Biogenic Amines as Hygienic Quality Indicators of Tuna. Relationships with Microbial Counts, ATP-Related Compounds, Volatile Amines, and Organoleptic Changes

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Changes in 10 biogenic amines throughout tuna storage at 0, 8, and 20 °C were studied. ATP-related compounds, volatile amines, microbial counts, and organoleptic assessment were also monitored. No statistical differences were found for those parameters between samples from different anatomical areas. Similar evolution profiles were observed for biogenic amines at the three temperatures, although the highest amounts were achieved, in general, in samples stored at 20 °C. Histamine was the prevailing biogenic amine throughout storage, and the defect action level of 50 μg/g was surpassed in samples stored at 8 or 20 °C before organoleptic rejection. A great increase in cadaverine and tyramine and a slight increase in putrescine were also observed. Formation of histamine, tyramine, and cadaverine seems to be related to mesophilic flora, Enterobacteriaceae and coliforms. Hygienic quality estimation by means of trimethylamine values and IMP ratio yielded inconsistent results in samples corresponding to the limit of organoleptic acceptance. In accordance with the results of this work, we proposed for tuna assessment the use of an index calculated from the sum of the contents of histamine, tyramine, cadaverine, and putrescine which showed good correlations with both time of storage and organoleptic assessment.

Keywords: *Biogenic amines; histamine; tuna spoilage; ATP-related compounds; volatile amines*

INTRODUCTION

Histamine intoxication is probably the best known sanitary problem associated with the high content of biogenic amines in fish (Taylor, 1986). Numerous cases of this food poisoning are the result of consumption of scombroid fish such as tuna (Murray et al., 1982). Although the exact mechanism remains uncertain, a high histamine content in fish has long been considered the most probable cause of this type of food poisoning (Taylor, 1986; López-Sabater et al., 1996). However, some cases of food poisoning from fish with low contents of histamine (Murray et al., 1982) indicate that other substances might be involved, as histamine toxicity potentiators. Hui and Taylor (1985) found that potentiation of histamine by inhibition of histamine-metabolizing enzymes occurs only at cadaverine/histamine or putrescine/histamine ratios of 5/1 or greater. In addition, biogenic amines in food have other toxicological implications, such as hypertensive crises in patients being treated with monoamine oxidase drugs (Tailor, 1994) or suffering from migraine headaches (Crook, 1981). Finally, in the presence of nitrites, some biogenic amines may produce compounds that can be endogenous precursors of *N*-nitrosamines (Rogowski and Dhola, 1984).

Fish freshness is traditionally judged by sensory methods, but several chemical indices have also been proposed. One of them is the trimethylamine (TMA) content in marine fish. TMA could be formed from the trimethylamine oxide (TMAO) as a result of bacterial enzyme activity. Therefore, high levels of TMA would be indicators of the degree of bacterial spoilage. Dimethylamine (DMA) is also produced from TMAO, but by the enzyme activity of fish flesh. Only when bacterial growth is halted (e.g. by freezing) does the DMA content increase (Sotelo et al., 1995). The adenosine triphosphate (ATP) breakdown process, from ATP to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx), can reflect the first changes in fish before bacterial growth. However, the formation of Hx from HxR can be favored by bacterial activity, and so they can also be useful as a fish hygienic quality indicator. Maximum values for Hx (Gallardo, 1978) or minimum values for IMP (Hattula et al., 1993) have been proposed for fish rejection, but it is generally recognized that a ratio of catabolites rather than the measurement of a single compound is much less prone to fish-to-fish or to species-to-species variability (Price et al., 1991). The traditional *K* value does not take into account whether HxR or Hx is the dominant metabolite in a particular fish species. For assessment of inosine-forming species such as tuna, the IMP ratio (IMP/IMP + HxR + Hx) has been proposed (Fujii et al., 1973; Gill et al., 1987).

Biogenic amines can also be useful in estimating freshness or degree of spoilage of fish because these compounds are found at very low levels in fresh fish, and their formation is associated with bacterial spoilage (Fernández-Salguero and Mackie, 1987). A maximum average content of 100 μg/g has been established in the European Community for acceptance of tuna and other fish belonging to the Scombridae and Scomberesocidae families (Directiva CEE, 1991). Recently, the Food and Drug Administration (FDA) has revised the histamine defect action level to lower it from 100 to 50 μg/g and, in addition, it recommended the use of other scientific data to judge fish freshness, such as the presence of other biogenic amines associated with fish decomposition (FDA, 1995). Furthermore, biogenic amines can be

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useful as indicators of poor-quality raw material in preserved fish products because they are thermally stable compounds (Sims et al., 1992).

Several works about changes in histamine, putrescine, and cadaverine during storage and spoilage of fish belonging to the Scombridae family have been performed, but few data are available about changes in other biogenic amines. In this work we studied changes in 10 biogenic amines during the storage of tuna fish (*Thunnus thynnus*), which is largely consumed in Spain as fresh or canned fish. Three different anatomical areas of tuna were studied, because contradictory results exist about the histamine distribution in fish flesh. Lönberg (1980) and Frank et al. (1983) reported an uneven distribution of histamine, whereas Middlebrooks et al. (1988) reported similar increases of histamine, tyramine, and cadaverine contents for different anatomical zones of *Scomberomus maculatus*, a fish that also belongs to the Scombridae family. The aim of this work was to examine the effect of storage temperatures on levels of biogenic amines in tuna and to determine the prevailing biogenic amines formed and the relationship between the microbial flora developed and the biogenic amine levels. Special emphasis will put on finding the levels of histamine and other biogenic amines at the limit of the shelf life of fish. Therefore, other chemical spoilage indicators such as TMA-N and ATP-related compounds, commonly used for fish assessment, were also studied.

MATERIALS AND METHODS

Analytical Methods. 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 using a high-performance liquid chromatographic (HPLC) method based on ion-pair chromatographic partition in a C_{18} reversed phase column, involving a postcolumn reaction with *o*-phthaldehyde to form fluorescent derivatives with amines (Veciana-Nogués et al., 1995). Trimethylamine (TMA-N) and dimethylamine (DMA-N) values were obtained by gas−liquid chromatography using a glass colum packed with 200 cm of Carbowax 20M + 0.8% KOH on Carbopack B and a flame ionization detector according the procedure described by Veciana-Nogués et al. (1996a). ATP and related compounds (ADP, AMP, IMP, HxR, and Hx) were also determined by an ion-pair chromatographic method which used a C_{18} reversed phase column and UV detection according to the method proposed by Veciana-Nogués et al. (1997). All chromatographic methods used in this work were statistically validated in terms of linearity, precision, recovery, and sensitivity.

Organoleptic fish assessment was performed by six trained panelists on the basis of the Soudan score (Sainclivier, 1983) as modified by López-Sabater et al. (1996). The appearance of the skin and peritoneum, slime on the skin, general odor, and texture and color of the muscle were scored. The score ranged from 1 for the best quality to 6 for very poor quality, and samples were rejected when a mean value of 3 or higher was obtained.

Counts of mesophilic flora, psychrotrophic flora, Enterobacteriaceae, total coliforms, and histamine-forming bacteria were performed according to the procedures described by López-Sabater et al. (1996).

Sampling. Fresh tuna (45 kg) belonging to the *T. thynnus* species was obtained in the wholesale fish market of Barcelona and immediately transported to the laboratory in crushed ice. Fish was divided into three anatomical zones (anterior, middle, and posterior). Each anatomical zone was divided into three portions (approximately 10−15 cm thick, 3−4 kg weight, including skin), which were placed in separate sterile polyethylene bags (Eurotube). One piece from each anatomical area was stored at 0, 8, and 20 °C, for 21, 9, and 4 days, respectively. Chemical and microbiological analysis and sensory assessment were made at zero time, every 3 days in portions stored at 0 °C, daily in portions stored at 8 °C, and every 12 h in those stored at 20 °C.

Statistical Analysis. All statistical tests were performed using the statistical package SYSTAT (Systat, Inc., Evanston, IL). One-way ANOVA for repeated measures was carried out to study differences in relation to the anatomical area, and regression analyses were used to study the suitability of biogenic amines as fish quality indicators.

RESULTS AND DISCUSSION

Results of this work were expressed as the average values obtained from samples corresponding to the three anatomical areas (anterior, middle, and posterior), because no statistical differences ($p > 0.05$) were found in ATP-related compounds, TMA-N, DMA-N, biogenic amines, microbial counts, and organoleptic values among the three anatomical zones in the tuna studied.

In samples from zero time contents of ATP, ADP, and AMP were <0.05 μmol/g. IMP, HxR, and Hx were the main ATP-related compounds found, showing contents of 4.65 ± 0.70 μmol/g, 3.33 ± 1.01 μg/g, and 0.23 ± 0.01 μmol/g, respectively. In general, IMP is the main ATP-related compound in fresh fish since ATP, ADP, and AMP can only be found at relatively high levels after killing of fish with minimal struggle (Izquierdo-Pulido et al., 1992). The content of TMA-N was 0.9 ± 0.05 mg of N/100 g of fish at zero time and, according Castell's classical criterion, samples should be graded as excellent (Sotelo et al., 1995). Mesophilic flora was 3.7 ± 0.2 log CFU, and psychrotrophic flora was 4.0 ± 0.1 log CFU, whereas Enterobacteriaceae, coliforms, and histamine-forming bacteria were not found. Therefore, samples were judged to be fresh on the basis of their microbiological counts and also on the basis of sensory properties showing a Soudans value of 0.

SD and SM were the prevailing biogenic amines found in fresh tuna samples from zero time. PU and CA were also found, but at very low levels (<0.5 μg/g), and the other biogenic amines were not detected. Both SD and SM are ubiquitous in fish flesh (Ababouch et al., 1991) since they play an important role in both vegetal and animal cellular growth (Bardocz, 1995). PU and CA are biogenic amines commonly related to fish spoilage (Middlebrooks et al., 1988); however, low levels of those amines and especially of PU have also been reported as natural amines in animal and vegetal tissues (Bardocz, 1995).

Throughout the time storage, IMP decreased, reaching very low values at the end of the study at the three temperatures (Figure 1). Contents of HxR increased during ca. 1.5 days in samples stored at 20 °C, ca. 3 days in samples stored at 8 °C, and ca. 9 days in those stored at 0 °C. Tuna is traditionally classified as an inosine-forming fish and, therefore, HxR should be the main ATP-related compound found in spoiled samples. However, contents of Hx in samples stored at 8 and 20 °C were higher than those of HxR after 5 and 2 days of storage, respectively. Only at 0 °C were the contents of HxR always higher than those of Hx. In addition, although significative ($p < 0.001$) correlations between IMP ratio and time of storage were obtained for samples stored at the three studied temperatures, the coefficients of correlation were slightly better in samples stored at 0 °C ($r = 0.9833$) than in samples stored at 8 or 20 °C ($r = 0.9322$ and 0.9221, respectively). The bacterial growth, which is more favored at 8 and 20 °C than at 0 °C, seems to be related to the increase of the

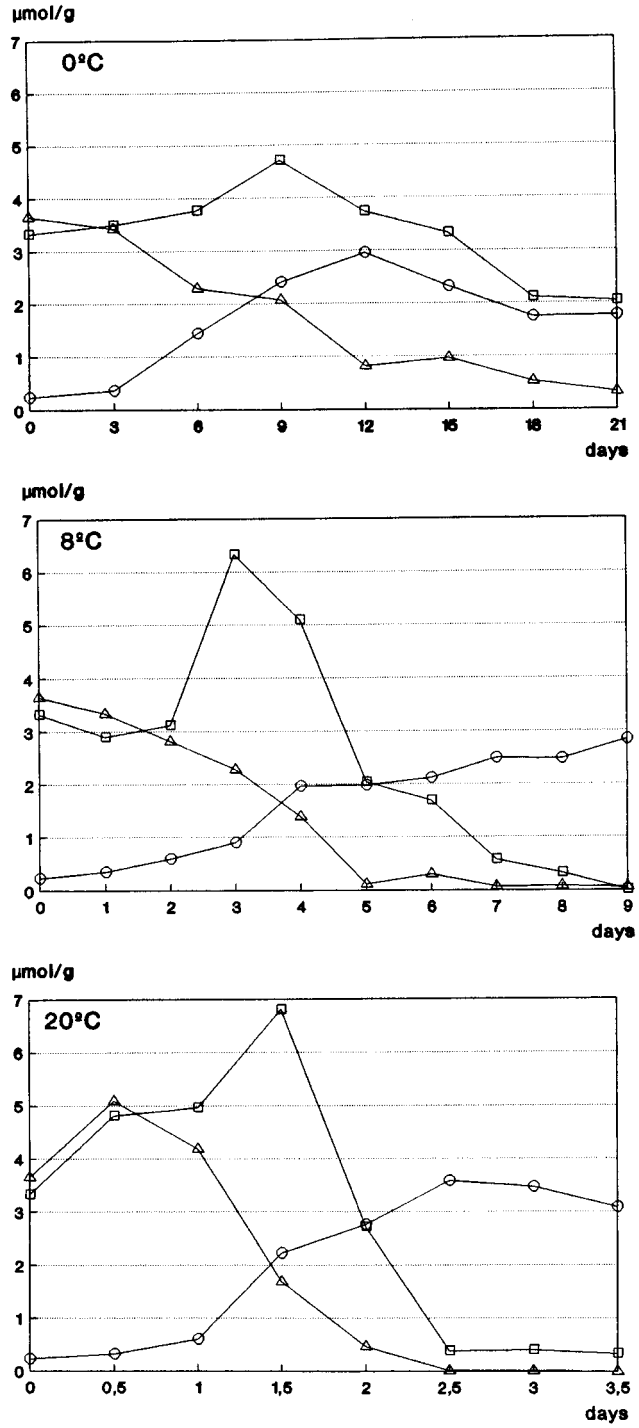

Figure 1. Changes of IMP (△), HxR (□), and Hx (○) in tuna samples stored at 0, 8, and 20 °C.

formation of Hx from HxR (Gallardo, 1978; Fletcher and Stathan, 1988).

Formation of the TMA has been traditionally more related to psychrotrophic bacteria than to mesophilic bacteria (Huss, 1988). The increase in TMA-N throughout the time of storage was higher and earlier in samples stored at 20 °C than in samples at 8 or 0 °C (Figure 2). In contrast, contents of DMA-N remained constant throughout the time, showing similar average values at the three studied temperatures (1.1 ± 0.2, 1.9 ± 0.4, and 1.6 ± 0.1 mg of N/100 g for samples stored at 0, 8, or 20 °C, respectively). TMA-N results from bacterial enzyme activity, whereas increases of DMA-N result from the enzyme activity of fish muscle (Huss, 1988). Our results confirmed that bacterial growth did not modify DMA contents.

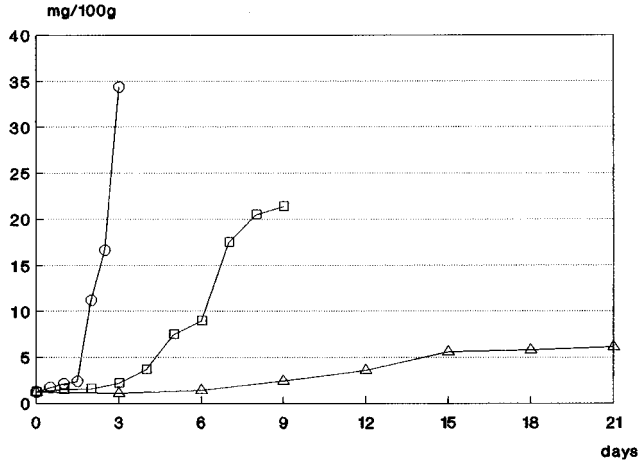

Figure 2. Changes of TMA-N in tuna samples stored at 0 (△), 8 (□), and 20 °C (○).

Counts of mesophilic flora, psychrotrophic flora, Enterobacteriaceae, coliforms, and histamine-forming bacteria increased throughout tuna spoilage. Only in samples stored at 20 °C did the counts of mesophilic flora reach the value of 8 log CFU at the end of the study, surpassing the level of 7 log CFU which has been reported as limit for fish rejection (ICMSF, 1974). In samples stored at 0 and 8 °C mesophilic flora reached counts of 5.6 log CFU and 6.5 log CFU, respectively. Increases of Enterobacteriaceae and coliforms were also higher in samples stored at 20 °C than in samples stored at 8 °C, but those microorganisms were not found in samples stored at 0 °C. In contrast, the increases of psychrotrophic flora throughout storage time were similar at the three temperatures, reaching ca. 8 log CFU at the end of the study. Histamine-forming bacteria, which were not detected in samples from zero time, were found after 12 h of tuna storage at 20 °C, after 2 days at 8 °C, and after 3 days at 0 °C. It should be noted that histamine formation in samples stored at 8 °C was earlier than the detection of histamine-forming bacteria.

Changes in biogenic amines were different depending on the amine, but were relatively similar for each amine at the three studied temperatures. Contents of SD remained constant during storage, and similar values were observed at the three studied temperatures, showing an average value of 5.44 ± 1.5 µg/g. Contents of SM decreased slightly from the initial value of 14.6 ± 2.1 to 10.2 ± 1.9 µg/g after 21 days of storage at 0 °C, to 10.9 ± 2.0 µg/g after 9 days of storage at 8 °C, and to 12.2 ± 2.1 µg/g on the fourth day at 20 ± 1 °C. Therefore, tuna spoilage had little influence on SD and SM contents. Similar results in tuna and other pelagic fish species were reported by Fernández-Salguero and Mackie (1987), Ababouch et al. (1991), and Veciana-Nogués et al. (1996b).

Changes of AG showed a singular profile. First, the content of AG increased from nondetected to a maximum level of ca. 6, 11, and 13 µg/g after 9, 5, and 0.5 days of storage at 0, 8, and 20 °C, respectively. Then, contents decreased until reaching nondetected levels at the three temperatures at the end of the study (Figure 3). No previous data were found about the AG evolution during tuna storage or spoilage studies, but similar profiles for AG contents have been reported during storage of other pelagic fish species, such as mackerel,

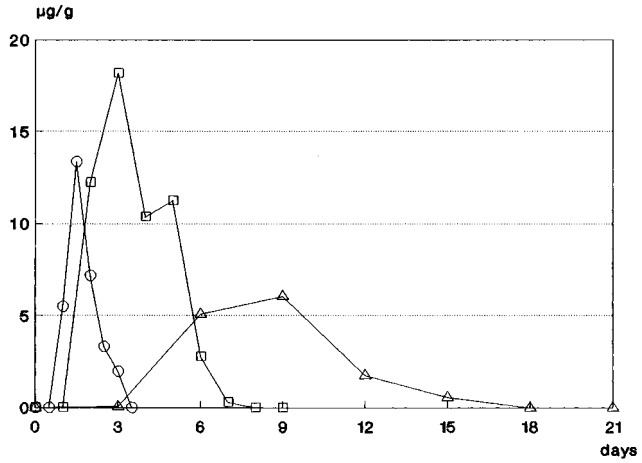

Figure 3. Changes of agmatine in tuna samples stored at 0 (△), 8 (□), and 20 °C (○).

Table 1. Contents (Micrograms per Gram of Fish) of Histamine (HI), Tyramine (TY), β-Phenylethylamine (PHE), Putrescine (PU), and Cadaverine (CA) in Tuna Samples Stored at 0 °C

time (days)	amine contents[a]				
	HI	TY	PHE	PU	CA
0	nd[b]	nd	nd	0.4 ± 0.1	0.2 ± 0.1
3	0.1 ± 0.2	nd	nd	0.3 ± 0.1	0.2 ± 0.1
6	0.9 ± 0.2	0.7 ± 0.1	nd	0.5 ± 0.2	1.0 ± 6.0
9	4.9 ± 5.9	1.3 ± 0.5	nd	1.3 ± 0.2	4.1 ± 2.0
12	19.9 ± 11.9	5.7 ± 1.8	nd	1.5 ± 1.2	13.8 ± 2.0
15	134.6 ± 29.0	5.5 ± 2.8	nd	4.6 ± 1.4	15.1 ± 3.5
18	93.3 ± 4.8	9.4 ± 1.8	nd	5.3 ± 1.8	19.1 ± 7.5
21	107.7 ± 6.1	13.8 ± 3.9	nd	5.2 ± 1.9	24.4 ± 6.6

[a] Mean value ± standard deviation obtained from the three anatomical areas studied. [b] Not detected.

Table 2. Contents (Micrograms per Gram of Fish) of Histamine (HI), Tyramine (TY), β-Phenylethylamine (PHE), Putrescine (PU), and Cadaverine (CA) in Tuna Samples Stored at 8 °C

time (days)	amine contents[a]				
	HI	TY	PHE	PU	CA
0	nd[b]	nd	nd	0.4 ± 0.1	0.2 ± 0.1
1	0.5 ± 0.8	nd	nd	0.4 ± 0.1	0.2 ± 0.1
2	11.5 ± 6.3	1.5 ± 0.2	nd	0.6 ± 0.2	6.2 ± 0.8
3	52.0 ± 23.3	3.7 ± 0.2	nd	1.6 ± 0.4	16.0 ± 4.8
4	103.6 ± 10.9	7.0 ± 0.7	nd	2.2 ± 0.9	29.0 ± 0.1
5	110.1 ± 29.9	9.1 ± 16.9	nd	2.3 ± 1.3	28.8 ± 0.2
6	350.7 ± 17.2	16.1 ± 5.1	nd	4.5 ± 1.6	46.5 ± 6.4
7	505.5 ± 30.0	16.1 ± 3.8	0.8 ± 0.8	5.2 ± 1.3	52.4 ± 6.4
8	908.1 ± 2.1	25.0 ± 4.4	1.6 ± 1.1	3.5 ± 1.7	54.1 ± 4.3
9	3681.9 ± 88.4	32.5 ± 8.5	6.9 ± 7.2	11.1 ± 1.2	56.2 ± 2.7

[a] Mean value ± standard deviation obtained from the three anatomical areas studied. [b] Not detected.

saury pike, or anchovies (Yamanaka and Matsumoto, 1989; Veciana-Nogués et al., 1996b).

Great changes in contents of PU, CA, TY, and HI were observed throughout the storage of tuna at the three temperatures (Tables 1−3). Similar evolution profiles were observed for those amines at the three temperatures, but the amine increases were great when storage was at high temperature. SE and TR were not detected in any sample and PHE, which was also not detected in samples kept at 0 °C, appeared after 2.5 days at 20 °C and after 7 days at 8 °C. Contents of PHE increased progressively throughout the time, but levels reached at the end of the study were much lower than HI, PU, CA, and TY. Histamine was the prevailing biogenic amine during tuna storage at 0, 8, or 20 °C. Formation of CA was higher than that of TY, and formation of TY was higher than that of PU. A similar order for biogenic amine formation was observed by Nagayama et al. (1985) during spoilage of tuna stored at room temperature. HI was also the prevailing biogenic amine found during spoilage of sardine (Ababouch et al., 1991), mackerel (Okuzumi et al., 1990), and anchovy (Veciana-Nogués et al., 1996b), whereas in other pelagic fish species the formation of PU or CA during spoilage was similar or higher than the formation of HI (Nagayama et al., 1985; Fernández-Salguero and Mackie, 1987; Middlebrooks et al., 1988). No decreases were observed for HI, PU, CA, and TY when samples were stored at 0 or 8 °C, but great decreases in HI and CA were observed after 3 days of storage at 20 °C, when fish samples were very spoiled. Decreases of HI in advanced fish spoilage were previously reported and should be linked to growth of microorganisms with histaminolytic activity (Schulze and Zimmerman, 1982). No data were found about CA decreases during storage or spoilage of any species of fish.

Table 3. Contents (Micrograms per Gram of Fish) of Histamine (HI), Tyramine (TY), β-Phenylethylamine (PHE), Putrescine (PU), and Cadaverine (CA) in Tuna Samples Stored at 20 °C

time (days)	amine contents[a]				
	HI	TY	PHE	PU	CA
0	nd[b]	nd	nd	0.4 ± 0.1	0.2 ± 0.1
0.5	2.1 ± 2.2	nd	nd	0.3 ± 0.1	0.4 ± 0.2
1	20.6 ± 5.6	2.8 ± 0.5	nd	0.3 ± 0.1	14.7 ± 2.8
1.5	924.3 ± 20.9	8.4 ± 3.8	nd	1.2 ± 0.2	29.9 ± 5.8
2	3103.2 ± 48.0	15.4 ± 1.1	nd	3.0 ± 0.7	44.2 ± 5.8
2.5	6549.2 ± 33.5	17.5 ± 1.1	2.6 ± 2.2	3.3 ± 0.5	80.8 ± 15.6
3	6869.3 ± 40.7	17.1 ± 1.2	8.1 ± 0.6	4.5 ± 1.4	108.4 ± 34.4
3.5	983.4 ± 210.2	25.9 ± 5.0	17.6 ± 5.7	5.6 ± 2.8	64.5 ± 3.3

[a] Mean value ± standard deviation obtained from the three anatomical areas studied. [b] Not detected.

Frank et al. (1985) reported that the biogenic amine formation is more related to the activity of mesophilic than psychrotrophic bacteria, which could explain the more extensive formation of those amines at 20 °C. However, according to our results, it should be emphasized that formation of TY, CA, and PU can also occur in samples stored at 0 °C. In this work HI contents in samples stored at 8 or 20 °C soon exceeded the defect level of 50 µg/g, which indicates that potential toxicological hazard could occur if cold chain networks are broken during commercial distribution of tuna. Formation of HI, TY, and CA seems to be related to mesophilic flora, Enterobacterioaceae, and coliforms since the highest amine contents were found in samples that also showed the highest counts of those microorganism ($r > 0.660$, $p < 0.001$). Formation of PU cannot be clearly related with mesophilic flora because the increases of this amine were similar at the three temperatures.

According to the Soudan score, the limit for organoleptic acceptance of fish is the value of 3. In tuna samples this value was reached after 12 days of storage at 0 °C, after 5 days at 8 °C, and after 1.5 days at 20 °C. Table 4 shows the values obtained for the analytical parameters studied in this work, as well as the microbial counts, in tuna samples after days of storage necessary to exceed the value of 3 according to the Soudans score. Contradictory results for tuna assessment were obtained depending on the parameter considered, and differences were also observed among samples stored at different temperatures. Thus, samples stored at 0 or 20 °C showed an IMP ratio very close to the range of 0.153−0.114 proposed by Fujii et al. (1973) as minimum value for fish acceptance, whereas samples

Table 4. Microbial Counts and Chemical Indicators in Tuna Samples at the Limit of Organoleptic Acceptance (Soudan Value of 3)

	0 °C, 12 days of storage	8 °C, 5 days of storage	20 °C, 1.5 days of storage
IMP ratio[a]	0.102	0.029	0.1584
TMA-N (mg/100 g)	3.5 ± 0.5	7.4 ± 0.5	2.3 ± 0.6
histamine (μg/g)	19.9 ± 11.9	110.1 ± 29.9	924.3 ± 20.9
tyramine (μg/g)	5.7 ± 1.8	9.1 ± 16.9	8.4 ± 3.8
putrescine (μg/g)	1.5 ± 1.2	2.3 ± 1.3	1.2 ± 0.2
cadaverine (μg/g)	13.8 ± 2.0	28.8 ± 0.2	29.9 ± 5.8
mesophilic flora (log CFU)	5.7 ± 0.1	6.6 ± 0.1	6.6 ± 0.2
psychrotrophic flora (log CFU)	8.1 ± 0.3	7.9 ± 0.8	7.0 ± 0.2
Enterobacteriaceae (log CFU)	1.4 ± 0.2	4.2 ± 0.2	4.1 ± 0.4
coliforms (log CFU)	nd[b]	nd	2.9 ± 0.5
histamine-forming bacteria (log CFU)	1.1 ± 0.4	1.5 ± 0.6	1.6 ± 0.1
Mietz and Karmas index[c]	2	6.7	51.0
biogenic amine index[d]	43.3	150.3	963.9

[a] IMP/IMP + HxR + HX. [b] Not detected [c] HI + PU + CA/(1 + SD + SM). [d] HI + CA + TY + PU.

stored at 8 °C showed a much lower value and would be clearly rejected. TMA-N values in samples with a Soudan value of 3 suggested that the classical TMA-N levels for fish rejection, ranging from 5 to 10 mg of TMA-N/100 g of fish (Ludorff and Meyer, 1978), should be revised for tuna, since the value of 10 was not exceeded at any temperature and only in samples kept at 8 °C was the TMA-N content >5 mg of TMA-N/100 g of fish.

Regarding biogenic amines, PHE was not detected in any sample before the organoleptic rejection, indicating that its presence was related only to advanced stages of tuna spoilage. The particular evolution of AG contents, which began to decrease before the limit of organoleptical acceptance, implies that AG could only be used as a freshness indicator in the first stages of tuna storage. Contents of HI in samples stored at 8 and 20 °C were, at the limit of the organoleptic acceptance, much higher than the maximum average content of 50 μg/g proposed by the FDA and also higher than the level of 100 μg/g established by European regulations. In contrast, mesophilic flora counts were lower than the maximum value of 7 log CFU proposed for fish rejection (ICMSF, 1974) on samples stored at the three temperatures studied. Counts of histamine-forming bacteria were similar at the three temperatures studied, although great differences were observed in HI formation when samples were stored at different temperatures. Thus, according to our results histamine formation not only depends on these particular microbial counts but seems to be mainly dependent on the storage temperature.

The biogenic amine index of Mietz and Karmas (1977) is the more classic criterion to evaluate fish spoilage on the basis of biogenic amine contents. The value of 10 proposed by Mietz and Karmas as the limit of fish acceptability was not reached in samples stored at 8 °C at the end of their organoleptic acceptance. Also, the content of HI in those samples was above 100 μg/g, which is higher than allowed by both FDA and CEE regulations. In addition, the results of this work and also those of previous papers (Nagayama et al., 1985; Abadouch et al., 1991; Veciana-Nogués et al., 1996b) suggested that it would be advisable to also monitor contents of TY, which are not included in the Mietz and Karmas index. For those reasons, we proposed the use of the values obtained from the sum HI + CA + TY + PU as a biogenic amine index (BAI). Table 5 summarizes the results of correlation studies between time of storage or organoleptic value and the Mietz and Karmas index or the index proposed by us. Results showed that at the three studied temperatures r values obtained and their statistical significance were slightly higher when the sum of HI, TY, PU, and CA was used. The value of 50 μg/g for HI + CA + TY + PU in BAI was not exceeded in samples stored at 0 °C before organoleptic rejection and, therefore, according to these results it could be a guiding limit value for tuna acceptance. However, it is necessary to carry out more studies to eventually corroborate this limit, because the appearence of fish spoilage is a function of many variables such as handling procedures and spoilage flora.

Table 5. Coefficient of Correlation (r) and of Determination (r^2) between Time of Storage or Soudan Value and Mietz and Karmas Index or the Biogenic Amine Index

temp (°C) of storage (n)	Mietz and Karmas index[a]				biogenic amine index[b]			
	time		SV[c]		time		SV	
	r	r^2	r	r^2	r	r^2	r	r^2
0 (24)	0.886[d]	0.786	0.685	0.469	0.889	0.791	0.877	0.769
8 (30)	0.668	0.446	0.740	0.547	0.702	0.493	0.784	0.615
20 (24)	0.624	0.385	0.723	0.522	0.626	0.392	0.783	0.614

[a] HI + PU + CA/(1 + SD + SM). [b] HI + CA + TY + PU. [c] Soudan's value. [d] Correlation coefficient always showed a $p < 0.001$.

Few data are available in the literature for TY during fish spoilage, and no data on the evolution of TY when tuna is stored at refrigeration or frozen temperatures have been previously reported. We note the importance of considering the levels of TY since high contents of this amine were often related to some toxicological problems. Intakes of TY >100−125 mg have been related with migraine headaches (Crook, 1981; Forsythe and Redmond, 1974), and severe hypertensive crises linked to interaction with classical MAOI drugs have been reported when the total ingestion of TY was >6 mg (Tailor, 1994). According to our results, the consumption of 150 g of tuna stored at refrigeration temperature (8 °C), after 2 days of storage, could provoke hypertensive crises in patients under classical MAOI drug therapy.

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