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Changes in free amino acids and biogenic amines of Egyptian salted-fermented fish (Feseekh) during ripening and storage

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ABSTRACT

The aim of this study was to investigate changes in the formation of amino acids and biogenic amines in Egyptian salted-fermented fish (Feseekh) during ripening (20 days) and storage (40–60 days). The total concentration of free amino acids increased from 8 (dry weight; DW) to 72 g/kg (DW) after 60 days of storage. The predominant free amino acids were leucine, glutamic acid, lysine, alanine, valine, aspartic acid, isoleucine and citrulline. Their concentrations accounted for 68% of the total concentration of amino acids after 60 days. The total contents of biogenic amines ranged from 84 to 1633 mg/kg (DW) during the investigated period. Cadaverine was the major amine detected in Feseekh at all sampling stages and its concentration varied between 21 and 997 mg/kg (DW). The histamine content (211 mg/kg DW) only exceeded the maximum tolerance level (200 mg/kg) after 60 days. It could be concluded that Feseekh can be consumed without any health risks between 20 and 40 days but it can be hazardous after 60 days due to the biogenic amine content.

1. Introduction

Feseekh is the traditional name for the salted-fermented Bouri fish (*Mugil cephlus*) produced in Egypt. It is popular not only as an appetiser, but also as the main dish at some feasts in Egypt (El-Sebaiy & Metwalli, 1989). There are two types of Feseekh on the Egyptian market, the first type having a low salt content and being suitable for consumption after 15–20 days of maturing, whilst the second has a high salt content and can be eaten after 2–3 months of storage. From the nutritional point of view, Feseekh is a rich source of high quality protein, essential amino acids, vitamins, and minerals.

Fish is known to have an excellent essential amino acid composition, which is why it is recommended for a balanced, healthy diet. The amino acid composition of foodstuffs does not vary greatly; however, the free amino acid composition changes considerably after processing and storage.

The concentration of amino acids depends on the harvesting season and manner of feeding prior to capture. For example, large quantities of lysine and arginine can be found in fish harvested in the summer season (Aksnes & Brekken, 1988).

Biogenic amines are naturally occurring, low molecular weight organic bases, ubiquitous in animals and plants. Putrescine, cadaverine, spermidine and spermine have an aliphatic structure, histamine and tryptamine have a heterocyclic structure and tyramine and phenylethylamine have an aromatic structure.

The formation of high levels of biogenic amines, especially histamine, in fish products may be rapid, and their development depends on the number of microorganisms present.

Several genera are involved in toxicity, such as *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Shigella*, Photobacterium and the lactic acid bacteria (*Lactobacillus*, *Pediococcus*, and *Streptococcus*). These bacteria are capable of producing hazardous amounts of histamine in a very short period of time when the fish are kept at elevated temperatures (Omura, Price, & Olcott, 1978).

From the toxicological point of view, the consumption of an excessive amount of biogenic amines can represent a health risk for sensitive individuals. The typical symptoms are nausea, hot flushes, cold sweat, palpitations, headaches, red rash and high or low blood pressure. It is thus important to monitor the levels of biogenic amines in foodstuffs and beverages from food safety aspects.

Mietz and Karmas (1977) found that the histamine content varied extensively with the fish species and they created a biogenic amine index, calculated using the formula BAI = (mg/kg putrescine + mg/kg cadaverine + mg/kg histamine)/(1 + mg/kg spermidine + mg/kg spermine). They found that BAI values exceeding 10 can be considered as an indication of the extent of quality loss. This opinion was confirmed by Mendes (1999), who examined the histamine formation in sardines and mackerel.

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Yamanaka, Shiomi, and Kikuchi (1989) proposed cadaverine as the best indicator of chum salmon spoilage. Both putrescine and cadaverine seem to be good quality markers, as they were in the best correlation with both sensory levels and total microbial counts in the vacuum-packed and non-vacuum-packed flesh of carp (Krizek, Vachá, Vorlová, Lukásová, & Cupáková, 2004). In addition, putrescine and cadaverine can potentiate the toxicity of histamine and react with nitrites to form nitrosamines (Cinquina et al., 2004).

The consumption of foods with high levels of histamine can cause allergy-like symptoms in sensitive humans (Lehane & Olley, 2000; Taylor, 1985).

The European Union set the maximum average value of histamine in fish and canned fish at 100 mg/kg; whereas in ripened products like anchovies, the average histamine content must be lower than 200 mg/kg (EEC, 1991).

Although, numerous outbreaks of food poisoning from eating Feseekh occur in Egypt every year, there are no accurate figures for the number of deaths.

Little data is available on the occurrence of free amino acids and biogenic amines in Feseekh. Therefore, the objective of this study was to provide information on the concentration of these components in salted-fermented fish during ripening and storage.

2. Materials and methods

2.1. Fish samples

Fresh Bouri fish (*Mugil Cephalus*) was purchased early in the morning from the local market in Zagazig, Egypt. The fish were cleaned thoroughly and left at room temperature in the laboratory until they acquired the right degree of distension and were then piled in a glass jar, with plenty of salt between the layers, till the jar was packed. It was then tightly sealed and left for 60 days at room temperature. The concentration of both amino acids and biogenic amines was determined after 20 days of ripening and at 40 and 60 days of storage.

2.2. Determination of dry weight

Dry weight measurements were made on 3 g samples of Feseekh dried in an oven (MLW, WS 50, Germany) at 100–105 °C until constant weight according to the Hungarian standard (MSZ EN ISO 1666:2000).

2.3. Extraction of amino acids and biogenic amines

The amino acids and biogenic amines in the Feseekh samples were extracted using the method described by Simon-Sarkadi and Holzapfel (1994). To extract free amino acids and biogenic amines, 10 ml of 10% trichloroacetic acid (TCA) was added to 3 g samples of Feseekh and the mixture was shaken for 1 h using a Laboshake (Gerhardt Ls 500i, Germany). The extract was filtered through Whatman No. 1 filter paper. To remove the fat content, the samples were kept at −20 °C for one day, and then centrifuged (MLW, T 24, Germany) at 7000g for 15 min. Supernatants were collected and filtered through a 0.25-μm membrane filter (NALGENE, USA).

2.4. Determination of amino acids and biogenic amines

The analysis of amino acids and biogenic amines was performed with an AAA 400 amino acid analyser (Ingos Ltd., Czech Republic) equipped with a Watrex Polymer 8 ion-exchange column (20 × 3.7 cm) for amino acids and an Ostion LG ANB ion-exchange column (6 × 3.7 cm) for biogenic amines. Free amino acids and biogenic amines were separated by stepwise gradient elution using Li^+ buffer systems for amino acids and using Na^+/K^+ buffer system for biogenic amines. Colorimetric detection was accomplished at 570 and 440 nm after post-column derivatisation with ninhydrin reagent (Csomos & Simon-Sarkadi, 2002).

The analyses were done in duplicate. The average values are given in Figs. 1–5. The relative standard deviations of the data were below 5%.

3. Results and discussion

3.1. Amino acids

Salted-fermented fish (Feseekh) contained 21 free amino acids. The total concentration of free amino acids increased 1.4-fold after the ripening period (8–11 g/kg DW) and reached 72 g/kg

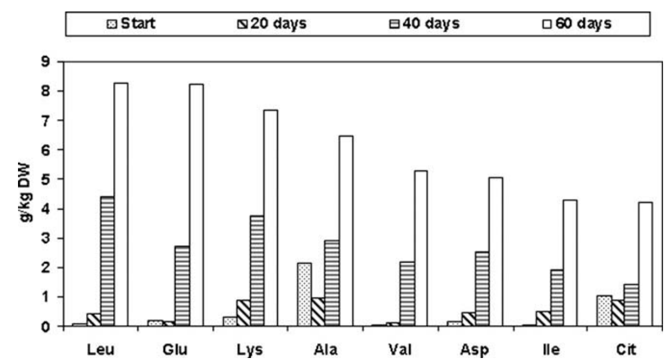

Fig. 1. Changes in the contents of dominant free amino acids in Egyptian salted-fermented fish during ripening and storage.

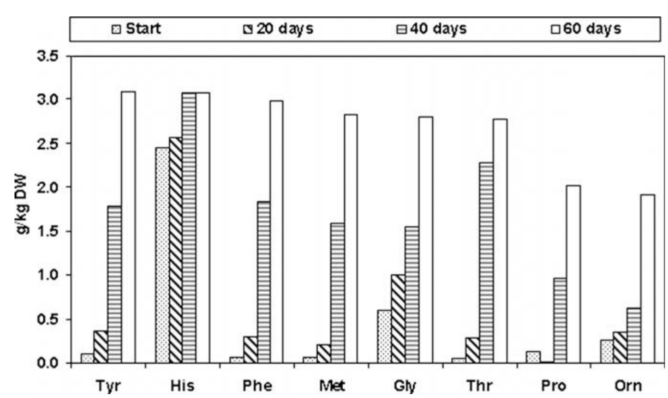

Fig. 2. Changes in the contents of the second group of amino acids in Egyptian salted-fermented fish during ripening and storage.

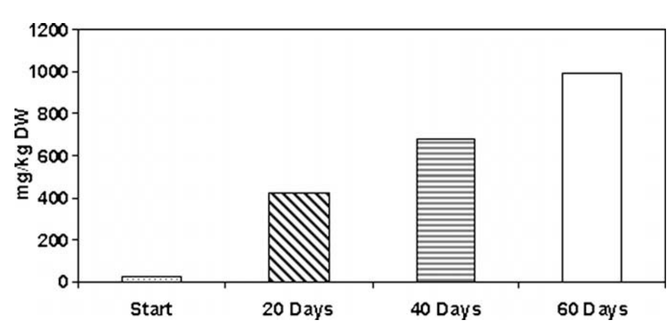

Fig. 3. Changes in the cadaverine content in Egyptian salted-fermented fish during ripening and storage.

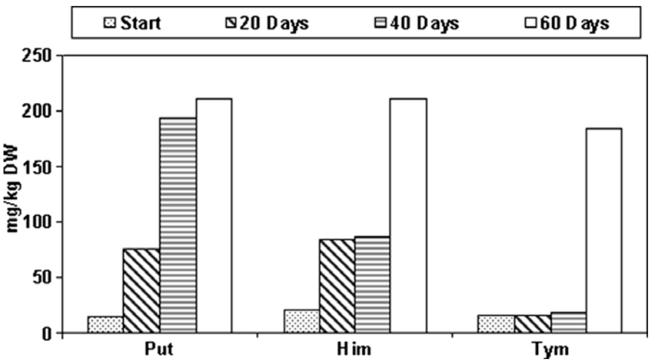

Fig. 4. Changes in the histamine, tyramine and putrescine contents in Egyptian salted-fermented fish during ripening and storage.

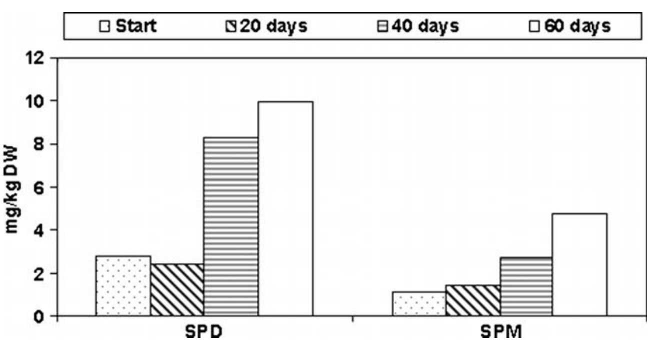

Fig. 5. Changes in the spermidine and spermine contents in Egyptian salted-fermented fish during ripening and storage.

(DW) after 60 days of storage (data not shown). The main free amino acids were leucine (0.11–8.25 g/kg DW), glutamic acid (0.18–8.21 g/kg DW), lysine (0.34–7.34 g/kg DW), alanine (2.16–6.49 g/kg DW), valine (0.07–5.29 g/kg DW), aspartic acid (0.17–5.06 g/kg DW), isoleucine (0.05–4.28 g/kg DW) and citrulline (1.03–4.22 g/kg DW) as shown in Fig. 1. These amino acids represented 68% of the total concentration of amino acids after 60 days of storage. Kim, Shin, Kang, and Byun (2003) obtained similar results. They found that after 3 months the major amino acids of salted-fermented shrimp were aspartic acid, glutamic acid, alanine, leucine and lysine. In addition, taurine can be considered as a major amino acid in Philippine salted-fermented shrimp (Peralta et al., 2005).

The percentage of glutamic acid in Feseekh samples after 60 days (16.7%) was in agreement with that found by Sanni, Asieduw, and Ayernorw (2002), who reported that the percentage of glutamic acid in Ghanaian fermented fish condiment samples was 12.4–14.5%.

The second group of free amino acids in Feseekh samples included tyrosine (0.10–3.09 g/kg DW), histidine (2.46–3.08 g/kg DW), phenylalanine (0.06–2.98 g/kg DW), methionine (0.06–2.83 g/kg DW), glycine (0.06–2.81 g/kg DW), threonine (0.05–2.78 g/kg DW), proline (0.13–2.02 g/kg DW) and ornithine (0.25–1.92 g/kg DW), as shown in Fig. 2. They accounted for 30% of the total free amino acids after 60 days.

The remaining amino acids had the following concentrations: serine (0.04–1.01 g/kg DW), glutamine (0.13–0.15 g/kg DW), cystathionine (0.03–0.06 g/kg DW), γ-amino butyric acid (0.01–0.14 g/kg DW) and arginine (0.10–0.21 g/kg DW). These amino acids were detected in low concentrations and represented 2% of the total biogenic amine after 60 days (data not shown).

Among the amino acids, lysine, tyrosine and histidine are the main precursors for biogenic amines. All these amino acids showed considerable changes during storage, exhibiting increases of 31-, 22- and 1.3-fold for tyrosine, lysine and histidine, respectively (Figs. 1 and 2).

3.2. Biogenic amines

Salted-fermented fish (Feseekh) contained six biogenic amines, namely, histamine, tyramine, putrescine, cadaverine, spermidine, and spermine. The total concentration of biogenic amines increased around 7-fold during the ripening period (83.63–608.72 mg/kg DW), reaching a concentration of 1633.02 mg/kg (DW) after 60 days of storage (data not shown).

As presented in Fig. 3, cadaverine was found to be the main biogenic amine in Egyptian salted-fermented fish during ripening and storage, representing about 61% of the total biogenic amine content. Cadaverine originates from the decarboxylation of lysine and has been associated with Enterobacteriaceae (Halász, Barath, Simon-Sarkadi, & Holzapfel, 1994). Lakshmanan, Shakila, and Jeyasekaran (2002) observed that the bacteria that produce cadaverine and putrescine survive and multiply rapidly between 9 and 12 days, and contribute to the formation of amines after the ice storage of emperor fish and of shrimp.

Fig. 3 also shows changes in the cadaverine concentration during ripening and storage. The increase in the concentration of cadaverine (21–997 mg/kg DW) was found to be 48-fold after 60 days of storage. This result was lower than that obtained by Jae-Hyung, Hyung-Kee, Young-Jun, Man-Goo, and Han-Joon (2002), who reported that the concentration of cadaverine increased from 480 to 1083–1205 mg/kg after 20 days in Korean salted and fermented fish products.

Putrescine (Fig. 4) was found to be the second dominant amine in Egyptian salted-fermented fish samples, increasing from an initial level of 15 mg/kg (DW) to a 5-fold value during 20 days of ripening. After 40 and 60 days of storage the increase was even more pronounced, 13- and 14-fold, respectively. Valle, Malle, and Bouquelet (1996) found that when the fish became inedible the putrescine contents of herring stored at 0 °C was 110 mg/kg. Recently, Krizek, Pavlicek, and Vacha (2002) proposed putrescine values lower than 10 mg/kg for good quality carp meat, values between 10 and 20 mg/kg for acceptable quality and values over 20 mg/kg for poor quality carp meat based on sensory scores.

Fig. 4 shows that the concentration of histamine increased 4-fold during 20 days of ripening. This concentration remained fairly constant until 40 days and only exceeded the maximum tolerance level (200 mg/kg) (EEC, 1991) after 60 days (211 mg/kg DW). Histamine levels are especially important, since they are a potential health hazard.

However, Egyptian salted-fermented fish samples are not hazardous in the case of normal consumption after 20–40 days.

As illustrated in Fig. 4, tyramine was found to be present in low concentrations of 16 and 17 mg/kg (DW) after 20 and 40 days, but exhibited a sharp increase (about 12-fold) after 60 days. The concentration of tyramine was two times higher than that recorded for fresh water carp (<10 mg/kg) by Krizek et al. (2004). Yen and Hsieh (1991) also reported low contents of tyramine in canned tuna. However, the maximum recommended level of tyramine in food should be in the range of 100–800 mg/kg (Brink, Damnic, Joosten, & Huis In't Veld, 1990).

Spermine (2–10 mg/kg DW) and spermidine (8–20 mg/kg DW) were present at low concentrations in Feseekh samples (Fig. 5). These low levels of spermine and spermidine in Feseekh samples are in agreement with the results reported for aerobically stored gilt-headed sea bream (Koutsoumanis, Lambropoulou, & Nychas, 1999) and for various sea fish species, including salmon, rockfish, lobster and shrimp (Mietz & Karmas, 1977). The results were lower than those reported for spermidine by Jae-Hyung et al. (2002), who found that spermidine contents increased from 43 to 309–351 mg/kg during the first 5 days of ripening in Korean salted-fermented fish products, but decreased thereafter.

Further studies will be needed to control or reduce the concentrations of biogenic amines in Feseekh during storage, since these contents tended to increase as the ripening process advanced.

4. Conclusion

The results of this study showed that the free amino acid and biogenic amine content of Feseekh fish increased significantly during ripening and storage.

It could be concluded that Egyptian salted-fermented fish (Feseekh) can be consumed without any health risks between 20 and 40 days, but it could be hazardous after 60 days, due to the increased biogenic amine content.

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