Kısa Bilimsel Araştırma Makalesi

DETERMINATION HISTAMINE IN SALTED ANCHOVY (ENGRAULIS ENCRASICOLUS) BY ELISA*

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Tuzlanmış Hamsi Balığında (Engraulis Encrasicholus) Histamin Düzeyinin ELISA ile Belirlenmesi*

Özet: Histamin, mikrobiyel aktivite sonucu amino asitlerin dekarboksilasyonu veya aldehit ve ketonların aminasyonu ile oluşan düşük molekül ağırlıklı organik bir bileşiktir. Balık ve balık ürünleri, et ve fermentle et ürünleri, tavuk, peynir, şarap ve bira gibi bir çok gıdannın normal bileşiminde bulunan histamin, histinin amino asidinin, enzimatik dekarboksilasyonu sonucu oluşur. Histamin gıdalarla birlikte yüksek düzeyde alındığı zaman bulantu, kusma, karnı ağırsı, ishal, kaşıntı, kizarıklık, yanma, ödeme, baş ağrısı ve hipotansiyon gibi çeşitli semptomlarla karakterize gıda zehirlenmelerine neden olur. Bu çalışmada, tuzlanmış hamsi (Engraulis encrasicholus) balığında depolama süresince histamin düzeyinin değişimi araştırılmıştır. Bu amaçla, balıklar deneySEL olarak kuru tuzlama yöntemi ile tuzlanıktan sonra 1 yıl oda sıcaklığında muhafaza edilmiştir. Balıklardan 0, 2, 4, 6, 8, 10 ve 12. aylarda örnekler alınarak Ridascreen Histamin ELISA test kiti ile histamin miktarı ölçülmüştür. Yapılan analizler sonucunda balıklardaki histamin düzeyi belirlenilen aylarda sırasıyla 0, 32, 35, 64, 91, 95 ve 136 mg/kg olarak bulunmuştur. Sonuç olarak, tuzlanarak 12 ay muhafaza edilen hamsi balığı örneklerindeki histamin düzeyi, Türk Gıda Kodeksi-Su Ürünleri Yönetmeliği'nde belirtilen kabul edilebilir maksimum limit olan 200 mg/kg'ın altında saptanmıştır.

Anahtar Kelimeler: ELISA, hamsi, histamin, tuzlama

Abstract: Histamine is an organic compound with low molecule weight which is formed by the decarboxylation of amino acids as a result of microbiological activity or by animation of aldehydes and ketones. Histamin is present in normally compound of various foods such as fish and fish products, meat and fermented meat products, chicken, cheese, wine and beer. Histamine is formed as a result of the enzymatic decarboxylation of histidine. Histamine causes food

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poisoning when intake high levels in foods characterized by various symptoms such as nausea, vomiting, abdominal cramps, diarrhea, itching, rash, burning, edema, headache and hypotension. In this study, it was examined the variation of histamine levels in dry salted anchovy (*Engraulis encrasicolus*) during the storage period. For this purpose, fish samples were stored under room temperature for one year after salting with dry salt method. Samples were taken in 0, 2, 4, 6, 8, 10 and 12th months and histamine levels were determined with Ridascreen Histamine ELISA kit. As a result of the analyses, histamine levels were found as 0, 32, 35, 64, 91, 95 and 136 mg/kg in defined months. In conclusion, histamine level in the anchovy samples which were stored as 12 month as salted were found under the maximum permissible limit of 200 mg/kg of Turkish Food Codex and Seafood Regulations.

**Key Words:** ELISA, anchovy, histamine, salted

**Introduction**

Fishery is an important source of income in the Black Sea Region. In 1997, total 265,551 tons of fish were fished in the Black Sea Region; and from that 213,780 tons were anchovy. In 2006, total fishing was reached to 280,514 tons, and from that 212,081 tons were anchovy. Anchovy has the percentage of 80% of total fishing. At the same time, together with the increased fishing in the Black Sea, the amount of consumed fish per person has risen from 7.6 kg to 8.1 kg in the last 10 years (5).

Anchovy is taken place in the family of *Engraulidae* and consumed in our country with enjoy. Species of anchovies in the Black Sea are *Engraulis encrasicolus ponticus* and *Engraulis encrasicolus maeticus*. Among them, *Engraulis encrasicolus ponticus* is known as “Black Sea anchovy” and its height is reached to 18-20 cm. The other species named as *Engraulis encrasicolus maeticus* is known as “Anchovy of Azov”. The height of the anchovy of azov is a bit smaller, its head is shorter, it has flat nose and bigger eyes (26).

The best fishing period of anchovy is the months of November, December, January and February. When anchovies were fished in winter season abundantly, they were stored for long period of time with drying, smoking, salting, and marinating, and thus it can be consumed in seasons which anchovy is not consumed. Generally, 3 basic methods are used in salting of fishes. These are dry salting, brine salting, and modified salt solution (11, 31). Salt has a flavoring character in foods and also prevent the production of pathogenic microorganisms and putrefaction bacteria through diffusion and osmosis in the product.

Depending upon the amount of salt used, it provides the removal of water in the fish. At the same time, salt increases osmotic pressure and decrease the water activity in the medium. Additionally, it has been indicated that salt inhibits the activity of histidine decarboxylase which has an effect on the formation of histamine. Histamine is a biogenic amine forming as a result of decarboxilation of the amino acid of histidine with
the enzyme of L histidine decarboxylase. Histamine can be found in mast cells, basophiles, hypothalamus, and gastric mucosa. Bacteria belong to the Enterobacteriaceae are responsible for the decarboxilation of histamine. In the histamine intoxication, Morganella morganii, Raoultella planticola (Klebsiella pneumoniae), Hafnia spp. and Photobacterium phosphoreum are isolated from fishes mostly, and Proteus spp., Serratia spp., Enterobacter spp., Staphylococcus spp. and Citrobacter spp. isolated a little (18).

In the biotransformation of histamine, two enzymes named as “Diamine Oxidase” (DAO, histaminases) and “Histamine N-Methyl Transferase” has an important role. These two enzymes detoxify histamine in the intestinal system and prevent absorption. Most of histamine degraded by the enzyme named as “Histamine N-Methyl Transferase”, and the product formed is transferred to methyl imidazol acetic acid. Other part of histamine is degraded by converting into imidazoloacetaldehyde with enzyme “Diamine Oxidase”. Small amount of histamine is not metabolized. When histamine is taken with foods more than 500-1000 mg/kg, it causes allergic reactions. The symptoms arise as itching, urticaria, headache, nausea, diarrhea, and forniculation between a couple of minutes and a few hours, and in serious cases it causes constriction in bronchus and dyspnea depending upon the amount of the digested histamine (21).

The factors influencing histidine decarboxylase activity were incubation temperature, salt, water activity, pH and histidine concentration. A naturally large amount of histidine in scombroid fish and temperature of fish itself were particularly the cause of histamine formation (20). Histamine producing bacteria had been isolated from various species of marine fish but fish stored in saturated brine had not been mentioned (15). Chakrabarti (7) also found that the histamine content in salted and fried whole mackerel was above 10 mg/100 g. In addition, acidic conditions bring about degradation of proteins in fish tissue into amino acids. Gokoglu (14) and Kilinc and Cakli (17) were reported that histamine levels were lower than the toxic limit (100 mg/kg) during the marination and storage period of sardine marinades.

The use of a modified atmosphere with carbon dioxide has been shown to extend the shelf-life of foods by inhibiting microbial growth (12). Ozogul and Ozogul (25) found that the amount of histamine sardines (Sardina pilchardus) increased during the storage period and reached 20.3 ± 1.3 mg/100 g for air storage, 14.0 ± 1.2 mg/100 g for VP and 10.5 ± 1.2 mg/100 g for MAP. In addition to this, Erkan et al. (10) determined that histamine was 52 and 45 ppm by 9th day in sardine packaged in O₂/CO₂/N₂, 5/35/60 (%) and O₂/CO₂/N₂, 5/70/25 (%) respectively, in sardine samples, stored in modified atmospheres at 4 °C for 9 days.

Changes in biogenic amine contents during canning were not clear. 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 (33). Since histamine is heat resistant, it can remain intact in canned or other processed fish products (23). The use of poor quality fish as raw material for canning, or defective handling techniques of high quality fish during processing results in the presence of toxic levels of histamine in canned products (27).

In this study, it was aimed to determine the changes occurred in the histamine value during the storage of anchovy (*Engraulis encrasischolus*) in room temperature after salting.

**Material and Method**

**Sampling:** It was used anchovy (*Engraulis encrasischolus*, L., 1758) as a research material. 10 kg anchovy was supplied as fresh from fishing boats in Samsun region on December 2005. Anchovies were brought to the laboratory in one hour in ice (fish:ice, 1:2) (0 ±1 °C).

In the experimental study, firstly heads of fish samples were cut off, and internal organs were cleaned and washed. Salting procedure was made in traditional method (24). For this purpose, rock salt was placed under the sterile glass jar in 1 cm thickness, and anchovies were arranged as one layer anchovy and one level salt. 10 anchovies were arranged in the first layer of jar (approximately 80 g.), and salt adequate for covering them (nearly) 40-50 g was added. This arrangement was applied to each layer. 900-1000 g (fish:salt=1.5:1) rock salt was used for approximately 1.5 kg fish. Salted anchovies with the method of dry salting were stored in room temperature (22±1 °C). Histamine analyses were carried out as 2 repetitions through taking samples 0, 2, 4, 6, 8, 10, and 12th months.

**ELISA test procedure:** The histamine analysis in fish samples were carried out by using Ridascreen Histamine ELISA test kit (R-Biopharm A.G. Kit, Art. No. R 1604, Darmstadt, Germany). According to the ELISA test kit procedure one g of sample was taken and 200 ml distilled water was added. Then it was homogenized for 15 minutes in blender and the mixture was centrifuged for 5 minutes at 2500 g in room temperature (20-25 °C). The supernatant was diluted appropriately, and analysis was carried out according to the method mentioned in the test kit. The extracts were read in 405 nm wave length in DAS RS 232 brand ELISA spectrophotometry.

**pH determination:** 10 g of sample was taken and 10 ml distilled water was added. Then the mixture was homogenized in a stomacher. The pH of this mixture was measured a digital pH-meter (Inolab pH730, Germany) (6).
Statistical Analyses: All statistical analyses were performed by using SPSS 13.0 package software (SPSS, Chicago, IL, USA). Statistical evaluation has been carried out with one-way ANOVA and Turkey multiple-comparison test with two repetitions. The correlation between the histamine concentration and time is carried out by using analyses of pearson correlation test and linear regression test. The results were expressed as mean±standard error. The importance was expressed as \( p<0.05 \) (30).

Result

In this study, it was examined the formation of histamine during the storage period of anchovy. As a result of histamine analyses made with ELISA method, the level of histamine in the months of 0, 2, 4, 6, 8, 10, and 12\(^{th}\) months were found as 0, \(32\pm0.57\), \(35\pm1.15\), \(64\pm0.57\), \(91\pm1.15\), \(95\pm1.15\) and \(136\pm1.15\) ppm respectively (Figure 1). Difference of histamine level between months in fish samples was significant (\( p<0.05 \), ANOVA). As a result of pearson correlation test and linear regression analyses, it was observed a strong positive correlation between the storage period and histamine level (\( r=0.98 \), \( p<0.01 \)) and the equation of this correlation was determined as \( r=21.071-19.571x \) (Figure 2). In the study, the initial pH value was 6.0 in raw material (anchovy), and at the end of 12 months, it was determined as 4.9.

Figure 1: Mean level of histamine in salted anchovy (ppm).
Şekil 1: Tuzlanmış hamsi balığında ortalama histamin düzeyi (ppm).
Discussion

Salting is the oldest processing method known in food preservation. Salt gives aroma specific to the product in food products, increases the hardness of product through drawing water of tissue, and besides increases the shelf life with conservative effect. In the study, the initial pH value was 6.0 in raw material (anchovy), and at the end of 12 months, it was determined as 4.9. The enzyme named as cathepsin, one of the proteases, breaks off the peptide chains of proteins, and provides the formation of aroma specific for marinate. The application of acetic acid and salt applied during the marination procedure in fresh fish, proteins in fish were denaturized, the bounded water was removed, and accordingly the pH was decreased (16). Gokoglu (14) reported that histamine content of fresh sardine was 14.23 mg/kg. After dipping in the solutions of 2 and 4% acetic acid, histamine contents of fish decreased in 1 h but histamine levels at the end of the maturing process was higher than those found in fresh fish. On the other hand Kilinc and Cakli (17) stated that the histamine content of fresh sardine was 20.14 mg/kg. Even though at the beginning of a marination process histamine content was found to be 10.21 mg/kg, it was found to be 22.08 mg/kg at the end of the marination process.

When the amount of histamine taken with foods exceeded some limits, it caused light, medium, and heavy poisoning in humans. These values differ according to the type of foods and from country to country. The maximum acceptable limit for histamine is 5 mg/100 g (50 ppm), according to the data of FDA (1), 20 mg/100g (200 ppm) in
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Australia (2), 10 mg/100g (100 ppm) in the European Union (4) and 200 mg/kg in Turkey (3).

Anchovies (*Engraulis encrasicholus*) are very sensitive for the formation of histamine by reason of high amount of free histidine included in muscles of anchovies (32). Histamine levels of salted anchovy were given in the Figure 1. The average level of histamine in 0, 2, 4, 6, 8, 10, and 12th months were found as 0.32±0.57, 35±1.15, 64±0.57, 91±1.15, 95±1.15 and 136±1.15 ppm, respectively. In the study, it was observed that the level of histamine was not exceeded 200 mg/kg at the end of 12th month. On the other hand In the Philippines, 17-33 mg/100 g, 2.1 mg/100 g and 3.9 mg/100 g of histamine contents were found in dried squid, salted and dried herring and shrimp paste, respectively. Kongpun and Suwansakornkul (20) found the histamine increased from 1.87 mg/100 g to 115.52, 158.28 and 125.10 mg/100 g on the 6th day of salting of Spanish Mackerel with salt ratio of 1:1, 2:1 and 3:1, respectively. After 6 days, it decreased markedly in all samples until the end of the process.

Proteolytic enzymes, microbial contamination, temperature, pH, and oxygen are the factors affecting the formation of histamine in foods. Most of bacteria developed mostly in room temperature, and some bacteria developed in lower temperatures. The main factor in the formation of histamine was the high amount of histidine in foods, and the existence of bacteria having the activity of histidine decarboxylase (22).

Histamine was determined more frequently in scombroid fishes with respect to other fishes. Chuan et al. (8) founded histamine level as 63 ppm in packaged tuna fish, and as 75 ppm in trout in Taiwan. The histamine level in fishes change with temperature, and it was reported by Shakila and Vasundhara (29) that histamine level in fresh-water fishes in India exceeded maximum acceptable limits (20 mg/100g) in 18 hours at 30 °C, and in 5 days in fishes stored at 5 °C. In our study, it was seen that the histamine level in anchovy stored at 20-25 °C hardly reached to 32 ppm in the 2nd month. The reason is that removal of water by salt in the fish and lowering water activity, and preventing the production of bacteria. As a result, it was considered that the bacteria caused the production of halophilic bacteria and histamine were not, developed, and the shelf life of fish was extended.

Erkan et al. (9) tested biogenic amines in 39 cans of sardine, tuna, sardel, mackerel, pelamide and trout. They found that four fish samples contained higher amounts of biogenic amines (three sardine samples contained more than 1000 mg/kg histamine) while all other samples were acceptable. Windyga et al. (34) determined the content of histamine in 79 samples of imported canned fish products (sardines, mackerel). The levels of histamine were exceeded 20 mg/100g of product in 18% of tested products (sardines).
As distinct from the findings of our study, histamine level in fermented fish samples was found higher (13). Sanceda et al. (28), found the histamine level in sauced anchovy as 1380 mg/kg, and Kirschbaum et al. (19), between 721 and 757 mg/kg.

As lower findings from our study, histamine level in salted mackerel in Malaysia was found between 0.7 and 1.6 mg/100 g., in salted herring in Philippines was found 2.1 mg/100 g., in dried anchovy in Hong Kong 1.2 mg/100 g (35). It is estimated that the difference between these histamine levels may be caused between the differences between the methods used, bacterial load in the medium, preservation temperature, and type of fishes. The histamine problem in fish products constitute a risk in fishes imported from tropical countries.

As a result, it should be considered that foods having high histamine content constituted a potential risk for public health. In order to prevent the formation of histamine in foods, it should be careful on selection of quality raw material and hygienic measures. It should be provided a longer preservation by selecting adequate preservation methods. In our study, it was determined that the salted and preserved anchovy was remained under the limits specified in Turkish Food Codex-Su Ürünleri Yönetmeliği (3) the acceptable limit of 200 mg/kg after 12th month.

References


