İstanbul Üniv. Vet. Fak. Derg.
35 (2), 41-50, 2009

J. Fac. Vet. Med. Istanbul Univ.
35 (2), 41-50, 2009

Kısa Bilimsel Araştırma Makalesi

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

Göknur TERZİ [1**], Ali GÜCÜKOĞLU [1]

Geliş Tarihi : 25.03.2009
Kabul Tarihi : 17.07.2009

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 fermente et ürünleri, tavuk, peynir, şarap ve bira gibi bir çok gıdanın normal bileşiminde bulunan histamin, histidin amino asidinin, enzimatik dekarboksilasyonu sonucu oluşur. Histamin gıdalarla birlikte yüksek düzeyde alındığı zaman bulantı, kusma, karın ağrısı, ishal, kaşıntı, kızarıklık, yanma, ödem, 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 tuzlandıktan 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 belirtilen 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

[1] Ondokuz Mayıs Üniversitesi, Veteriner Fakültesi, Besin Hijyeni ve Teknolojisi Anabilim Dalı, Samsun.
** This research was supported by Unit of Scientific Research Project of Ondokuz Mayis University (Project Number: Vet-010, 2005)

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 encrasicholus*) 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 definited 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 tones, 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 encrasicholus ponticus* and *Engraulis encrasicholus maeticus*. Among them, *Engraulis encrasicholus ponticus* is known as "Black Sea anchovy" and its height is reached to 18-20 cm. The other species named as *Engraulis encrasicholus 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 belonged 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 imidazoloacetaldehyte 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, nauseation, diarrhea, and formication 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 $O_2/CO_2/N_2$, 5/35/60 (%) and $O_2/CO_2/N_2$, 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 encrasicholus*) in room temperature after salting.

Material and Method

Sampling: It was used anchovy (*Engraulis encrasicholus,* 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 $\pm$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$\pm$1 °C). Histamine analyses were carried out as 2 repetitions through taking samples 0, 2, 4, 6, 8, 10, and 12^{th} 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, Darmstad, 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 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 (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.

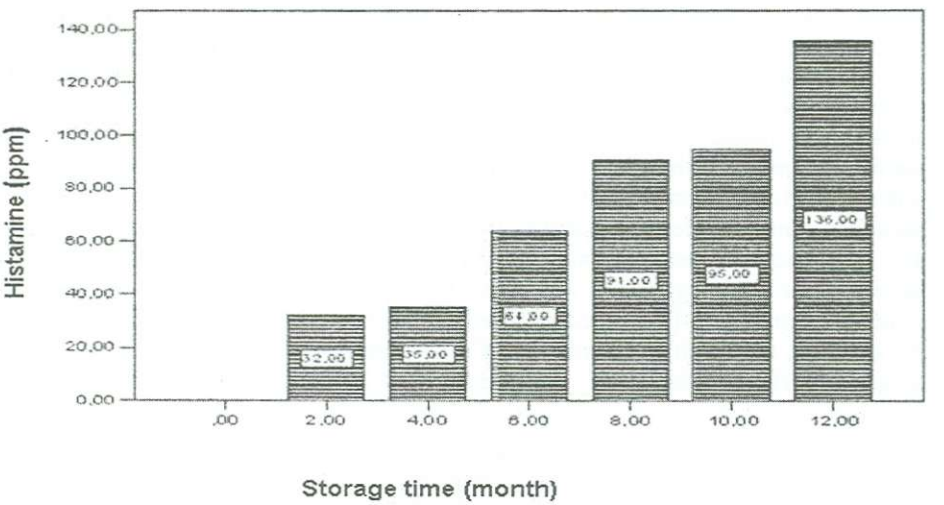

Figure 1: Mean level of histamine in salted anchovy (ppm).
Şekil 1: Tuzlanmış hamsi balığında ortalama histamin düzeyi (ppm).

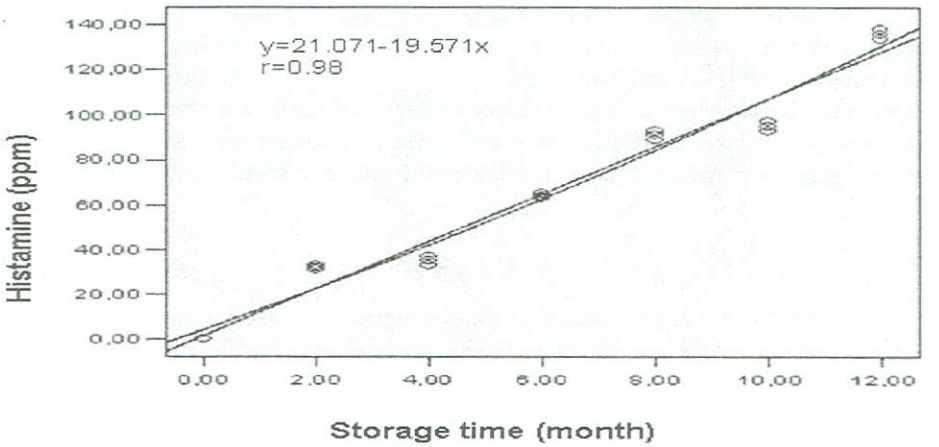

Figure 2: Relationship between the histamine formed and storage time.
Şekil 2: Depolama süresi ile histamin oluşumu arasındaki ilişki

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

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 12^{th} months were found as $0,32\pm0.57$, 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 12^{th} 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 Urunleri Yönetmeliği **(3)** the acceptable limit of 200 mg/kg after 12th month.

References

1. **Anonysmus:** FDA and EPA guidance levels. In Fish and fishery products hazards and controls guide (pp. 245-248, Appendix 5). Department of Human Health and Human Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood, Washington, DC, 1998.
2. **Anonysmus:** Australian Food Standards Part D: Fish and fish products, Standards D1 and D2, Version 18, 2001.
3. **Anonysmus:** Turkish Food Codex-Seafood Regulation.09.08.2005, 2005/25901.
4. **Anonysmus:** Commision recommendation of 10 January 2003 concerning a coordinated programme for the official control of foodstuffs for 2003 (2003/10/EC), OJEC, 7: 76-81, 2003.
5. **Anonysmus:** 1997-2006 yıllarına ait Su Ürünleri İstatistikleri. Türkiye İstatistik Kurumu, 2007.
6. **AOAC:** Official Methods of Analysis of the Association of Offical Analytical Chemists, 16th, 4th revision, AOAC ınternational, Washington, DC, USA, 1998.
7. **Chakrabarti, R.:** Histamine content in dried fish products from Kakinada coast. Fishery Technol, 1991; 78:59-62.
8. **Chuan, Y.Y., Shin-Jung, L., Deng-Fwu. H.:** Biogenic amines, histamine and label of dressed fried fish meat products in Taiwan. Food Control, 2006; 17: 423-428.

9. **Erkan, N., Helle, N., Ozden, O.:** The content of biogenic amines in canned fish from the Turkish market. Berl Munch Tierarztl Wochenschr, 2001; 114 (7-8): 241-245.
10. **Erkan, N., Ozden, O., Alakavuk, D.U., Yildirim, S.Y., Inugur, M.:** Spoilage and shelf life of sardines (Sardina pilchardus) packed in modified atmosphere. Eur Food Res Technol, 2006; 222: 667-673.
11. **Ertaş, H.:** Balıkların soğutma-dondurma ve salamura metotları ile muhafazası. Gıda,1978; 3 (6): 237-246.
12. **Farber, J. M.:** Microbiological aspects of modified atmosphere packing technology-A review. J Food Protec, 1991; 54 (1): 58-70.
13. **Fardiaz, D., Markakis, P.:** Amine in fermented fish paste. J Food Sci, 1979; 1562-1563.
14. **Gokoglu, N.:** Changes in biogenic amines during maturation of sardine *Sardina pilchardus* marinade. Fish Sci, 2003; 69: 823-829.
15. **James, D., Olley, J.:** Summary and future research needs. In: Histamine in marine products: production of bacteria, measurement and prediction of formation. FAO Fisheries Technical paper 252, Sun Pan, B. and James, D. 1985, pp. 47-50.
16. **Karl, H., Schreiber, W.:** Salz-und Suregehalt von marinaden: eine status-quo-untersuchung. Deut Lebensm-Rundsch,1990; 286-288.
17. **Kilinc, B., Cakli, S.:** Chemical, enzymatical and textural changes during marination and storage period of sardine (Sardina pilchardus) marinades. Eur Food Res Technol, 2005; 221: 821-827.
18. **Kim, S.H., Wei, C.I., Clemens, R.A., An, H.:** Review:Histamine accumulation in seafoods and its control to prevent outbreaks of scombroid poisoning. J Aquat Food Product Technol,2004; 13 (4): 81-100.
19. **Kirschbaum, J., Rebscher, K., Bruckner, H.:** Liquid chromatographic determination of biogenic amines in fermented foods after derivatization with 3,5-dinitrobenzoyl chloride. J Chromatogr A, 2000; 517-530.
20. **Kongpun, O., Suwansakornkul, P.:** Histamine Formation During Salting of Spanish Mackerel (Scomberomorus commerson). J Aquat Food Prod Tech, 2000; 9 (1): 21-30.
21. **Lehane, L., Olley, J.:** Histamine (Scombroid) fish poisoning:a review in risk assessment framework, National office of animal and plant health, Canberrege, 1999.
22. **Lehane, L., Olley, J.:** Histamine fish poisoning revisited. Int J Food Microbiol, 2000; 1-37.
23. **Lopez-Sabater E.I., Rodriguez-Jerez J.J., Hernandez-Herrero, M., Roig-Sagues. A.X., Mora-Ventura, M.T.:** Sensory quality and histamine formation during controlled decomposition of tuna (Thunnus thynnus). J Food Protect, 1996; 59: 167-174.

24. **Mol, S., Özden, Ö.:** Tuzlama Teknolojisi. Ed: Varlık, C., Su Ürünleri İşleme Teknolojisi Kitabı, İ.Ü. Yayın No: 4465, ISBN: 975-404-715-4, 2004.
25. **Ozugul, F., Ozugul,Y.:** Biogenic amine content and biogenic amine quality indices of sardines (Sardina pilchardus) stored in modified atmosphere packaging and vacuum packaging. Food Chem, 2006; 99: 574-578.
26. **Örek, H., Bingel, F.:** Karadeniz hamsimiz ve hamsigiller. Bilim Teknik Derg, Temmuz, 2000.
27. **Price, R. J., Melvin, E. F.:** Recommendations for on Board Handling of Albacore Tuna. Sea Grant Extension Program, 1994, Food Science and Technology Extension, University of California Davis.
28. **Sanceda, N.G., Kurata, T., Arakawa, N.:** Accelerated fermentation process for the manufacture of fish sauce using histidine. J Food Sci, 1996; 61: 220-225.
29. **Shakila, R.J., Vasundhara, T.S.:** Formation of histamine and other biogenic amines during storage of freshwater fish chunks. Asian Fish Sci, 2002; 15: 1-6.
30. **Sümbüllüoğlu, K., Sümbüllüoğlu, V.:** Biyoistatistik. Hatipoğlu Yayın Evi. Ankara, 1997.
31. **Tunalı, Ş.:** Balık tuzlama metotları. Et ve Balık Endüstri Derg, 1976; 1 (4): 36-40.
32. **Veciana-Nogue´s, M.T, Vidal-Carou, M.C., Mariné-Font, A.:** Histamine and tyramine in preserved and semi-preserved fish products. J Food Sci, 1989; 54: 1653-65.
33. **Veciana-Nogue´s, M.T., Marine´-Font, A., Vidal-Carou, M.C.:** Biogenic Amines in Fresh and Canned Tuna. Effects of Canning on Biogenic Amine Contents. J Agric Food Chem 1997; 45: 4324-432.
34. **Windyga, B., Grochowska, A., Sciezyńska, H., Górecka, K., Fonberg-Broczek, M.:** Determination of histamine content in canned fish products determined by the colorimetry method of Hardy and Smith. Rocz Panstw Zakl Hig, 1992; 43 (2):193-199.
35. **Wootton, M., Silalahi, J., Wills, R.B.H.:** Amines levels in some Asian seafoods product. J Sci Food Agr, 1989; 49: 503-506.