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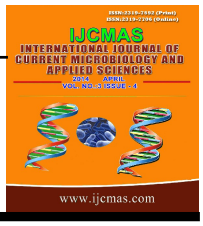

Original Research Article

The study of four biogenic amines (Spermidine, Putrescine, Histamine and Tyramine) changes in Shanak yellow fin fish *(Acanthopagruslatus)* within ice storage by HPLC

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A B S T R A C T

Keywords

Biogenic amines, Histamine, Spermidine, Putrescine and Tyramine, Shanak yellow fin fish, HPLC

Given the importance of fish and another marine products as a valuable protein source available and the rapid perishable of this products , 21 Shanak yellow fin fish samples were collected from the Bahrekan coastal fishing, Hendijan. This study was carried out In order to study of the changes of biogenic amines during storage in ice.In this research,the biogenic amines was evaluated every 3 days , by using HPLC.The mean data were compared by conducted of one-way variance analysis (ANOVA) with confidence coefficient (p=0.05). In this research four amines as:Histamine ,Spermidine , Putrescine and Tyramine were studied . The results showed that the average rate of them were lower than accepted world standards . The highest value of Histamine were reach to 3.4 mg/g in all samples, which is less than the minimum level approved by the Europe FDA (20 ppm). As well as,the value of Spermidine was 0.76 mg/g and the value of Tyramine was 0.56mg/g. HPLC was not able to detect Putrescine.

Introduction

Biogenic amines

Biogenic amines are a group of nitrogen compounds that formed by the decarboxylation effect of amino acids or amination and transamination of aldehydes and ketones. These compounds actually are the organic bases with low molecular weight that Synthesis by microbial, plant and animal metabolisms. The chemical structure of biogenic amines are aliphatic (Putrescine, Kadaverin, Spermine and Spermidine), or aromatic (Tyramine and Phenyl ethyl amine) or as a heterocyclic (Histamine and Tryptamine).

The amines such as polyamines, Spermidine , Spermine, Putrescine and Kadaverine are necessary compounds in live cells and they are important for

regulation of nucleic acids function and synthesis of protein and probably in stability of cell membranes

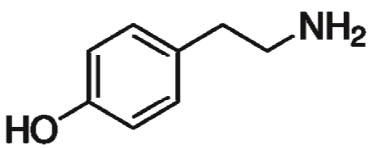
Biogenic amines may be produced by two ways as follows:
1) Endogenouse or internal decarboxylation that including the production of amines by decarboxylase enzymes found in fish and another marines internal cells .
2) Exogenouse or external decarboxylation occur by microorganisms that can produce external cell decarboxylase (Shalaby1996).

Histamine in fish

Histamine in fish is resistant to heat and cannot remove by processes such as the cooking, canning, freezing and smoking (Becker et al., 2001 and Kim et al., 2001). Fresh water fish are not considered for the presence of Histamine as a threat to human health (Dalgaard et al., 2008 and Rabie et al., 2009). The levels of Histidine in this category of foods are the important factor influencing the formation of Histamine in fish and its products (Chamberlain, 2001). Therefore there is also present the possibility of risk during long-term storage of fish (*Lehane et al. ,2000).*

Tyramine:

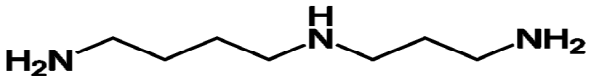
Tyramine naturally found in some foods. Production of this compound is due to breakage of protein chain of old foods. Tyramine produced by effect of tyrosine decarboxylase enzyme and decarboxylation of tyrosine via biochemical way.



Tyraminestructure

Spermidine :
Spermidine is one of the polyamines that Involved cell methabolism. ts known effects are as followed:

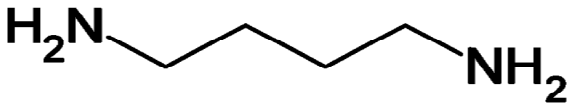
1-regulation of growth
2- prevention of aging
3-inhibition of nitric oxidesynthetase enzyme
4-Participation in the copy Process of RNA
5-stimulation of poly nucleutid kinase of T4 and T7
6-use in purification of proteins joint to DNA



Spermidine structure

Putrescine:

Putrescineis an organic chemical compound with bad odor that produced with Kadaverin from breakage of amino acids in living or dead organisms. Putrescine is the cause of bad odor due to putrefaction and decay of protein(meat).Putrescine is very toxic. Putrescine can change to spermidine by decarboxylation of S-adenozylmethionin .The polyamines (putrescine is the simplest one of them) is major growth factor for cell division. Characteristics of Putrescine was first discovered in 1885 by Berlin ,the German physician.



Putrescine structure

Advantages in maintaining and sustaining the quality of fish:

Ice does not just cold fish but all fixtures, equipment and the environment that it is in contact with them. When the ice melts continuously bacteria, blood and slime from the fish body wash and thus greatly reduces surface contamination Best results are achieved when small pieces of ice are insufficient contact with fish.

Chilling effect on the chemical and biochemical activities

Chemical and biochemical reaction rates to vary logarithmically with temperature. Therefore reduce the product temperature decreases enzyme activities, especially in the fresh products.

Literature review

Study of biogenic amines and the possibility of their use as quality control indicators carried out on Heik fish(Merlucciusmerluccius) during keeping in ice, exhibited that the amount of biogenic amines during keeping in ice was progressive increased and was associated with the change rate of trimethyl amine (Ruiz-Capillas & Moral, 2001). Rossano et al., (2006) studies the effect of storage temperature and freezing time on the rate of Histamine in Europian Ancho fish by use of capillary electrophoresis method. The results showed that the rate of Histamine production was considerably increased at 20 °C while clearly, freezing stops or slows the process.

Materials and Methods

Standard solutions of biogenic amines

Store standard solution

Standard solution was prepared by mixing of 0.1g of Histamine standard in 10 ml TCA 5%. The concentration of solution has been achieved to10000 µg/ml. This solution was stored in the refrigerator.

Working standard solution

1 ml of the store standard solution was removed and its volume reached to 10 ml by adding distilled water, so working standard solution has been achieved to the concentration of 1000µg/ml.This solution was stored in refrigerator on a weekly basis.

Fish samples

21 pcs fresh Shanak yellow fin fish were taken directly from Hendijan Port. After washing, the fishes were put in the boxes containing crushed ice (the alternating layers of ice and fish) and transported to the laboratory. The common crushed ice rate for transporting and keeping of fish is three parts of ice and one part of fish in England. Thus, the ratio of 3 to 1 Shanak yellow fin fish and ice was fixed during total period by adding alternative crushed ice daily. The wastewater due defrast of ice was drained from the bottom of box. The changes in temperature cause changes in the amount of bacteria. The four chemicals were evaluated at regular intervals in times of zero, 3, 6, 9, 12, 15 and 18days.

Extraction

Biogenic amines extraction according to the method proposed by Mietz and Karmas (1978) that also is adjusted for other amines, as follows:

50 g of fish sample mixed with 75 ml of TCA 5% (5 g in 100 mL water) for 2 minutes, and were centrifuged at 4000rpm for 10 min. The upper phase (supernatant) was filtered by cone and cellulose filter, and then transferred to250mL volumetric flask. The remained sediment remixed together by adding of 75 ml of TCA 5%

and above steps was repeated. The extraction also took for the third time to reach the total volume to 225 ml. After washing the funnel with some TCA 5%, the volume of the solution reached to 250 ml. This mixture actually is the dilution 0.2 of the fish sample and capable in the refrigerator for 2-3 weeks.

NaCl 4g, NaOH 50% 1ml and chloroform-butanol (1 + 1) 5 ml were put into centrifuge tubes with glass cap, after that 10 ml of solution per sample (equivalent to 2g of sample) and 10 ml of TCA 5% (as a control), has been added to above centrifuge tubes . The tubes were severely shaked for 2 minutes and again centrifuged for 5 min at 3000rpm. The upper phase (organic) transferred to a 60 ml separating funnel. N- Heptane 15 ml and then HCl 0.2N 1 ml was added and severely shaked. This step also was repeated three times in total. In the next step by adding of distilled water 1ml to the funnel, the extraction process was continued for confidence of completely extraction of biogenic amines. Put the tubes in the oven (80 °C) to help dry the solution by gentle stream of air.

Derivatization

Derivatization is based on the method of Dawood and colleagues (1988) as described below:

Add 1 ml of NaOH 2M to the retained dry matter or the standards outlined in the previous section. Again, benzoylchloride 5 ml was added and well mixed by vortex. The solution was allowed to stand for 20 minutes. Then added 2ml of saturated NaCl solution to stop the derivatization process. Add 2 ml of diethyl ether, and well shaked. The solution was centrifuged at 2500 rpm for 5 min. Afterward the upper phase (ether) transferred to a clean tube and evaporated in a gentle stream of air to dry.

HPLC calibration

Calibration curves

Based on the concentration of Histamine in the original sample, enough Histamine standard from working standard solution transferred to a glass tube and dried with

AUFS(Absorbance Unit Full Scale)	0/200
Data mode	Absorbance
Wave Length	254 nm
Minimum Hight	1000
Threshold	100
Polarity	+
Ratio min (Au)	0/100
Minimum Area	15000
Filter type	Hamming

agentle streamofnitrogen, then this Histamine standard was injected to the device by two repeates according to the method as described in the derivatization process.

Calibration curve as a linear equation(y: is mean area under the curve of each concentration, based on $\mu V * \sec$ and X is mean area under the curve of each concentration, based on ppm) was calculated and plot with Lest Square method and by use of Excell software. The correlation coefficient(R^2) to check the linearity of the results calculated by this software that shown by device in the calibration curve.

Blank sample

By the parallel on chemical analysis of the samples, 10 ml of TCA 5% was tested beside the original samples. This sample was used as the zero standards.

Analysis by HPLC

This stage is based on the method carried out by Dawood & Colleagues (1988). Added methanol 200 ml to the remaining dry matter and filtered by Millipore 0.45 mm .20 micro liters of this solution was injected in to the device by Hamilton syringe. The measuring basis of biogenic amines separation is using the UV absorbance at 254 nm and a reversed-phase with the isocratic system of methanol and water (volume ratio 70 and 30) with a solvent flow of 1.1 ml per minute at room temperature. Other device settings are as follows:

Based on the matching of retention time of unknown samples with standard samples, biogenic amines were identified and their concentrations were determined by notice to the area under the curve and according to the standard curve.

Statistical Analysis

Statistical analysis of data due chemical indicators was performed with SPSS. The one way variance analysis procedure (ANOVA) is used to determine the presence or absence of a significant difference at95% level between the obtained values of each index at zero, 3, 6, 9, 12, 15, 18 days. Also, at least significant difference test was used in order to accurately determine the presence or absence of significant differences among treatments.

Results and Discussion

Measurement of Histamine level on different days:

The Histamine level was additive from first to sixth days and at the maximum level reach to ¾ mg/100g. Afterward, the Histamine level gradually was decreased to its lowest level in the study at 0.5 mg/100g with significant difference ($P<0.05$) on sixth to 12th days, because of the composition of Histamine with compo sable proteins. The Histamine level was additive after 12th days later and the increasing observed significant difference ($P <0.05$).This increasing level trend persisted until the end of experiment (18th day).Obtained results are as follow histogram:

Measurements of Tyramine on different days

At the beginning of the research and at the first day of Tyramine content was high and gradually reduced .This trend continued until the12th day and in this day, the amount of Tyramine reached to its lowest level at 0.14mg per 100 g and then slowly began to increase and from12th day until the final day increasing trend was observed. The amount of Tyramine was at the highest level and reached to0.56mgper 100 g during the final day of the investigation.

Measurement of Spermid in level on different days

The amount of Spermid in decreased from the first day to the ninth day, with no significant difference was found ($P<0.05$) the amount of Spermid in reached to its lowest level at 0.26mg per 100 g and then, from 9^{th} day the amount of Spermid in slowly began to increase and reached to

the highest level (0.76mgper 100g) until the final day of the investigation. Significant difference was found.

The survey of biogenic amines concentration on different days

In this study, after 18 days, the value for the highest amount of Histamine (3.4mg/100g) was obtained in the sixth day and the lowest amount (0.5mg/100g) was obtained in the 12th day. FDA (1998) confirmed the average concentration of Histamine in fish at 5mg/100g for health assurance of products, and more than this level is undesirable. Europe Union has suggested that the average concentration of Histamine in fish should not be more than 10 mg of Histamine per 100 g of fish muscle and it seems to be good for general health (Lehane and Olley, 2000). As well as the SABS (South African Boreau of Standard) and AFSC (Australian Food Standard Code), respectively, 10mg/100g and 20mg/100g of Histamine levels in fish muscle has been suggested as a limit. Thus, the results of this study are consistent with international references. Fresh fish usually have low amounts of Histamine in limit of 0.1mg/100g as well, red meat fishes, such as sardine and mackerel have higher levels of Histamine in compare with white meat fishes like cod and Hamour (Kagawa, 2000). It should be noted that the production of amines in muscles can be vary according to the muscle type (white or dark), different slices (in area of tail or near it) and environment temperature, fishing season and fish size. Also, environmental conditions such as temperature, rate of pollution, bacterial infestation of fish, etc. influences the amines production. In addition, the type and composition of Histamine-producing bacteria can be affected by other factors such as feeding behavior, geographic location, nets and fishing tools, time from fishing to freezing, temperatures used in freezing, ice quality, how to order fish on each other, the quality of the transportation and storage of fish baskets, fish transport while fishing and afterward , temperature and the salt content in water, the processing method used in the final product and the conditions of shopping centers such as the water used to supply fish to keep it moist and keep the fish in the time period offered to sell (Yoshinaga and Frank,1982; Smith et al., 1993; Sabater et al., 1996). Histamine is a composition that its consumption is actually dangerous for human and it was several reports of extended poisoning due to consumption of marine products that contain high levels of Histamine (Hwang et al., 2003). The amount of Histamine in pelagic fishes is greater than of benthic fishes ($P< 0.05$). The results of this study showed that the amount of Histamine in Pelagic fishes is 2.74±0.52 mg/kg and in benthic fishes is 1.39±0.28 mg/kg, which is a significant difference ($P<0.05$) between the amount of Histamine in pelagic and benthic fishes.

There is no reports of Histamine poisoning outbreak in Iran, that is may be because of the absence of an accurate diagnosis and recording (Hosseini et al., 2007 and Kamkar et al., 2003). Indicated that the maximum amount of Histamine, in the thick sections and mean sections of fresh fish Hoover was 5.24 and 8.53 mg/kg,

Figure.1 Comparison Chart of Histamine on different days (SD ± mean)

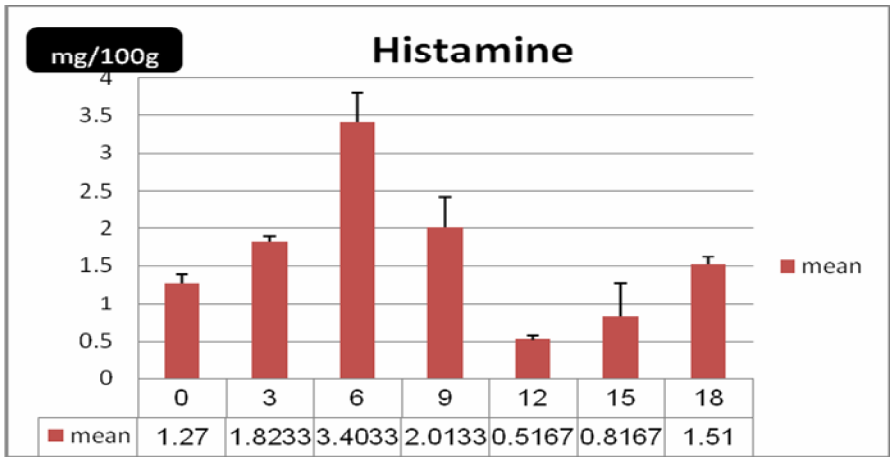


Figure.2 Comparison Chart of Tyramine on different days (SD ± mean)

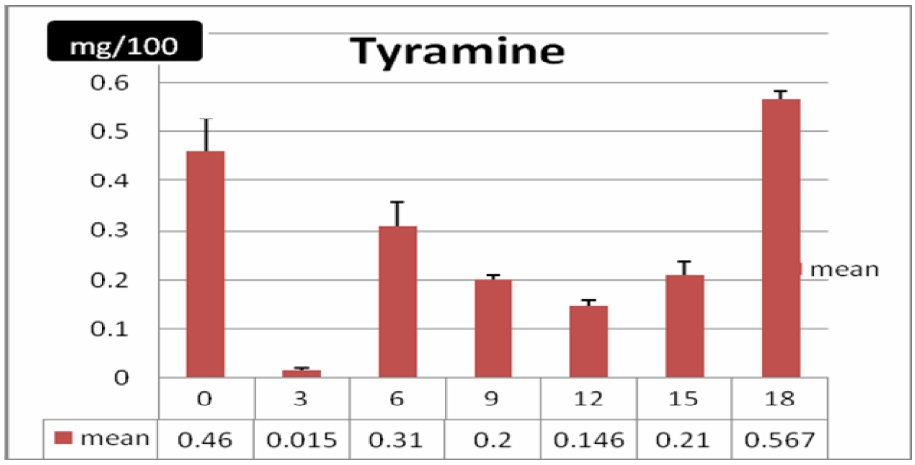
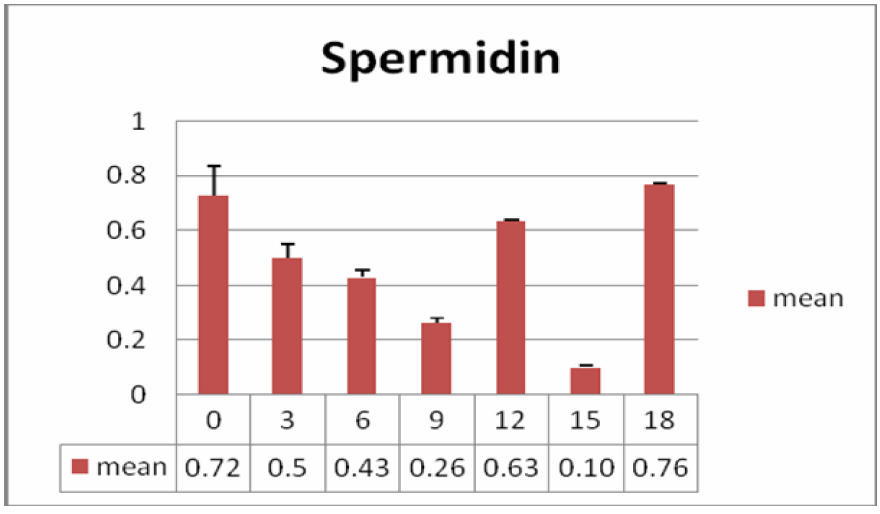
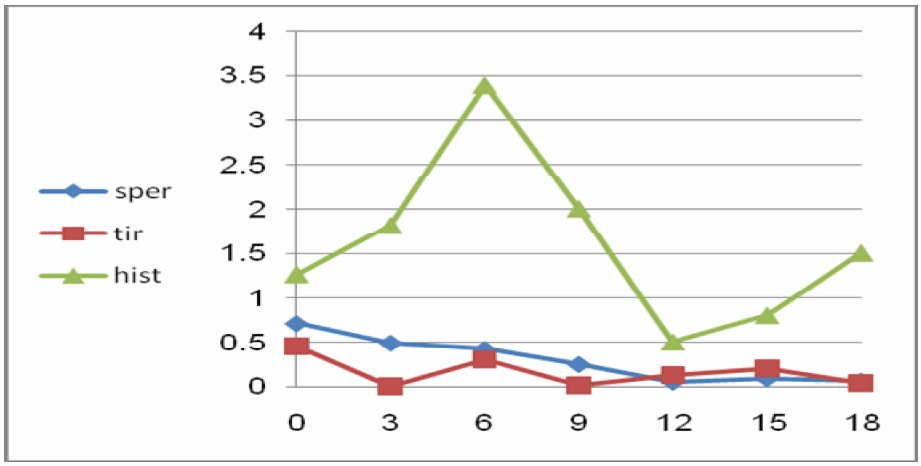


Figure.3 Comparison Chart of Spermidin on different days (SD ± mean)



Graph of general changes of biogenicamines in Shanak yellow fin fish



consequently (Mercogliano et al., 2008). According to the diferrent reports (Arnedo et al., 1989 and Anon, 1992) about Histamine poisoning outbreaks, only the Sword fish and Tuna fish were involved in Spain. The Histamine levels between 20 to50 ppm represent fish putrefaction .Values represent the amount of 50ppm of Histamine is considered as an active dose, that cause fish spoilage and such fish products are unusable (Codori and Marinopoulos, 2010; US FDA, 1998; Wenta& Liao, 1992; Windyga et al., 1992). The survey by FDA (1970) indicated that the amount of Histamine in fresh Tuna fish always was less than 1 ppm and in another fresh fishes was near to 5 ppm or maximum 20 ppm (Wenta and Liao, 1999 and Windyga et al., 1992). In another report, all 195samples of mackereland sardines studied, Histamine levels were below the limit (*Kasha and Noriens, 1988).* Gajewska et al (1991) were studied on Tyramine level in fish and dairy products and reported that Tyramine level in raw fishes were between 0 to2.6mg per100g (*Gajewska et al., 1991*).In present study, The amount of Tyramine on the 12th day, was at the lowest level (0.14mg per100g) and on the big eye tuna and yellow fin, it was shown 18th was at the highest evel(0.51mg per100grams), the amountis farless than the amount listed in above reference.

In a study that carried out by researchers on the corruption of tuna fish and detection of corruption index they introduced Putrescine, Tyramine and Kadaver in as biogenicamines index or quality index. They Stated that the total amount of them (all three biogenicamines) should not be increase from 10.1mgper 100 g (Veciana et al., 1997).

In a study was carried out in Italy during the years of 1988-1995, it was shown that Histamine levels in 41 samples of fresh fish, tuna and mackerel, was below the limit (Donn, 1991). The study was carried out on the different fishes and tuna reported that the amount of Histamine level in fresh fishes was less than 3 mg/100 g (Laurent et al., 1995). Another researcher's survey the amount of Histamine level in tuna and reported that all samples contain Histamine was not higher than the European standard level (Sabater et al., 1996). As well as in another study was done by Chamberlin on three species of fishes, including Albakur, that, there is no significant difference in

the amount of Histamine values among the three studied species. Histamine levels in all samples stored at low temperature, was at lower limit and by increasing temperature, the amount of Histamine was increased (Chamberlin, 2001). Effect of the temperature on Histamine production and the freshness of the yellow fin fish have been investigated. The results showed that the Histamine levels at zero °C, was 2.78mg/100g and with increasing temperature of fish storage, also increased the amount of produced Histamine as well as fish putrefaction (Guizani et al., 2004). The report states that in none of the 29 samples of yellow fin fish and dolphins, Histamine levels were not over than 2ppm. These low values obtained are not unexpected, since similar results have already been obtained in the case of herring's (Chamberlin, 2001 and Salguero & Mackie, 1987). As noted, the quality of marines is influenced by the type and storage conditions of them and according to the survey results of some researchers can be expressed that production of biogenicaminesis dependent on biogenic factors, especially bacteria in every case.

Some bacteria have decarboxylase enzyme, are capable of breaking down free amino acids and will change them into biogenic amines. The conditions of fish storage can be limited in some biogenic amines production as well as growth of some bacteria. Thus, the Histamine-forming is related to the bacteria activity in the upper temperature (mesophilic bacteria). In a research, Lukton and Olcott (1958) reported that higher Histamine production is in the red muscle of fishes such as mackerel fishes in compare to the white muscle of fishes such as cod fish, so cod fish have low level of Histamine and mackerel fishes have higher Histamine. In 1987, a study was took place on marine fishes by "Taylor" and colleagues, found that a large number of pelagic marine fishes especially those that have regularly and quickly swim, the proportion of their red muscle is more developed .These fishes were quickly exposed to putrefaction and poisoning by their consompotion will be more dangerous because of having red muscle and high concentration of the histidine amino acid. A study in Senegal by Laurent and colleagues has conducted on different kinds of fishes. Fish samples were collected from different regions of Senegal and evaluated. The results showed that the Histamine level in fresh fish samples was lower than their tuna (3mg /100g).This level is less than accepted limit (20mg /100g) in Senegal (Laurent et al; 1995) . A study conducted by Baranjy and colleagues on samples of tuna, sardines, salmon and mackerel showed that Histamine levels in samples were 10.500to 0.1547 mg/100 g, and the resulting values were below limit specified in Yugoslavia (20 mg/100g) (Baranji et al., 1997).

Not worthy that the values obtained inthis study, for Spermidine on the 9th day wasatthe lowest level (0.26mg per100g),And on the 18^{th} day was at the highest (0.76mg per 100 grams), That seems to be the appropriate valuesin the range of healthy for human consumption. Ina similar study on a full and fillet srain bow fish trout, the amount of Tyramine were reported on0.2and0.4microgramsper gram inthe 18thday. It is worth noting that in the investigations, no indication has been obtained for Putrescine in the tested samples. Should be noted that studies have shown that psychrophilic bacteria are the main causes of the formation of Putrescine (Chytiri et al., 2004).

Whatevere the fish is making a late frost

and improper the conditions of its transporting and keeping in ice, so autodigestion processes and biogenic amines decarboxylased bacteria act faster. The obtained Results of present study showed that the Histamine increased initially and then decreased and afterward increased .This reduction of Histamine is due to the growth of Histamine-breakup bacteria (Sato et al., 1994). The amount of Histamine in this investigation is lower than standard limit by the FDA. The amount of Tyramine in four species investigated was lower than the limitstated in the world.

Due to the importance of biogenic amines in fish's body and its relationship with human health, it is recommended that similar studies carry out on other species of fishes. Since the biogenic amines during the production process, especially canned fishery products and fish handling methods will increased, so it have been proposed the research on the amount of biogenic amines during the process of production of tuna. Since the storing of fish in non sanitation conditions especially as non freezing, strongly increased the concentration of biogenic amines, so it have been proposed that immediately keeping of fishes in the ice after fishing, or rapidity processed or freezing.

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