Biogenic Amines in Fresh and Canned Tuna. Effects of Canning on Biogenic Amine Contents

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The concentrations of 10 biogenic amines were determined in fresh tuna ($n = 20$) and canned tuna ($n = 38$) purchased in Spain and also in samples taken at 5 stages of 12 canning processes ($n = 60$). Spermine and spermidine were the only amines found in all samples but were lower in canned than in fresh tuna samples ($p < 0.05$). The Food and Drug Administration legal limit for histamine (50 μg/g) was not exceeded in any samples, and the levels of the other biogenic amines commonly related with fish spoilage were, in general, low in all samples studied; no significant difference was found between fresh and canned tuna. Results showed that both the inosine monophosphate ratio and certain biogenic amines could be useful as quality indicators for the raw fish used in canned tuna, since no change was observed throughout the canning process, except for spermine and spermidine. Decreases in both spermine and spermidine were significant only after the sterilization step, which is in agreement with the significant differences observed between fresh and canned tuna from the market.

Keywords: *Histamine; biogenic amines; fresh tuna; canned tuna*

INTRODUCTION

Biogenic amines can occur in a wide variety of foods such as cheese, meat, fishery products, wines, beer, or other fermented foods (Stratton et al., 1991; Yen and Hsieh, 1991; Izquierdo-Pulido et al., 1994; Hernández-Jover et al., 1996). Biogenic amines have been defined as aliphatic, alicyclic, and heterocyclic organic bases of low molecular weight. These substances are ubiquitous in biological materials; they are not only biosynthesized in animal and vegetal cells, but they are also produced by microbial decarboxylation enzymes (Stratton et al., 1991). Biogenic amines are either psychoactive or vasoactive substances, which may cause complications in some individuals (Yen and Hsieh, 1991). Various toxicological implications for biogenic amines in foods have been reported such as histamine intoxication (Hwang et al., 1995), migraine headaches (Joosten, 1988), or hypertensive crises in patients under monoamine oxidase inhibitor (MAOI) drug therapy (Tailor, 1994). Histamine intoxication, which was initially named scombroid poisoning, is probably the best known sanitary problem associated with biogenic amines in fish. Histamine is the main compound responsible for this intoxication, but other biogenic amines such as putrescine or cadaverine increase histamine toxicity (Stratton et al., 1991).

Biogenic amines can also be used to estimate freshness or degree of spoilage of fish, because they are found at very low levels in fresh fish and their presence is related with bacterial spoilage (Fernandez-Salguero and Mackie, 1987). To evaluate fish freshness, Yamanaka et al. (1989) proposed the use of cadaverine; Mietz and Karmas (1977) proposed an index based on histamine, putrescine, cadaverine, spermine, and spermidine contents, whereas Veciana-Nogues et al. (1997a) suggest monitoring the content of histamine, tyramine, cadaverine, and putrescine. However, histamine is the only biogenic amine for which maximum legal levels have been established for fish. Thus, 100 μg/g is the maximum average level established in the European Union for tuna and other fishes of the Scombridae and Scomberesocidae families (CEE, 1991). More recently, the Food and Drug Administration (FDA) has lowered the histamine defect action level from 100 to 50 μg/g and has recommended the use of other biogenic amines related with spoilage in fish evaluation (FDA, 1995).

The relationship between increase in some biogenic amines such as histamine, putrescine, cadaverine, or tyramine and fish spoilage has also been reported (Nagayama et al., 1985; Veciana-Nogués et al., 1996). Changes in biogenic amine contents during canning were not clear. It seems to be accepted that histamine is stable under the conditions used in the sterilization process for canned fish (Warne, 1985). Therefore, the concentration of this histamine in canned fish would be that of the raw fish prior to the sterilization process. However, changes of biogenic amines during canning were also reported. Thus, Pan (1985) reported that histamine formation could take place during the heating stage of the sterilization process, whereas Fernández Salguero and Mackie (1987) suggested that some losses of amines might be expected in drained liquors during the precooking step. Likewise, Sims et al. (1992) reported that the conditions of the precooking and the subsequent heat processing of canned products significantly lowered histamine, putrescine, and cadaverine.

Other compounds such as trimethylamine or adenosine triphosphate (ATP) related compounds have also been extensively used in fresh fish freshness assessment. Among them, hypoxanthine (Hx) has been proposed as a quality indicator of raw materials in canned fish because of its heat stability (Gallardo, 1978). Inosine monophosphate (IMP) and inosine (HxR) were also reported to be relatively stable at sterilization temperatures and, together with Hx, they have been suggested for the quality assessment of canned fish (Tokunaga et al., 1982; Gill et al., 1987). Several indices

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S0021-8561(97)00092-7 CCC: $14.00 © 1997 American Chemical Society

Table 1. Contents of Biogenic Amines (Micrograms per Gram) in Fresh and Canned Tuna Samples from Spain

	SD	SM	HI	TY	SE	AG	CA	PU	PHE	TR
fresh tuna	1.20−11.70[a]	7.30−37.00	nd[d]−9.50	nd−10.65	nd−3.65	nd−17.70	nd−9.53	nd−4.84	nd−1.70	nd−5.85
(n = 20)	6.82[b]*	22.35*	0.15*	0*	2.73*	1.75*	0.70*	0.27*	0.01*	0.01*
	(7.48)[c]	(18.04)	(1.98)	(0.27)	(2.73)	(4.21)	(1.81)	(0.85)	(0.20)	(0.01)
canned tuna	1.50−9.95	2.23−35.20	nd−40.5	nd−3.00	nd−8.40	nd−10.40	nd−12.05	nd−2.20	nd−7.3	nd−12.9
(n = 38)	4.02**	10.80**	0.42*	0*	0**	0**	0.62*	0.22*	0.00*	0.00*
	(2.65)	(7.30)	(1.55)	(0.23)	(2.42)	(1.12)	(1.21)	(0.80)	(0.03)	(0.00)

[a] Range. [b] Median; values in the same column bearing the same number of asterisks are not different ($p > 0.05$). [c] Deviation quartile. [d] nd, not detected (<0.25 μg/g for HI, PU, CA, AG, and PHE and <1 μg/g for SD, SM, TY, SE, and TR).

Table 2. Contents of Biogenic Amines (Micrograms per Gram) throughout Canning Process (n = 12)

	raw fish	before cooking	after cooking	after packing	end product
HI	0.32 (0.62)[a*]	0.55 (1.44)*	0.40 (0.65)*	0.54 (0.90)*	0.63 (0.83)*
TY	0.32 (0.67)*	0.08 (0.18)*	0.24 (0.63)*	0.17 (0.35)*	0.15(0.24)*
SE	1.80 (1.44)*	2.08 (2.06)*	2.32 (2.35)*	2.05 (2.42)*	1.80 (1.41)*
AG	0.94 (0.77)*	0.17 (0.44)*	0.15 (0.36)*	0.32 (0.53)*	0.40 (0.29)*
CA	0.25 (0.45)*	0.17 (0.31)*	0.33 (0.64)*	0.19 (0.32)*	0.21 (0.29)*
PU	0.29 (0.51)*	0.22(0.28)*	0.27 (0.13)*	0.13 (0.20)*	0.32 (0.57)*
PHE	nd[b]	nd	0.10 (0.30)	0.27 (0.90)	nd
TR	nd	0.11 (0.38)	0.12 (0.80)	0.01 (0.20)	0.12 (0.90)
SD	5.10 (1.74)*	4.31 (1.34)*	3.46 (1.21)*	3.51 (1.72)*	2.82 (1.29)**
SM	14.25(5.91)*	19.91 (5.55)*	11.89 (3.34)*	12.89 (3.65)*	8.32 (2.67)**

[a] Mean value and (standard deviation); values in the same column bearing the same number of asterisks are not different ($p > 0.05$); statistical comparisons were not performed for PHE and TR because these amines are found in only a low percentage of samples. [b] nd, not detected (<0.25 μg/g for HI, PU, CA, AG, and PHE and <1 μg/g for SD, SM, TY, SE, and TR).

obtained from contents of ATP-related compounds have been proposed for fish assessment. For tuna, which is an inosine-forming species, the use of the IMP ratio [IMP/(IMP + HxR + Hx)] seems to be the most appropriate (Gill et al., 1987).

The purpose of this study was to determine the concentration of 10 biogenic amines (histamine, tyramine, serotonin, β-phenilethylamine, tryptamine, putrescine, cadaverine, agmatine, spermine, and spermidine) in fresh and canned tuna samples available in the Spanish market, because, except for histamine, few data are available about the usual levels of biogenic amines in these products. In addition, the contents of biogenic amines together with some microbial counts and the contents of IMP, HxR, and Hx were determined in samples corresponding to different steps of the canning process to elucidate the potential usefulness of biogenic amines as quality indicators of raw fish used for canning.

MATERIALS AND METHODS

Samples. *(a) Market Samples.* Fresh tuna samples (n = 20) were obtained from several retail outlets in Barcelona, and canned tuna samples (n = 38) were received from different Spanish commercial brands. Fresh tuna samples were transported in ice to our laboratory and immediately analyzed after their collection.

(b) Canned Tuna Process. Sixty samples belonging 12 different batches of canned tuna packed in oil were obtained directly from a manufacturer in northwestern Spain. Tuna fish was caught in the Pacific ocean, frozen immediately, and transported at −12 °C to the processing plant. Each batch included samples from different steps of the canning process: (A) frozen tuna (raw material); (B) before cooking; (C) after cooking; (D) after packing, before oil addition; and (E) after sterilization step (end product). All samples were taken in duplicate, frozen at the factory using liquid nitrogen, shipped by plane in a liquid nitrogen container, and, at the laboratory, stored at −20 °C until analysis.

Analytical Methods. Biogenic amines were determined by high-performance liquid chromatography (HPLC), after extraction with 0.6 N perchloric acid according to the method of Veciana-Nogués et al. (1995). ATP metabolites [inosine monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx)] were determined according to the procedure of Veciana-Nogués et al. (1997b) based on the HPLC method proposed by Murray and Thomson (1983). In addition, counts of mesophilic bacteria, psychrotrophic bacteria, Enterobacteriaceae, and coliforms were performed according to the procedures of López-Sabater et al. (1994).

Statistical Analysis. All statistical tests were performed by the Statistical Software Package SPSS (SPSS, Inc., Chicago, IL). In samples from the market, where biogenic amine data fluctuated over a wide range, results are reported as median and the deviation quartile was used to describe variability (Altman, 1991). The nonparametric Mann−Whitney U test and the Kruskall−Wallis test were used, respectively, to compare biogenic amine contents of fresh and canned tuna samples and to compare the samples from different points during the canning process.

RESULTS AND DISCUSSION

Biogenic Amines in Fresh and Canned Tuna. SD and SM were found in all samples studied (Table 1). SD and SM are naturally occurring biogenic amines in food, and their formation is not related with bacterial spoilage (Maijala et al., 1995; Hernández Jover et al., 1996; Veciana-Nogués et al., 1996). Contents of SD were, in both kinds of sample, lower than contents of SM. Other authors also reported higher contents of SM than SD in foods of animal origin, whereas in foods of plant origin SD was the prevailing polyamine (Bardocz et al., 1995; Izquierdo-Pulido et al., 1994). The Mann−Whitney U test showed that average contents of both SD and SM were statistically higher ($p < 0.05$) in fresh than in canned tuna samples.

The other biogenic amines studied in this work were found in only some of the samples (Table 2). Concerning the contents of biogenic amines detected, no significant differences ($p > 0.05$) were found between fresh and canned tuna except for SE and AG. Contents of SE were always lower than 4 μg/g except in two samples of canned tuna, which showed SE contents of 8.4 and 7.3 μg/g. SE was detected in a higher percentage of samples of fresh tuna (85%) than of canned tuna (47%). No information is available about the possible decrease in SE related to heat treatments. Contents of SE were

extensively reported in foods from vegetal origin (Adrian, 1991; Kena et al., 1992), but very few data are available for foods from animal origin. According to previous papers, SE was not found in either fresh fish or fish products (Duerr et al., 1980; García and Mariné, 1983). The presence of SE in fish products (ripened anchovies) has only been reported once (Veciana-Nogués et al., 1996) and always at low concentration (<0.05 μg/g).

TR and PHE were found at low levels (<3.5 μg/g) in 15% and 20% of fresh samples and in 23% and 26% of the canned samples, respectively. Therefore, there appears to be a higher incidence of TR and PHE in canned than in fresh tuna. Yen and Hsien (1991) also reported low contents of TR and PHE in canned tuna. Few data are available about contents of these biogenic amines in fresh fish, but high contents of both amines have been reported in very spoiled fish (Veciana-Nogués et al., 1996) or in ripened fish products (Yen and Hsieh, 1991; Veciana-Nogués et al., 1996).

HI, PU, CA, and TY have been commonly related with fish spoilage, especially HI (Tokunaga et al., 1982; Middlebrooks et al., 1988; Ababouch et al., 1991; Veciana-Nogués et al., 1996). In both fresh and canned tuna studied here the contents of these four biogenic amines were, in general, very low. In both kinds of sample HI levels were always below the legal limit of 50 μg/g established by the FDA. Moreover, CA levels were, in general, higher than those of HI.

A wider range of AG concentration was found in fresh than in canned tuna, and, in addition, AG was found in a higher number of samples of fresh tuna than of canned tuna (85% versus 47%). The content of this biogenic amine was higher ($p < 0.05$) in fresh than in canned tuna. AG increases during the first stages of fish spoilage but decreases thereafter (Yamanaka and Matsumoto, 1989; Veciana-Nogués et al., 1996). Therefore, low levels of AG could be related to fresh fish and also to very spoiled fish.

Although no significant differences were found between HI, PU, CA, and TY contents in fresh and canned tuna, the percentage of canned samples that showed contents of these amines higher than the median value found in fresh tuna was >20% for PHE, TR, TY, >40% for PU, >50% for CA, and >60% for HI. The greater presence of biogenic amines in canned tuna compared with that in fresh tuna could be explained by the use of poor raw fish or, as Pan (1985) reported, by their formation during canning process.

Canning Process. In samples used as raw material the contents of biogenic amines (Table 2) were very low except for SD and SM, which, as in fresh tuna from the market, were detected in all samples. HI, CA, PU, and TY were found in only a low percentage of samples, and their levels never exceeded 1.5 μg/g. As in fresh tuna from the market, AG showed an average value slightly higher than those of the other biogenic amines related to spoilage. TR and PHE were not detected in any sample. Table 3 showed that the contents of IMP were in samples used as raw material higher than the contents of HxR and Hx, as occurs in fresh fish (Ryder et al., 1984; Shirai et al., 1988). The average value of the IMP ratio was 0.53 ± 0.12, and all individual IMP ratio values were always higher than the minimum value required for fish acceptance (Fujii et al., 1973; Gill et al., 1987). The low levels of biogenic amines related to tuna spoilage and high IMP ratio agree with the low microbial counts obtained in raw material samples.

Table 3. Contents of IMP, Inosine, and Hypoxanthine (Micromoles per Gram) throughout Canning Process ($n = 12$)

canning step	IMP	inosine	hypoxanthine	IMP ratio
raw fish	4.65 (0.50)[a]*	3.35 (1.25)*	0.80 (0.45)*	0.52 (0.11)*
before cooking	4.40 (0.90)*	2.70 (0.98)*	1.25 (0.85)*	0.51 (0.07)*
after cooking	4.35 (1.50)*	2.10 (0.70)*	1.05 (0.60)*	0.48 (0.16)*
after packing	4.45 (1.20)*	2.95 (0.90)*	1.30 (1.00)*	0.53 (0.13)*
end product	3.10 (1.20)*	2.75 (1.15)*	1.35 (0.50)*	0.48 (0.08)*

[a] Mean value and (standard deviation); values in the same column bearing the same number of asterisks are not different ($p > 0.05$).

Thus, mesophilic counts [3.00 ± 1.40 log colony-forming units (CFU)/g] were much lower than the value of 10^7 log CFU/g proposed for fish rejection (Huss, 1988). Counts of psychrotrophic bacteria (3.12 ± 1.70 log CFU/g) were also lower than the maximum value of 10^5 log CFU/g proposed for fresh fish acceptance (Pascual-Anderson, 1992). Enterobacteriaceae showed an average value of 3 log CFU/g, ranging from undetectable to 3.2 log CFU/g, and coliforms were not found. Microbial counts in these samples were lower than those that are usual in fresh fish, probably due to the practice of freezing of tuna immediately after it is caught (López-Sabater et al., 1994). According to all of these data, tuna used as raw material for canning showed a high degree of hygienic−sanitary quality.

Contents of biogenic amines in samples corresponding to the same canning step but belonging to different batches were similar (Table 2). Also in these samples SD and SM were the only ones always found throughout the canning process. SE was detected in a high percentage of the samples, ranging from 0.4 to 7.7 μg/g. The contents of SE were higher than those of other biogenic amines (except SM and SD) and were also, in general, higher than in samples from the market. However, no formation of SE was observed during the canning process, since no significant differences ($p > 0.05$) were found in contents of SE between samples corresponding to the five steps of the canning process studied. Very few data are available about SE contents in fish, and it has never been related with fish spoilage; therefore, differences in contents of SE could be linked to differences between individuals or fish species. HI, PU, CA, TY, and AG, were found at low levels (<2.5 μg/g), and no significant differences between samples corresponding to different steps of the canning process were found. These results do not agree with the decrease in HI, PU, and CA subsequent to heat treatment reported by Sims et al. (1992). Our results showed that these biogenic amines related with the fish spoilage can be useful for estimating the degree of freshness degree of tuna used as raw material for canned tuna.

The only changes in amine contents during canning were observed for SD and SM, which decreased after the heat treatments involved in both cooking and sterilization steps. However, only after the sterilization were the changes significant ($p < 0.01$ for SD and $p < 0.005$ for SM). Sayem-El-Dayer et al. (1984) also reported that heat treatment slightly decreased SD contents, but no data are available for SM.

Table 3 shows the contents of IMP, HxR, and Hx and the value of the IMP ratio [IMP/(IMP + HxR + Hx)] in samples corresponding to different steps of the canning tuna process line studied. By using a Kruskall−Wallis test, no significant differences ($p < 0.05$) were found between samples A, B, C, D, and E samples in contents of IMP, HxR, or Hx. Our results confirmed the heat

stability of these compounds reported by Gill et al. (1987), which pointed out high recovery values for the three compounds when they had been spiked to tuna before canning. The IMP ratio obtained in all samples studied was always much higher than 0.114, which has been proposed by Fujii et al. (1973) as a minimum value for fish acceptance. These results agree with the low contents of biogenic amines related with fish spoilage and confirm that the IMP ratio can also be used as a freshness indicator of the raw tuna used. Changes of IMP and HxR occur by autolytic enzyme activity without growth of microorganisms, whereas biogenic amine formation is related to bacterial development. Therefore, in frozen storage of fish, where bacterial growth is inhibited, changes in biogenic amine contents would not be expected, while IMP ratio values could be modified mainly if the frozen storage period is long.

In summary, the results of this work showed that biogenic amine contents in samples from fresh and canned fish tuna from the Spanish market were in general low. The legal limit (50 μg/g) for histamine was never exceeded. Regarding the potential toxic effects linked to biogenic amines in foods, samples examined in this study showed a very low or no risk. The highest content of HI was found in a canned tuna sample, which showed 40 μg/g. This value is much lower than the health hazard action level of 500 μg/g reported by the FDA. The contents of TY and PHE, which have been related to migraine attack or to hypertensive crises in patients taking MAOI drugs, were also low. According to our results, only changes in SM and SD were observed during canning, since both amines decrease after cooking and sterilization steps. Therefore, HI, TY, CA, and PU, biogenic amines related with fish spoilage in canned tuna, came from the fish used as raw material. IMP, HxR, Hx, HI, TY, CA, and PU seem to be appropriate as quality indices to assess the raw fish used in canned tuna, because none of these compounds was produced by heat treatments and also because their contents did not decrease with cooking or sterilization processes. On the contrary, SD and SM are not good as indicators of spoilage because they are affected by heat treatment.

ABBREVIATIONS USED

HI, histamine; TY, tyramine; SE, seritonin; PHE, β-phenylethylamine; TR, tryptamine; PU, putrescine; CA, cadaverine; AG, agmatine; SM, spermine; SD, spermidine.

ACKNOWLEDGMENT

We especially thank Dr. C. Pascual (Laboratorio de Tenología de Productos Pesqueros, Facultad de Biologíaa, Universidad de Santiago de Compostela) for provision of samples corresponding to the tuna elaboration processes and to Dr. Teresa Mora Ventura and Dr. Emilio Ignacio López-Sabater (Unidad de Higiene e Inspección de los Alimentos, Facultat de Veterinaria, Universidad Autonoma de Barcelona) for microbiological determinations. We also thank Robin Rycroft for correcting the English.

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Received for review January 28, 1997. Revised manuscript received July 23, 1997. Accepted August 13, 1997.[⊗] This work was carried out with financial aid from the Comisión Interministerial de Ciencia y Tecnologia (CICYT, Project ALI-89-0633-CO3-01) of the Ministerio de Educación y Ciencia (Spain).

JF970092K

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.