Changes in biogenic amine contents throughout storage of canned fish products

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ABSTRACT

The levels of biogenic amines were assessed throughout storage at 4°C, for up to 120 days, of canned sardines, anchovy and smoked fish. Histamine, tyramine, putrescine, cadaverin, spermidine and spermine levels increased in general with elapsing storage time; the concentrations of the former in sardine, anchovy and smoked fish reached maxima of 281.17 mg/kg dry weight (DW), 166.21 mg/kg DW and 104.51 mg/kg DW, respectively, by 120 days. Spermidine and spermine levels increased slightly, whereas significant differences were found (P<0.05) in the levels of cadaverine and putrescine throughout storage. The total amine contents (579.15 mg/kg DW) of anchovy were highest, followed by sardine (456.86 mg/kg DW) and smoked fish (210.79 mg/kg DW). Overall, canned anchovy and sardine appear to pose public health risks owing to their biogenic amine levels above accepted thresholds.

Keywords: Biogenic amines, canned fish, chemical, contaminants, food safety

INTRODUCTION

Fish is an important source of vitamins and minerals, and an excellent source of essential amino acids – yet deterioration thereof occurs fast upon bacterial contamination, and often generates biogenic amines that raise a public health issue (Rabie, et al. 2009). Typical symptoms of excessive ingestion of biogenic amines are nausea, hot flushes, cold sweat, palpitations, headaches,
red rash, and too high or low blood pressure (Hungerford, 2010).

Biogenic amines are low molecular weight organic bases with biological activity, they encompass cadaverine, putrescine, spermidine, spermine, tyramine, tryptamine and histamine, which they are usually formed in foods by microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes and ketones by amino acid transaminases (Zhai, et al. 2012). In view of the relevance of biogenic amines for food safety issues as emphasized above, it is important to monitor their levels in fish. Besides histamine, such secondary amines as putrescine and cadaverine are good indices of spoilage of marine fish (Mietz and Karmas, 1977). For instance, Haláz, et al. 1994; Rezaei, et al. 2007 reported that putrescine and cadaverine in refrigerated foods (e.g. chilled fresh fish stored in ice) can actively accumulated at high rates even during storage below 5°C; those two biogenic amines correlate well with both sensory levels and total microbial counts in vacuum-packed and non-vacuum-packed carp (Krizek, et al. 2004).

It has been reported that M. morganii, K. pneumoniae, and P. vulgaris are prolific histamine formers, producing >1000 mg/kg in the culture broth (Kim, et al. 2001). These species have rarely been detected in fresh fish, but have mostly been isolated from fish spoiled under controlled storage conditions, above 20°C (Kim, et al. 2001); Histamine, cadaverine and putrescine can be a good quality marker to show the quality of common carp specimens. Kržízek, et al. (2004) have suggested that PUT and CAD and the sum of both amines are useful quality indicators for common carp flesh. In addition, PUT values lower than 10 mg kg⁻¹ can represent the good quality of the common carp flesh, 10–20 mg kg⁻¹ as acceptable quality and the value over than 20 can indicate the poor quality established on sensory evaluation (Hernández, et al. 2010). Mietz and Karmas (1977) claimed a strong dependence of histamine content upon the fish species, and accordingly proposed a biogenic amine index, BAI, calculated as

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\text{BAI} = \frac{(C_{\text{putrescine}} + C_{\text{cadaverine}} + C_{\text{histamine}})}{(1+C_{\text{spermidine}}+C_{\text{spermine}})}
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where C denotes concentration expressed in mg/kg; they suggested that BAI values exceeding 10 are an indication of excessive quality loss, and said statement was corroborated by Mendes (1999) after examining histamine formation in sardines and mackerel.

According to the FDA guidelines (FDA, 2011), the toxicity and defect action levels of histamine, established for tuna, mahi-mahi, and related fish, are the 50 mg/100 g and 5 mg/100 g, respectively; the term defect action level
refers to the level of histamine naturally or inevitably occurring in foods without, however, presenting a considerable hazard for humans. The European Union has set the maximum average level of histamine in fish and canned fish at 100 mg/kg, but the content allowed rises to 200 mg/kg in such ripened product as anchovies (EEC, 2005). The European Food Safety Agency, EFSA (2011), has recently established a daily maximum intake of 50 mg of histamine and 600 mg of tyramine for healthy adults, although such thresholds may be drastically reduced in the case of reported intolerance or use of monoamine oxidase inhibitor drugs. The FDA has issued industry guidelines aiming at establishing procedures for the safe processing and importing of fish and fishery products based on the hazard analysis and critical control points (HACCP) approach (FDA, 2011). However, a maximum total BAs level of 750–900 mg/kg has been proposed (Ladero, et al. 2010). The levels of histamine are regulated differently among countries, e.g. 100 mg/kg in South Africa and Italy, or 200 mg/kg in Australia and Germany (Auerswald, et al. 2006; Lange, et al. 2002). Good quality fish should contain less than 10 mg/kg histamine: 10 and 30 mg/kg unfold a significant deterioration, whereas above 50 mg/kg is conclusive evidence of too extensive a decomposition (Oguri, et al. 2007). Unfortunately, values as high as 211 mg/kg have been found by Rabie et al. (2009) in sated fermented fish Feseekh after 60 days of storage, which constitutes a health hazard for consumption at large.

Fermentation of fish brings about favorable taste and flavor via controlled breakdown of proteins to free amino acids – but these compounds play, in turn, the role of substrates for microbial-mediated synthesis of biogenic amines. Traditional manufactures of fermented fish in the presence of low levels of salt potentiate this hazard – but no reliable and consistent data exist that convey a reliable picture of the problem. On the other hand, there is evidence of food poisoning events in Egypt along the years that are probably linked to eating salted fermented fish (usually sardine and anchovy). Therefore, this study was carried out to aid in assessing this serious food-related problem; it specifically departed from a survey of canned fish products available commercially in Egyptian fish markets throughout storage.

MATERIALS AND METHODS

Fish sampling

Three types of fermented fish sold in canned form in Egypt were considered: anchovy, sardine and smoked fish (Renga). A total of 30 samples (in triplicate) of each of those three fish products were accordingly obtained.
directly from manufacturers, at random, and kept refrigerated at 4 ºC; individual units were then sampled (also in triplicate) by 0, 30, 60, 90 and 120 days of storage – chosen so as to be representative of their expected shelf-life. Prior to analysis at our laboratory premises, the skin of said fish samples was removed, and muscle portions were carefully excised and ground to produce a sufficiently homogeneous fish mince.

**Determination of dry weight**

A reference drying method (MSZ EN ISO 1666:2000) was applied, using a WS 50 oven (MLW, Germany) to heat at 100-105 ºC until constant weight.

**Extraction of biogenic amines**

Extraction of biogenic amines from the samples was performed following the method by Simon-Sarkadi and Holzapfel (1994) and later tuned by Rabie, et al. (2009) for fish matrices: 10 ml of 10 % (v/v) trichloroacetic acid was added to 3 g of sample, the mixture was shaken for 1 h using a Laboshake Ls 500i (Gerhardt, Germany), and then it was filtered through Whatman No.1 filter paper. To remove fat, samples were kept at -20 ºC for 1 d, and then underwent centrifugation at 7000 g for 15 min using a T 24 apparatus (MLW). The supernatant was collected and filtered through 0.25-µm membrane filters (Nalgene, USA).

**Quantitation of biogenic amines**

Biogenic amines were ascertained using an AAA 400 amino acid analyser (Ingos, Czech Republic) – equipped with an Ostion LG ANB ion-exchange column (6 cm-long, 3.7 mm i.d.); 100 µL-aliquots injected were then separated by stepwise gradient elution at 0.30 mL.min\(^{-1}\) (60 ºC), using a Na\(^+\)/K\(^+\) buffer. Detection was by spectrophotometry at 570 and 440 nm, after post-column derivatisation (121 ºC) with ninhydrin supplied at 0.20 mL.min\(^{-1}\) (Csomos and Simon-Sarkadi, 2002). All analytical determinations of biogenic amines were done in duplicate, and the average of each set was taken as a datum point. Identification was by matching retention times of actual samples to those of chromatographic standards, and quantification proceeded using peak area after suitable calibration curves had been constructed.

**Statistical analyses**

All experimental values were reported as average ± standard deviation of triplicates. Statistical significance of the differences found between data was ascertained via Student’s t-tests, using P=0.05 as reference.

**RESULTS AND DISCUSSION**

Previous analyses have unfolded presence of Citrobacter, Clostridium, Escherichia, Proteus, Pseudomonas and Photobacterium spp., besides such lactic acid bacteria as...
Lactobacillus, Pediococcus and Streptococcus (Kim, et al. 2009; Rezaei, et al. 2007; Ozogul and Ozogul (2007) claimed that such genera are able to produce hazardous amounts of biogenic amines when fish is kept at warm temperatures.

The concentrations of all biogenic amines are depicted in Fig. 1; the highest concentration (678.15 mg/kg) was found in anchovy, followed by sardine (544.86 mg/kg); the lowest levels appeared in smoked fish, probably owing to the aseptic role of smoke upon inhibition of growth of amine decarboxylating bacteria. Rabie, et al. (2009) had previously studied total biogenic amine contents in a specific form of Egyptian salted fermented fish (Feseekh) by 60 days of storage: values ranged from 83.63 to 1633.02 mg/kg. Fish such as tuna have been reported to contain His at 2.040 to 5.000 mg/kg (Kalac, 2006).

Fermented products in which considerable amounts of biogenic amines can be found as a consequence of the use of poor quality raw materials, contamination and inappropriate conditions during processing and storage. Additionally, the microorganisms responsible for the fermentation process may contribute to biogenic amines accumulation (Latorre-Moratalla, et al. 2010).

The amount of post-harvest time at elevated temperatures (after proper chilling onboard the harvest vessel) to which a fish can be exposed (e.g., during processing, storage, and distribution) without adverse effects depends primarily on whether the fish was previously frozen (e.g., in the harvest vessel) or heat-processed sufficiently to destroy histamine-forming bacteria (FDA, 2011). Rossano, et al. (2006) studied the influence of storage temperature and time of freezing on histamine formation in anchovies, showing the ability of freezing to inhibit or slow down its formation; it should be emphasized that the finished products do not undergo any heat treatment, so the amine-forming microbial activity is not inhibited or even stabilized.

The evolution of concentration of each biogenic amine considered independently is available in Figs. 2, 3 and 4 for sardine, smoked fish and anchovy, respectively. The highest concentration of histamine (281.17 mg/kg) was recorded in sardine by 120 days (P<0.05); by the same time, its content in anchovy (166.21 mg/kg) was also significantly higher than that in smoked fish (P<0.05). Recall that histamine forms from histidine could continue even after bacterial autolysis. This was confirmed in experiments using recombinant histidine decarboxylase (HDCs) of the histamine-producing bacteria.
Figure 1: Changes in concentration of total biogenic amines in sardine, smoked fish and anchovy throughout storage time (average ± standard deviation, n=3).
Figure 2: Changes in concentration of several biogenic amines in sardine throughout storage time (average ± standard deviation, n=3).
Figure 3: Changes in concentration of several biogenic amines in smoked fish throughout storage (average ± standard deviation, n=3).
Figure 4: Changes in concentration of several biogenic amines in anchovy throughout storage time (average ± standard deviation, n=3).
Photobacterium phosphoreum, P. damselae, R. planticola, and M. morganii in which the bacteria themselves were absent (Kanki, et al. 2007).

Putrescine usually correlates with its precursor free amino acid, ornithine, via a synergistic deiminase-decarboxylase mechanism (Virgili, et al. 2007); this biogenic amine was dominant in anchovy (39.54 mg/kg), and its content increased 6-fold (P<0.05) as storage time elapsed. Putrescine was observed in sardine and smoked fish at lower concentrations, viz. 24.81 and 17.65 mg/kg$_{\text{DW}}$ by 0 days, respectively, but increased 3- and 6-fold until 120 days, respectively. Valle, et al. (1996) found putrescine levels of 110 mg/kg in herring stored at 0°C; more recently, Krizek, et al. (2002) reported putrescine contents below 10 mg/kg in good quality carp meat – based on sensory assessment; 10-20 mg/kg values still permitted carp to bear an acceptable quality, but poor quality was claimed above 20 mg/kg.

As illustrated in Fig. 4, the tyramine content remained also high in anchovy, with a 5-fold increase within 120 days (P<0.05); this is typically the major biogenic amine found in fermented products (Riebroy, et al. 2004). In the case of sardine, tyramine increased from 14.69 mg/kg by 0 days to 7-fold that value by 120 days of storage. On the other hand, tyramine was essentially absent in smoked fish throughout the storage period (Fig. 3).

Cadaverine originates from decarboxylation of lysine; its highest content (132.57 mg/kg) was found in anchovy by 120 days (Fig. 5) and the lowest content (10.83 mg/kg) in smoked fish by 0 d (P<0.05); its content in sardine was essentially constant between 0 and 30 days (25.05 and 28.31 mg/kg) – but an increasing tendency became apparent as time elapsed (see Fig. 2). Our results are substantially lower than those by Jae-Hyung, et al. (2002) – who reported levels that increased from 480 to 1083-1205 mg/kg within 20 days in Korean salted and fermented fish products. Lakshmanan, et al. (2002) claimed that the bacteria releasing cadaverine and putrescine multiply rapidly between 9 and 12 days, thus contributing to formation of that biogenic amine after ice storage of emperor fish and shrimp.

Finally, the maximum contents recorded of spermidine and spermine in smoked fish were 12.86 and 22.28 mg/kg, by 120 days; 8.05 and 6.44 mg/kg in sardine, by 120 days; and 3.66 and 8.71 mg/kg in anchovy, also by 120 days. These low levels are consistent with the results reported by Rabie, et al. 2009 to salted fermented fish (Feseekh), and for various sea fish species, e.g. salmon, rockfish, lobster and shrimp (Mietz and Karmas, 1977).
CONCLUSIONS

The concentration of total biogenic amines in canned fish increased significantly (P<0.05) during refrigerated storage up to 120 days. In particular, histamine in sardine attained a level that is almost 3-fold the maximum allowed (and enforced) by EC regulations for canned fish, and almost 50% above that for ripened fish; hence, especially health-compromised or otherwise susceptible individuals should exercise special care regarding consumption of this type of fermented fish.

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