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The Effects of Different Thawing Methods on the Hygienic Quality of the Canned Tuna

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ABSTRACT: The consumption of canned tuna in the country has been increasing and some factors are effective on the chemical and organoleptic qualities of this product during transport. The aim of this project is to study methods to reduce histamine and improve the organoleptic properties of the product. Three thawing methods consisted of immersion, cold water shower and open air has been considered. Beside, two storage conditions at -18 and 4 °C were chosen to examine histamine, TVN, pH and organoleptic properties of the canned tuna. The results indicated an increase in the microbial load at 4 °C as compared to -18 °C and also showed that the formation of biogenic amines is certainly affected by the temperature ($P < 0.05$). According to the results the presence of viscera led to accelerate the spoilage of the fish and organoleptic properties. According to the results cold water shower might be considered as the best way of thawing ($P < 0.05$).

Keywords: *Canned Tuna, Hygienic Quality, Thawing Conditions.*

Introduction

The ever growing population and the increasing demand for different kinds of food including protein foods have caused the problem of supplying healthy and enough food to become one of the critical issues in many countries, particularly in the developing countries. Sea food has high nutritional value and the demand for it has caused to expand the shelf life. After catching the fish, storage conditions greatly affect the quality, therefore, it seems necessary to gain a better understanding and deep knowledge of the origin of these changes particularly physicochemical and microbiological changes in fish and other aquatic animals in different stages of storage to determine the duration of storage and the shelf life (Bita *et al*., 2011; Tironi *et al*., 2010; Muela *et al*., 2014).

Fish is the most common food that leads the spread of histamine and has the most important role in the family of scombroidea. Family of scombroidea includes tuna, mackerel, bonito, etc. and due to their high consumption in the world, most cases of scombroid poisoning have been accounted for them. The volatile nitrogen is also considered as a spoilage indicator of marine products (Nordt and Pomeranz, 2016).

Despite the presence of valuable amino acids in tuna, histidine can act as a substrate for the enzyme histidine decarboxylase and as the result of the enzymatic activity of histidine is converted to histamine (Chen *et al*., 2008). The most important microorganisms that are able to produce enzyme histidine decarboxylase include proteus species, especially Morgagni which

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is also known morganellamorgagni, klebsiellapneumoniae, photobacterium species and pseudomonas species. The levels of histamine in spoiled fish products can significantly vary depending on the types of product, spoilage bacteria and storage conditions which lead to spoilage. The spread of histamine-forming bacteria is limited and histamine is not always produced in the spoilage process (Cinquina *et al*., 2004; Kovacova-Hansukova *et al.,* 2015).

Factors affecting histidine decarboxylase might vary, especially in a decaying fish. The variety might be related to the difference in species of fish, processes related to processing and the temperature. If the amount of histamine becomes more than 20 ppm in the muscle tissue of raw fish and 50 ppm in canned fish, the fish and their products will become undesirable for consumption (Anon, 2010). Moreover, there are patients with histamine intolerance and this matter doubles the importance of investigating the level of histamine in aquatic animals (Koohdar and Razavilor, 2012).

Katsuwonus pelamis belongs to scombroidea and the rate of catches has been increasing in recent years and is caught in the Persian Gulf and Oman Sea and were selected for this study. The fish contains more than 1,000 mg/100ghistidine in the flesh and can increase histamine poisoning in this type of fish (Shahnazyanes *et al*., 2015; Tao *et al*., 2011).

Several studies have been investigated concerned with the importance of the presence of histamine in fish including a review of the histamine content in tuna, mackerel and fish products (fish sauce and dried fish) in Germany, India, Thailand, Cambodia, the Philippines, Japan and China and histamine was detected in 21% of the samples at the levels of50 ppm and in 9% of the samples the concentration was higher than 500 ppm (Muscarella *et al*., 2013). Therefore, in this research it is aimed to investigate the amount of histamine, TVN and pH in different thermal and thawing conditions for the mentioned products.

Materials and Methods

- *Samples*

Katsuwonus pelamis samples were supplied from a factory producing canned tuna in Qazvin. The samples were subjected to one of thawing conditions (cold showers, immersion in cold water and ambient air) and different storage conditions (temperature -18 and 4 ˚C) were applied in order to determine the parameters mentioned earlier.

- *pH and volatile nitrogen determinations*

pH was determined by pH meter with a combined glass electrode and temperature probe (pH meter Micro pH 2002; Crison, Spain). The total volatile nitrogen is considered as an indicator of spoilage in marine products and was determined according to AOAC method number; 999.01 (AOAC 2000).

- Histamine *determinations*

The extraction of histamine was carried out according to the procedure described by Hwang et al., (2003), The extract was injected to a reverse Phenomenex column: Luna C8 column (250 mm length); of HPLC equipped with fluorescence detector that was set at of 343 and 445 nm, respectively. The column temperature was set at 40 ˚C. The mobile phase was passed at a speed of 4.0 mm per minute by the model K-120 pump connected to the HPLC instrument. The mobile phase consisted of solutions A and B where solution A consisted of 0.01 M phosphate and 0.002 M sodium 1-decanesulfonate and solution B contained ultra pure acetonitrile. The mobile phase was prepared by combination of 80% solution A and 20% solution B.

- *Sensory evaluation*

Sensory characteristics such as color, odor and texture were assessed by 8 trained assessors filling out a questionnaire using Hedonic method. The products were marked between one (undesirable) and nine (very desirable) scores as assessed by the assessors (Herald *et al.*, 2008).

- *Statistical analysis*

The statistical analyses were carried out using SPSS software version 19. One-way analysis of variance at 5% was employed to assess the presence or absence of significant differences between the obtained values.

Results and Discussion

The results of the total numbers of microorganisms in fish samples transported by different methods are shown in Table 1.

Table 1. Fish samples transported by different methods

Transport Condition	Microbial load (CFU)
transported at -18 °C	4×10^3
transported at 4 °C	8×10^4

The data are means of triplicate measurements

The mean of microbial load in the transportation at 4 °C had higher growth and survival rate as compared to the transportation at -18 °C. In this study, biogenic amines were used as chemical indicators of spoilage in transported tuna samples at -18 and 4 °C. Extracts from muscle tissue of each fish was injected into the HPLC device and histamine concentration was calculated by a linear equation from the calibrated standard curve, Figures 1, 2, 3 and 4present the chromatograms concerned with standard, positive, negative samples and calibration curve. Among the separation methods, high-performance liquid chromatography had the best performance due to the high sensitivity, high accuracy, and the ability to analyze non-volatile and temperature-sensitive samples. Extensive studies have been carried out on different species of fish and fish products (Hoseini *et al*., 2006; Kohdar & Razavilar, 2012).

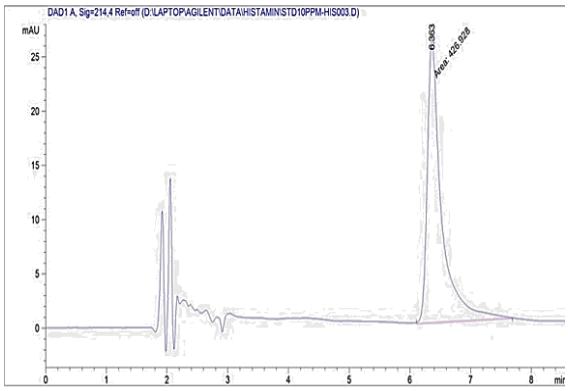

Fig. 1. Standard chromatogram

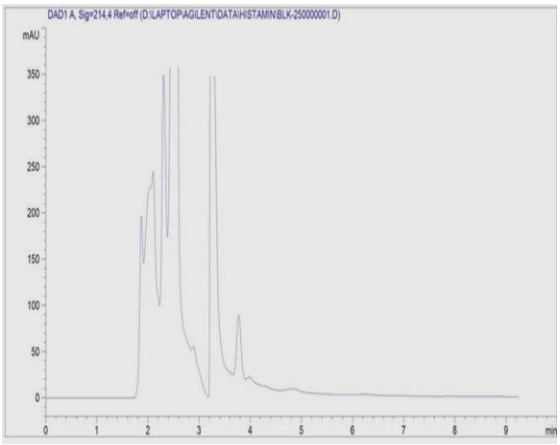

Fig. 2. Negative chromatography

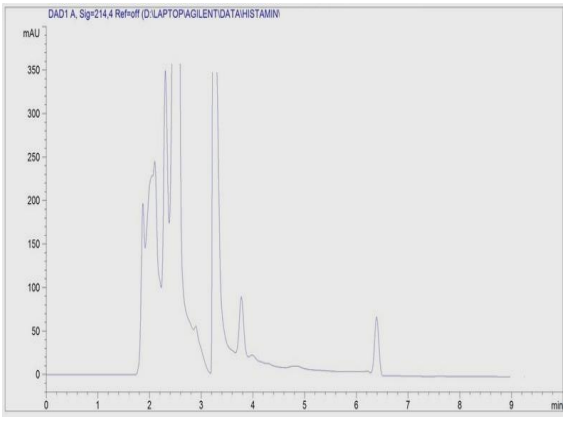

Fig. 3. Positive chromatography

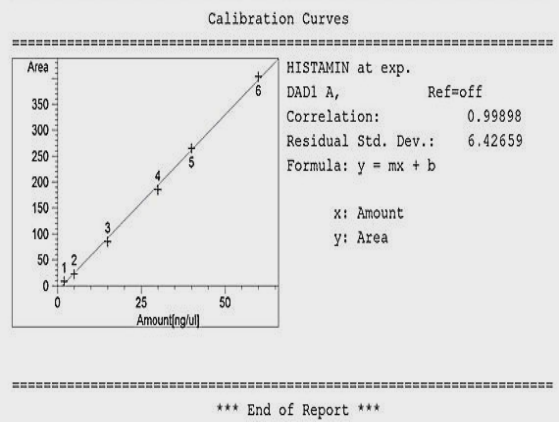

Fig. 4. Calibration curve

The mean of histamine, TVN and pH concentrations are given in Table 2.

As presented in Table 2, when the fish is un eviscerated, the temperature of transportation and preservation affect the histamine and TVN with minimal effect on the pH. Eviscerating and canning process with viscera doesn't have a significant effect on the pH but when there are viscera, the level of histamine and TVN is higher than when it is eviscerated completely. This suggests the influence of intestinal bacteria on the concentration of histamine and volatile nitrogen. This part of the results is in agreement with the research conducted by Chang *et al*. (2008) and Hu *et al*. (2012) who showed that during storage at high temperature, the level of mesophilic bacteria increases and has a direct relationship with the formation of biogenic amines. According to the report by Kamkar *et al*. (2004), the level of histamine was higher than the permissible limit of 50 ppm in 41.25%of canned tuna fish samples. In general, it can be expected that thawing by immersion cannot prevent histamine from increasing as well as thawing by cold water shower but it is more appropriate than thawing in room temperature and evisceration helps to reduce amino acid decarboxylation process and consequently the formation of histamine. As a result, according to this study, the level of histamine is less in thawing and thermal conditions when the fish is eviscerated before being cooked. The level of TVN is affected by the temperature and the higher the temperature the higher is the spoilage of fish and as the result the level of TVNis increased.

Table 2. Discriptional statistics of variables in different thawing methods

Method	Temperature (C°)		Histamine (mg/100gr)	TVN (ppm)	pH
Immersion	-18	With viscera	2.07^{ef}	5.82^{fg}	5.66
		Without viscera	0.33^{h}	5.58^{h}	5.66
	4	With viscera	2.49^{e}	6.60^{e}	5.60
		Without viscera	1.09^{g}	5.94^{f}	5.66
Cold water shower	-18	With viscera	1.89^{f}	5.20^{i}	5.53
		Without viscera	0.00^{h}	4.91^{j}	5.63
	4	With viscera	2.06^{ef}	5.77^{fg}	5.83
		Without viscera	0.87^{g}	5.67^{gh}	5.66
Thawing in room temperature (21°C)	-18	With viscera	10.42^{b}	11.56^{b}	5.86
		Without viscera	3.90^{d}	10.63^{d}	5.66
	4	With viscera	42.63^{a}	14.63^{a}	5.63
		Without viscera	4.43^{c}	11.20^{c}	5.63

The data are means of triplicate measurements. Values with different superscript upper case letters in a column are statistically significant at $P < 0.05$.

When thawing is carried out by with cold water shower and evisceration, the level of histamine, TVN and pH is significantly correlated with increasing temperature and when thawing is conducted when uneviscerated, the increase in temperature will increase the amount of histamine and TVN but it does not have effect on the pH changes. These results are in good agreement with the results of Salmani *et al*. (2008) who described that the increase in temperature will increase the level of histamine. It is considered that thawing with cold water shower when uneviscerated has higher level of histamine and TVN than when it is eviscerated completely. This indicates that the presence of intestinal bacteria has an effect on the level of histamine and TVN. Torido *et al*. (2016) indicated the increase in the level of histamine at 4 ˚C in 5 days storage of fish, which corresponds with the results of this study.

The results of thawing in room temperature represent a significant increase in the level of histamine as compared with two other methods. The level of histamine and TVN also indicates the effect of rising temperature on fish spoilage but there is not a logical process regarding the pH .According to Oliveira *et al*. (2012) the storage conditions have great effects on maintaining the quality of fish. Due to the fact that the fish caught spend a long time concerning transportation to reach the customer, the storage conditions have a strong influence on maintaining the quality of fish. A study carried out by chen *et al.* (2008) the fish were washed immediately after capture form sea and kept in low temperature with ice and no histamine was detected in 95% of the samples; and in 5% of the samples, histamine was reported between 4.95 – 6.90 ppm and it was concluded that the storage conditions have great influence on the keeping quality of the fish, and this is in agreement with our study. Salmani *et al*. (2008) investigated methods of fish storage in a tank of chilled sea water and ice in different seasons of the year and compared it with the traditional method and found that the concentration of histamine until 10 hours after the capture during four seasons in traditional method is much greater than the storage method in a tank of cold water and crushed ice, which this is also in line with our findings.

Considering Tables 2, there are significant differences that indicate various treatments in the chemical variables because the amount of Fischer test is more than 3 and significance level is lower than 0.05 and only in the case of pH there is not a significant difference because of one way variance comparison and the probability level is more than 0.05 and Ficher is less than 3.

The temperature increase using thawing by immersion in water has negative impact on organoleptic factors and has lower overall acceptability. The difference lies in the color of products since the temperature leading to the spoilage significantly affects the color of the product while the texture is the least affected. A study carried out by Chamberlin (2001) on three types of tuna including albacore tuna, big eye tuna and yellow fin tuna and it was concluded that there are not significant differences in the concentrations of histamine in these three products. The amount of histamine was lower than the permissible limit in all the samples stored at low temperature and rising the storage temperature of fish increased the amount of histamine.

According to the data in Table 3, the viscera and inappropriate storage temperature influenced negatively the organoleptic test. This study clearly shows the effects of temperature increase on the concentration of histamine and TVN. The increased amount of TVN and particularly histamine has unfavorable impact on the flavor of the final product.

Table 3. Sensory properties of fish samples

Method	Temperature (C°)		Color	Odor	Texture	Overall acceptability
Immersion	-18	With viscera	7.00^{b}	6.80^{a}	5.65^{c}	5.83^{d}
		Without viscera	7.50^{a}	6.83^{a}	6.00^{b}	6.80^{a}
	4	With viscera	3.00^{g}	3.37^{d}	5.22^{c}	4.50^{fg}
		Without viscera	4.37^{e}	3.51^{d}	3.71^{e}	6.00^{d}
Cold water shower	-18	With viscera	7.00^{b}	6.71^{a}	6.85^{a}	6.28^{c}
		Without viscera	6.87^{b}	7.00^{a}	6.50^{a}	6.50^{b}
	4	With viscera	4.56 ^{de}	5.10^{b}	5.50^{c}	5.00^{e}
		Without viscera	4.50 ^{de}	5.30^{b}	5.80^{c}	6.00^{d}
Thawing in room temperature (21°C)	-18	With viscera	4.60^{d}	3.20^{d}	5.40^{c}	4.60^{fg}
		Without viscera	4.83^{c}	4.83^{c}	3.66^{e}	4.66^{f}
	4	With viscera	4.00^{f}	3.00^{d}	3.00^{e}	4.42^{g}
		Without viscera	3.98^{f}	3.37^{d}	4.80^{d}	3.71^{h}

The data are means of triplicate measurements. Values with different superscript upper case letters in a column are statistically significant at $P < 0.05$.

The results indicate the negative influence of viscera on the overall acceptability, odor, color, and texture. The viscera accelerate the product spoilage and as the result cause a sharp pepper-like taste in the product and the color of the product become pinkish. In addition, the product spoilage causes the fish to give an unpleasant odor after cooking process and also the spoiled product loses its texture.

Comparing the results of organoleptic tests concerned with the temperature of 4 ˚C and -18 ˚C, it can be concluded that the viscera in both thermal conditions leads to the reduction of the product quality and undesirable effect on color, odor, texture, and in general negative effect on the final acceptability of the product by the customer. Thawing by cold water shower helps to keep the shape of the fish and its texture consistency and spoilage is not observed in the fish appearance.

The results of thawing at room temperature indicate that this method is the most inappropriate method for thawing. Because it is time consuming and the period of thawing is very long, the activity of histamine producing bacteria is twice as much as other two thawing methods, which influences the quality of the final product. All of the product's factors like color, odor and texture at temperature of 4 ˚C are less favorable than in -18 ˚C. Moreover, the study by Auerswald *et al*., (2006) in South Africa showed that the time is an important factor in the production of histamine. The results of the study represented that one-month storage from the first day of production increased histamine from 1.2 to 3.32 ppm. More favorable results were achieved in room temperature without viscera in terms of lack of intestinal bacteria to speed up the spoilage of histamine as compared to the condition when it is eviscerated; however, the obtained results are not desirable in comparison with other thawing conditions. In the case of inappropriate conditions of fish storage, histamine production can continue after canning and this process takes place faster as time passes. This might be due to the amount of residual enzyme histidine decarboxylase that is effective in the production of histamine or other chemical reactions.

Conclusion

It might be concluded that the best

method for thawing is the cold water shower and evisceration before cooking has positive effect on the concentration of histamine and on the other hand viscera accelerate the spoilage. The results indicated that evisceration before cooking has positive effect on the amount of histamine in *Katsuwonus pelamis* thawed by cold water. Thus, it is recommended to use cold water to thaw the fish.

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