)
	SE	ND	ND	ND	4.85 (0.30)	23.73 (0.72)
	PU	2.99 (0.01)	1.07 (0.89)	3.68 (0.12)	0.51 (0.27)	4.84 (0.25)
	CA	0.18 (0.01)	2.78 (0.35)	1.88 (0.10)	0.37 (0.33)	6.86 (0.52)
	HI	0.84 (0.06)	ND	0.25 (0.01)	0.50 (0.18)	1.37 (0.63)
	AG	ND	10.25 (2.92)	ND	1.43 (0.10)	2.73 (0.15)
	PHE	ND	ND	ND	ND	ND
	TR	ND	ND	ND	ND	ND
	SD	ND	2.98 (0.66)	ND	2.15 (0.29)	1.12 (0.61)
	SM	ND	2.72 (0.13)	ND	2.61 (0.16)	2.57 (0.94)

[a] Data are given as mean (standard deviations) of two batches. [b] Not detected.

Phenylethylamine and tryptamine, absent in fresh fish, remained undetected in marinated samples. In fact, these amines have been reported in fish only when the levels of other biogenic amines were very high (*22*). Spermine and spermidine were quite constant throughout the marinating process, showing average values of 2.63 ± 0.07 mg/kg for spermine and 2.08 ± 0.93 mg/kg for spermidine. These amines, regarded as physiological biogenic amines, are typically detected in fish because they are required for cellular growth (*33*) and are not related to bacterial spoilage (*34*).

Certain biogenic amines (octopamine, tyramine, putrescine, and cadaverine) were detected in the vinegar corresponding to just packed anchovies. The fact that putrescine and cadaverine levels decreased in fish flesh during the marinating process, in

Table 2. Concentrations (Milligrams per Kilogram) of Putrescine (PU), Cadaverine (CA), Phenylethylamine (PHE), Tryptamine (TR), Spermidine (SD), and Spermine (SM) in Fish and Vinegar during the Marinating Process of Anchovies Manufactured from Fresh (Batch F) and Spoiled (Batch S) Fish as Raw Material[a]

		batch F				batch S			
		0 days	3 days	8 days	14 days	0 days	3 days	8 days	14 days
PU	fish	2.07 (1.32)	2.60 (0.12)	2.61 (0.21)	2.56 (0.27)	47.89 (2.40)	16.75 (2.80)	15.34 (0.10)	14.52 (0.20)
	vinegar	*6.41 (0.01)*	*5.38 (0.58)*	*5.35 (0.59)*	*4.97 (0.40)*	*6.41 (0.02)*	*19.71 (1.02)*	*20.21 (0.50)*	*19.24 (1.22)*
	total	**8.48 (1.58)**	**7.98 (0.15)**	**7.96 (0.25)**	**7.53 (0.33)**	**54.3 (1.50)**	**36.46 (1.90)**	**35.55 (0.35)**	**33.76 (0.75)**
CA	fish	2.11 (1.49)	0.36 (0.16)	0.70 (0.26)	1.72 (0.99)	222.49 (6.70)	66.90 (3.22)	72.51 (5.25)	70.56 (5.25)
	vinegar	*0.15 (0.01)*	*0.88 (0.08)*	*1.60 (0.54)*	*2.92 (1.13)*	*0.15 (0.01)*	*107.59 (2.30)*	*104.14 (5.20)*	*136.23 (3.25)*
	total	**2.26 (1.70)**	**1.24 (0.19)**	**2.30 (0.32)**	**4.64 (1.22)**	**222.64 (3.50)**	**174.59 (3.70)**	**176.75 (3.25)**	**206.79 (4.77)**
PHE	fish	0.50 (0.25)	0.34 (0.01)	0.44 (0.50)	ND	19.83 (1.31)	8.10 (0.7)	7.52 (0.40)	6.52 (0.01)
	vinegar	*ND*[b]	*ND*	*ND*	*ND*	*0.16 (0.02)*	*8.07 (0.05)*	*8.59 (1.00)*	*8.79 (0.30)*
	total	**0.50 (0.25)**	**0.34 (0.01)**	**0.44 (0.50)**	**ND**	**19.19 (1.80)**	**16.17 (0.30)**	**16.11 (0.52)**	**15.31 (0.20)**
TR	fish	ND	ND	ND	ND	2.20 (0.11)	1.27 (0.01)	0.87 (0.04)	1.77 (0.07)
	vinegar	*ND*	*ND*	*ND*	*ND*	*ND*	*1.75 (0.02)*	*1.50 (0.05)*	*1.60 (0.06)*
	total					**2.2 (0.11)**	**3.02 (0.15)**	**2.37 (0.35)**	**3.37 (0.60)**
SD	fish	2.09 (0.38)	1.09 (0.27)	0.97 (0.57)	1.87 (0.27)	2.3 (0.31)	1.27 (0.03)	1.26 (0.20)	2.05 (0.30)
	vinegar	*ND*	*0.95 (0.45)*	*1.8 (0.01)*	*2.54 (0.01)*	*ND*	*3.25 (1.20)*	*3.09 (0.90)*	*2.30 (0.05)*
	total	**2.09 (0.38)**	**2.04(0.33)**	**2.77 (0.69)**	**4.41(0.38)**	**2.3 (0.31)**	**4.52 (0.60)**	**4.35 (0.55)**	**4.35 (0.90)**
SM	fish	3.46 (0.52)	2.36 (1.36)	2.31(0.23)	1.62 (0.43)	5.55 (0.01)	6.60 (0.01)	1.83 (0.21)	2.22 (0.06)
	vinegar	*ND*	*0.63 (0.01)*	*3.07 (1.68)*	*3.96 (0.13)*	*ND*	*1.10 (0.02)*	*5.56 (0.90)*	*2.03 (1.30)*
	total	**3.46 (0.52)**	**2.99 (1.65)**	**5.38 (0.27)**	**5.58 (0.52)**	**5.55 (0.01)**	**7.70 (0.50)**	**7.39 (0.15)**	**4.25 (0.70)**

[a] Data are given as mean (standard deviations) of two batches. [b] Not detected.

tandem with their increased levels in vinegar, suggests that this acidic medium acts as an extractive solvent.

During the storage of marinated products, increases in octopamine, tyramine, serotonin, putrescine, cadaverine, agmatine, and histamine levels were observed. These amines are commonly linked to fish spoilage with the exception of octopamine and serotonin, for which little information is available on fish products. Spermidine and spermine levels remained constant during storage. Although histamine levels increased during the storage of marinated anchovies (up to 1.37 $\pm$ 0.63 mg/kg), they were still much lower than the maximum values permitted by the EU for raw and ripened anchovies (*35*).

Secondary bacterial contamination during the filleting process is probably responsible for the appearance, or increase, of biogenic amines in marinated anchovies during their 3-month refrigerated storage. However, such increased biogenic amine levels were still considerably lower than those reported for salt-ripened anchovies, which had also been filleted, immersed in oil, and packed in glass jars (*36*, *37*).

The low levels of amines (both volatile and biogenic) detected in just-packed marinated products could reflect the extractive effect of vinegar and not only a low-level amine formation during the marinating process. To know which of these hypotheses is true, two laboratory trials were performed simulating the industrial marinating process. TMA-N, TVB-N, and biogenic amine levels during the marinating of anchovies were similar in duplicate trials ($P > 0.05$). Our results confirm that no formation of TMA-N and TVB-N occurs during anchovy marinating and demonstrate the effect of vinegar as a solvent extractor of these compounds (**Figure 2**). Consequently, high TMA-N or TVB-N levels should not be expected in anchovies marinated with vinegar. On the contrary, the presence of high TMA-N and TVB-N levels in vinegar can instead be linked to the use of poor freshness quality or spoiled fish as raw material (batch S). Thus, the volatile amine levels in vinegar from batches F (made from fresh anchovies of Extra quality) remained very low, consistent with the low levels detected in raw fish.

Biogenic amine levels in fish and vinegar, as well as total biogenic amine levels (as content in fish plus content in vinegar),

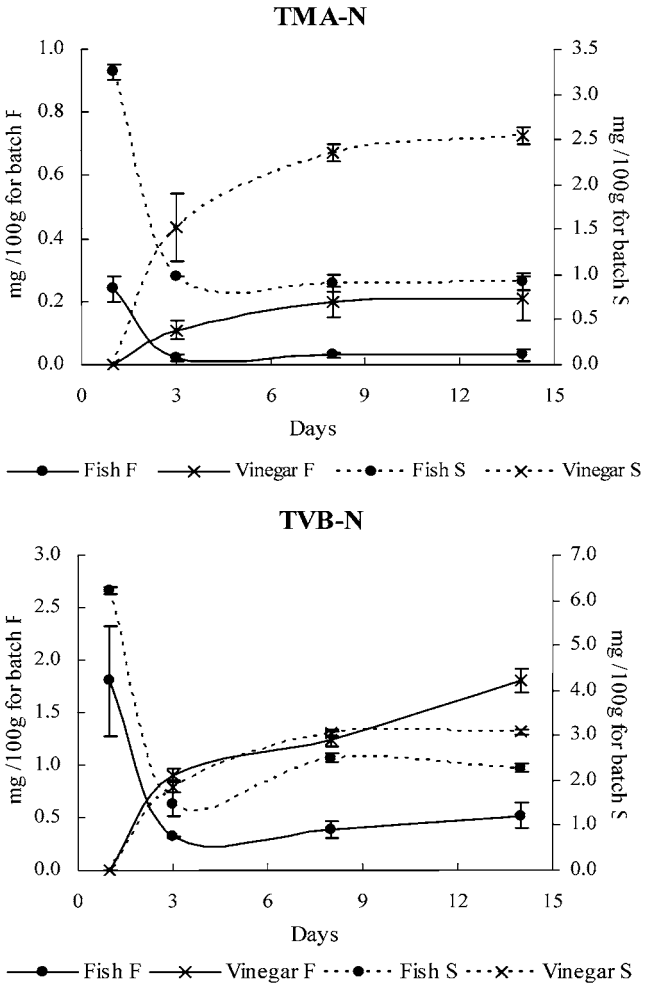

Figure 2. Evolution of TMA-N and TVB-N (milligrams per 100 g) in vinegar-marinated anchovies manufactured from fresh (batch F) and spoiled (batch S) fish as raw material.

detected in the laboratory trial samples are shown in **Tables 2** and **3**. Biogenic amine levels in batches F were very similar to

Table 3. Concentrations (Milligrams per Kilogram) of Octopamine (OC), Serotonin (SE), Histamine (HI), Tyramine (TY), and Agmatine (AG) in Fish and Vinegar during the Marinating Process of Anchovies Manufactured from Fresh (Batch F) and Spoiled (Batch S) Fish as Raw Material[a]

		batch F				batch S			
		0 days	3 days	8 days	14 days	0 days	3 days	8 days	14 days
OC	fish	ND[a]	ND	2.17 (0.23)	3.82 (0.96)	ND	0.89 (0.01)	2.77 (0.01)	3.53 (0.01)
	vinegar	*ND*	*0.06 (0.01)*	*1.80 (0.01)*	*3.15 (0.21)*	*ND*	*0.64 (0.03)*	*1.99 (0.01)*	*3.56 (0.02)*
	total		**0.06 (0.01)**	**3.97 (0.28)**	**4.97 (1.73)**		**1.53 (0.06)**	**4.76 (0.01)**	**7.09 (0.20)**
SE	fish	ND	1.90 (0.18)	5.22 (0.71)	9.87 (1.14)	ND	1.88 (0.05)	7.15 (0.02)	10.69 (1.14)
	vinegar	*ND*	*0.94 (0.36)*	*5.17 (0.32)*	*9.32 (0.03)*	*ND*	*1.86 (0.02)*	*5.14 (1.30)*	*7.93 (1.25)*
	total		**2.84 (0.20)**	**10.39 (0.62)**	**19.19 (1.39)**		**3.74 (0.40)**	**12.19 (1.20)**	**18.62 (0.95)**
HI	fish	ND	0.22 (0.02)	0.50 (0.04)	0.54 (0.05)	164.33 (8.60)	110.26 (1.00)	126.09 (4.10)	117.76 (4.00)
	vinegar	*0.51 (0.05)*	*0.62 (0.04)*	*0.69 (0.21)*	*0.48 (0.15)*	*0.51(1.00)*	*83.35 (2.30)*	*118.78 (2.50)*	*166.88(2.50)*
	total	**0.51 (0.05)**	**0.84 (0.01)**	**1.19 (0.03)**	**1.02 (0.01)**	**164.84 (1.20)**	**193.61 (1.35)**	**244.87 (3.30)**	**284.64 (4.25)**
TY	fish	0.74 (0.68)	1.55 (0.36)	3.43 (1.10)	7.81 (3.59)	98.67 (5.09)	63.64 (0.80)	57.13 (7.90)	55.43 (1.20)
	vinegar	*1.19 (0.02)*	*1.54 (0.28)*	*3.76 (1.45)*	*8.48(3.03)*	*1.19 (0.50)*	*51.95 (1.25)*	*52.37 (8.00)*	*71.18 (2.23)*
	total	**1.93 (0.80)**	**3.09 (0.45)**	**7.19 (1.35)**	**16.29 (4.39)**	**99.86 (3.20)**	**115.59 (2.30)**	**109.5 (6.25)**	**126.61 (4.20)**
AG	fish	3.59 (1.49)	1.10 (0.52)	1.55 (0.32)	1.82 (0.51)	153.83 (.5.50)	71.68 (2.20)	92.96 (6.50)	102.32 (1.70)
	vinegar	*0.02 (0.01)*	*1.20 (0.61)*	*1.53 (0.99)*	*2.08 (0.96)*	*0.02 (0.01)*	*86.97 (2.10)*	*96.37 (3.20)*	*139.6 (5.80)*
	total	**3.61 (2.27)**	**2.31 (0.62)**	**3.08 (0.38)**	**3.9 (0.63)**	**153.85 (2.30)**	**158.65 (2.20)**	**189.33 (7.20)**	**241.92 (4.65)**

[a] Data are given as mean (standard deviations) of two batches. [b] Not detected.

those found in the factory samples. Biogenic amine levels in batches S were very high, according to the sensory classification of anchovies used as raw material as inedible fish (class C). Cadaverine and histamine were the major amines in those samples, followed by agmatine, tyramine, and putrescine, whereas phenylethylamine and tryptamine were detected at lower amounts and octopamine and serotonin not at all. This amine profile matches the biogenic amine accumulation pattern reported for spoiled *E. encrasicholus* (*22*). As expected, spermidine and spermine levels in batches S were similar to those of batches F, due to their nonbacterial origin. Throughout the marinating process, the biogenic amine levels in fish increased, decreased, or remained quite constant, depending on the amine or the quality of fish used as raw material. However, biogenic amine levels in vinegar always increased throughout, demonstrating that vinegar effectively acts as an extractive medium for biogenic amines.

Taking into account total biogenic amine levels (amount in fish plus amount in vinegar), changes throughout marinating showed two evolution patterns: putrescine, cadaverine, phenylethylamine, tryptamine, spermidine, and spermine either slightly increased or remained quite constant (**Table 2**), whereas octopamine, serotonin, histamine, tyramine, and agmatine clearly increased (**Table 3**). Octopamine and serotonin increased independently of the state of the raw material. In batches F, serotonin and tyramine were the biogenic amines showing the highest formation as occurred in factory samples, whereas in batches S the highest formation was found for histamine and agmatine. Thus, the quality of the fish used as raw material affects not only the total amount of biogenic amines found in the end product but also their distribution pattern, with the major amine varying accordingly.

Percentages of total levels retained by fish and extracted by vinegar were calculated for each biogenic amine at the end of the marinating process (**Figure 3**). The percentages of biogenic amine retention in fish were very similar in both batches F and S, the extractive efficiency of vinegar proving to be the same regardless of the total amine level originally present. The percentage of biogenic amines extracted in vinegar ranged from 43 to 71%, the average value being ~55%. Concerning histamine, at the end of the marinating process nearly 60% of the total amount was found in the vinegar. Histamine is the only biogenic amine for which maximum levels in fish and fish

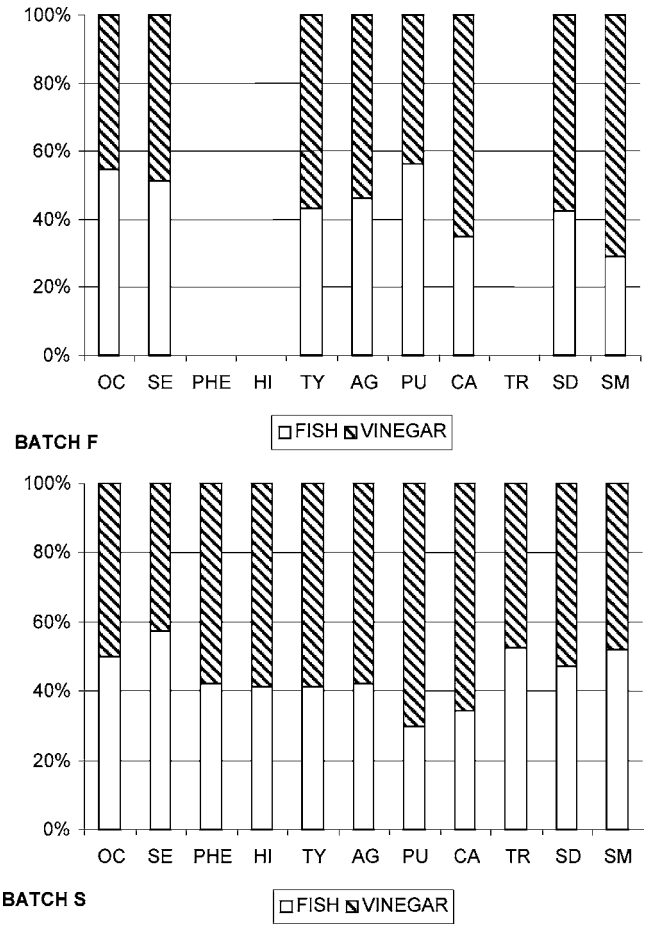

Figure 3. Distribution of biogenic amines between fish and vinegar detected at the end of the marinating process in anchovies manufactured from fresh (batch F) and spoiled (batch S) fish as raw material.

products are regulated by the EU, with a maximum average value of 100 mg/kg and nearly twice this value for certain salt-ripened fish products (*35*). There is, however, no maximum histamine level specified for derivatives obtained by marinating in vinegar.

To sum up, the results of this study suggest that the current limits of histamine in salt-ripening anchovies are not appropriate

for marinated anchovies. Taking into account the extractive effects of vinegar, the current 200 mg/kg maximum set for salt-ripened anchovies should be reduced for marinated anchovies. In addition, measurements of histamine and other biogenic amines in the vinegar of marinated fish samples could serve as a tool to assess the freshness of fish used as raw material, because these vinegar amine levels remained low in batch F. In a previous work (*38*), a biogenic amine index (BAI, as the sum of histamine, tyramine, putrescine, and cadaverine) was proposed to assess the freshness of anchovy, setting a threshold limit of acceptability at 15 mg/kg. On the basis of the present results, for the particular case of marinated anchovies, a limit of 30 mg/kg could be suggested. However, further studies are needed to study the suitability of this maximum in anchovies marinated in vinegar and even to consider the levels of biogenic amines in vinegar to assess the quality of raw fish used.

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