Jordan Journal of Agricultural Sciences, Volume **18**, No.**1 2022**
DOI: https://doi.org/10.35516/jjas.v18i1.104

Impact of Climate Change and Dry-Salting Preservation on Histamine Levels in Three Marine Fish Species

Ashraf Omar Khashroum*

*Department of Plant Production and Protection Faculty of Agriculture, Jerash University.

Received on 20/3/2021 and Accepted for Publication on 24/9/2021.

ABSTRACT

This study was conducted to estimate the concentrations of histamine in three species of marine fish (fresh and preserved with dry salting). No significant differences ($p \geq 0.05$) in histamine concentrations were found among the Chirocenterus dorab, Scomberoides commersonianus, and Xiphias gladius fish species during summer or winter. On the other hand, the mean concentrations of histamine differed significantly ($p < 0.05$) between summer and winter in the fresh samples of the aforementioned fish species. In the case of the salted fish species, no significant differences ($p \geq 0.05$) in histamine concentrations were detected in Chirocentrus dorab under all salt treatments (5%, 10%, 15%, and 20%). In contrast, significant differences ($p < 0.05$) in histamine concentrations were found between the two NaCl treatments of 5% and 20% in the Scomberoides commersonianus fish samples.

Keywords: Fish meat, histamine, biogenic amines, dried fish, salting, climate change

INTRODUCTION

Fish constitutes food for human beings and various aquatic and marine fauna. Nearly quarters-three of the Earth is covered with water. Therefore, aquatic and marine fish were an important part of the human diet for almost all countries of the world from immemorial time (Mohanty, 2011). Of all aquatic and marine plant and animal species, fish is the largest source of highly valuable, nutritious food products. About one thousand species of fish are caught commercially and used for food production. Fish can be eaten fresh, frozen, salted, dried, smoked, and canned (Sándor et al., 2011).

Fish is an important source of protein for human nutrition. Its proteins have higher nutritional value than the proteins derived from other sources like meat, milk, or eggs. In addition, it is an important source of omega-3, calcium, phosphorus, iron, and trace minerals such as copper. It also has appreciable vitamin B content.

Salt drying of fish is an old method of preservation (Prester, 2011). The process includes back cutting, degutting, salting, and drying-sun (Huang et al., 2010).

*Corresponding Author: E-mail: askshb@yahoo.com;

Salting is a Physico-chemical dewatering process whereby salt transfuses into the body of the fish with dehydration of the muscles, which makes the fish material salty and unsuitable for microbial growth. The salting process leads to a reduction in the protein content of fish due to the release of water from their tissues and a concomitant reduction in the activity of lipolysis enzymes, besides a reduction in the number of lipolysis bacteria through a drop in the amount of free fatty acids in the fish tissues after the addition of salt. In this preservation process, salting is usually followed by drying, which is one of the oldest food preservation methods. Drying removes water from the food material, which reduces the percentage of moisture to the point where it becomes extremely difficult for organisms to live and practice biological activities that may give rise to damage to the food material and spoilage (e.g., rotting) and deterioration in its quality (Al-Rubai,y, et al., 2020).

Histamine is one of the biogenic amines. It is formed by the activity of exogenous decarboxylase enzymes that are produced by microorganisms and flora in seawater. It is the most toxic amine in foodstuffs such as fish, cheese, and meat products. This amine acts as an essential mediator in the regulation of several essential activities in allergy and immunity diseases in the body (Al-Abdulnebi et al., 2013).

A variety of sources of biological hazards can be found in seafood, including the biogenic amines (Visciano et al., 2012; Sedaghati and Mooraki, 2019). Histamine is a biogenic amine formed in fish muscles via decarboxylation of free histidine by exogenous decarboxylases that are released by bacteria (Visciano et al., 2014). High levels of histamine are undesirable for the safety and quality of foodstuffs like fish sauce, fish-fermented products, and some fish species such as Scombridae and Scomberesocidae. Often, histamine poisoning occurs after minutes or hours of eating food that contains abnormally high levels of this compound. For this reason, the term Scombridae is frequently used to describe the species of fish that cause food poisoning, and the term histamine fish poisoning or scombrotoxicosis is used to describe this poisoning.

It is known that fish can be decomposed when the histamine level reaches 500 mg/kg and that histamine poisoning occurs when the concentration of histamine in fish reaches this value. Human intake of histamine at concentrations higher than 1000 mg/kg of fish may give rise to heart palpitations, while concentrations of 4 to 40 mg/kg of fish may induce cutaneous reaction (Saewan et al., 2018).

Scombroid histamine poisoning is typically a mild disease with a range of symptoms, including skin rash, hives, nausea, vomiting, diarrhea, flushing, tingling, and itching. The severity of the symptoms may differ greatly with the amount of histamine taken and the sensitivity of the individual to histamine (Kung et al., 2015). Therefore, different countries have set legal limits for histamine levels that are considered safe for human consumption. Examples include 200 mg/kg in Australia and 100 mg/kg in Europe and South Africa. However, the United States Food and Drug Administration (US FDA) adopts the concentration of 50 mg/kg in dried fish as an indicator of their decomposition (Simora and Peralta, 2018).

The preservation effect of the salt is due to the role of the salt in suppressing microbial activity, reducing the tissue water content, and improving certain functional properties, which are all factors that contribute to an increase in shelf life. However, while salt can prolong storage, high salt concentrations can cause oxidation of highly-unsaturated lipids, production of objectionable odor, denaturation of proteins, and non-desirable changes in texture and taste. It is, therefore, necessary to assess the effects of different concentrations of preservation salt (NaCl) during and after storage on fish quality (Wang et al., 2014). On this account, this study was conducted to assess the impact of environmental changes and varying concentrations of preservation salt on the concentrations of histamine in three marine fish species; Chirocenterus

dorab, Scomberoides commersonianus, and Xiphias gladius.

Materials and methods

Collecting and Preparing Fish Samples

Fresh samples of three marine fish species (Chirocenterus dorab, Scomberoides commersonianus, and Xiphias gladius) were purchased from the market and kept in ice-filled containers until reaching the laboratory. These samples were washed with tap water, then with distilled water, and dried using drying paper. Afterward, they were weighed and their lengths were measured. The masses of these fish samples ranged from 338 to 660 g and their total lengths ranged from 33.6 to 60 cm. Then, the viscera and gills were removed using clean, sterile knives and tools. Histamine concentrations in these fish samples were measured once during winter and once during summer.

The Chirocenterus dorab and Scomberoides commersonianus fish were treated with dry salt by adding NaCl at the concentrations of 5%, 10%, 15%, and 20%. Each sample was placed in a clean, sterile basket and left to dry under the shade in the laboratory. Dried samples of Scomberoides commersonianus were bought from the local market to be used in comparisons. For each fish species, three replicates of each treatment were made.

Extraction and Estimation of Histamine by ELISA

Assessment of histamine concentrations in fish was made according to the procedure recommended by the manufacturers of the DEE3100 ELISA kit and EIA ELISA kit. Ten grams of fish (fresh and dried) were homogenized in 90 ml of deionized water for 1-2 min in a blender. Then, one milliliter of the suspension was pipetted into an Eppendorf tube and centrifuged for 5 min at maximum speed. The lipid layer was sucked out. Then, twenty microliters of the supernatant were taken and diluted with 10 ml of deionized water. The rest of the steps of histamine concentration estimation were performed as illustrated in the ELISA kit. The concentration of histamine was measured by using Bio ELISA Reader (Ele 800, USA) and a histamine standard curve (Figure 1).

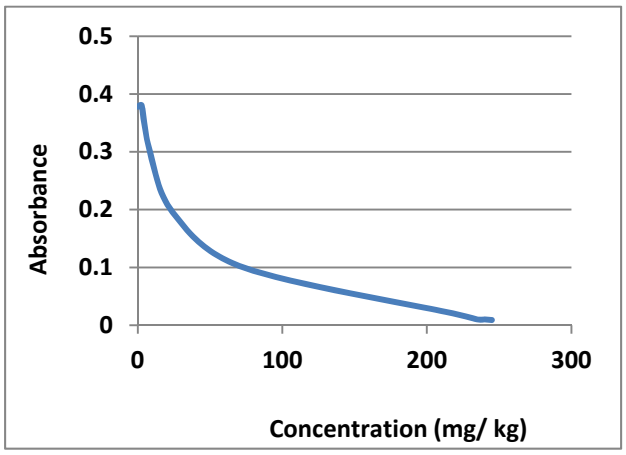

Fig.1 Histamine Standard Curve

Statistical Analyses

The histamine concentrations were subjected to one-way analysis of variance (ANOVA) using the XLSTAT software. When analysis of variance reveals statistical differences in means, Duncan's multiple range test is used as a post-hoc test to identify the different pairs of means. The significance level (α) was set at 0.05. The results are presented in the form of mean ± standard deviation. Duncan's

Results and Discussion

Histamine in Fresh Fish

Histamine is a toxic amine and agent for fish histamine intoxication. It is possible to use histamine as an indicator in the analysis of fresh fish quality (Wang et al., 2014). Table 1 shows the mean concentrations of histamine in muscles of fresh Chirocenterus dorab, ScomberoidesI commersonianus, and Xiphias gladius fish during summer and winter. No significant differences ($p \geq 0.05$) in histamine concentration among the three fish

species were found during summer or winter. On the other hand, the mean concentrations of histamine in Summer differed significantly ($p < 0.05$) than in winter in the fresh Chirocenterus dorab, Scomberoides commersonianus, and Xiphias gladius fish species. The mean histamine concentrations also differed significantly between these fish species in Winter. Chirocenterus nudus and Scomberoides commersonianus had close mean histamine concentrations (7.005 and 7.078 mg/kg, respectively) in the winter season. However, the mean histamine concentration was lower in Xiphias gladius meat (5.913 mg/kg) than in meat of these two species (Table 1).

Table 1. Mean Concentrations of Histamine in Fresh Fish (mg/kg)

Fish Species	**Mean Conc. of Histamine [1]**	
	Summer	Winter
Chirocentrus dorab	16.905 ± 3.345	7.005 ± 0.838
Scomberoides commersonianus	15.568 ± 1.639	7.078 ± 0.277
Xiphias gladius	14.238 ± 1.707	5.913 ± 1.968

[1] Mean concentration ± standard deviation

In summer, Chirocentrus dorab had the highest mean concentration of histamine; 16.905 mg/kg, while Xiphias gladius had the lowest mean histamine concentration; 14.283 mg/kg. These results are close to some of the results of Yeh et al. (2004) on fillets of Marlin, Mackerel, and Anchovy in winter and summer. These researchers reported that in spoiled fish, histamine is highly variable. The holding time, temperature, fish body part, fish species, and the types and counts of microorganisms present are influential factors. Yeh et al. (2004) concluded that the studied samples might have had low post-catching contamination or might have originally contained low concentrations of histamine.

In winter, all the Chirocentrus dorab, Scomberoides commersonianus, and Xiphias gladius fillet samples had significantly fewer biogenic amines than in summer ($p < 0.05$). This finding can be ascribed to the low temperature during fish sample handling that led to a reduction in the rate of formation of biogenic amines since microorganisms are responsible for the formation of biogenic amines and their biological activity drops with temperature (Yeh et al., 2004).

Variations in histamine concentrations during winter and summer are expected to be a result of the variations in intrinsic enzyme activity. The pre-spawning fish were captured in summer while they are still at an active feeding state. Accordingly, proteases could have been more active in this fish than in the fish caught in late September, shortly after spawning (Mansur and Horner, 1998)

Histamine in the Salted Fish

Inhibition of food or bacterial decarboxylase enzymatic activity and prevention of bacterial growth and activity are important factors for the control of amine formation. The efficacy of salt in the inhibition of amine formation can be explained by the fact that both enzymatic activity and bacterial growth are inhibited by the salt, thus contributing to the achievement of the goal of stopping biogenic amine formation (Wang et al., 2014).

Table 2 presents the mean concentrations of histamine in the three fish species under consideration. Chirocentrus dorab and Scomberoides commersonianus were preserved by dry salting using four salts (NaCl) treatments: 5%, 10%, 15%, and 20%. In the meantime,

Scomberoides commersonianus was dry-salted commercially.

Statistical analysis uncovered lack of statistically-significant differences ($p \geq 0.05$) in histamine concentrations in the Chirocentrus dorab fish between the four salt treatments (5.0%, 10.0%, 15.0%, and 20.0%). In contrast, significant differences ($p < 0.05$) in histamine concentrations in the Scomberoides commersonianus fish samples were found between the two NaCl treatments of 5.0% and 20.0%.

The mean concentration of histamine in the studied samples decreased with the increase in the salt concentration used for preservation. It was found (Table 2) that the highest mean concentrations of histamine were 6.543 mg/kg in Chirocentrus dorab and 7.044 mg/kg in Scomberoides commersonianus at the salt concentration of 5%, while the lowest mean concentrations of histamine were obtained in two fish species (Chirocentrus dorab (4.480 mg/kg) and Scomberoides commersonianus (4.542 mg/kg)) at the salt concentration of 20%. These results indicate that the higher the concentration of the preservation salt, the lower the histamine concentration in the fish tissues. However, it should be highlighted that the histamine concentrations reported in all fresh and dried fish samples in the present study did not exceed the legal limit of 50 mg/kg set by the US FDA. Thereupon, all these fish samples were safe for human consumption because histamine, which is the most toxic biogenic amine (Ruiz-Capillas et al., 2019; Saewan et al., 2021), is found in these samples at concentrations far below the safety threshold of 50 mg/kg.

The foregoing results agree with the results of Mansur and Horner (1998) who pointed out that high salt concentrations tend to be effective in delaying the production of histamine in herring. Accordingly, we should be aware that histamine poisoning may be associated with dried fish, depending on preservation method and salt concentration. Proper and hygienic fish processing methods can inhibit the activity of histamine-forming bacteria and reduce the concentration of histamine during cutting, salting, drying, and packaging of these dried products (Simora and Peralta, 2018).

Table 2. Mean Concentration of Histamine in Salted Fish (mg/kg)

Fish Species	NaCl (%)	Mean Histamine Conc. (mg/kg)
Chirocentrus dorab	5.0%	$6.543 \pm 1.156^{a,b}$
	10.0%	$6.223 \pm 1.049^{a,b}$
	15.0%	$5.964 \pm 0.299^{a,b}$
	20.0%	4.480 ± 0.005^{b}
Scomberoides commersonianus	5.0%	7.044 ± 0.552^{a}
	10.0%	$6.227 \pm 0.570^{a,b}$
	15.0%	$5.868 \pm 0.052^{a,b}$
	20.0%	4.542 ± 0.272^{b}
Scomberoides commersonianus	Unknown	5.913

On the other hand, the foregoing results differ from the results of Chong et al. (2011) who reported that with the increase in preservation salt concentration, the histamine concentration increased in Spanish mackerel and reached the highest concentration in the salt concentration range of 13-15%. These researchers expected that this finding may be explained by a rise in the overall viable count of histamine-forming bacteria with the increase in the salt concentration.

Huang et al. (2010) found that a traditionally-dried fish (Scomberoides commersonianus) had the highest mean concentration of histamine (9.804 mg/kg). The fish samples were dried for several days under the sun and kept at room temperature. Under these circumstances, flies may easily contaminate the fish. Through rupture of the belly walls during the sun-drying process, histamine-forming bacteria can penetrate the intestines and migrate to the inner muscles of the fish (Moori et al., 1988). Huang et al. (2010) clarified that significant quantities of histamine have often been found in India's commercial fish products, particularly the salt-dried products that do not receive the same heat treatment as in Taiwan, which may cause certain outbreaks of histamine. Furthermore, cross-contamination can happen because, during manual processing, the fish are eviscerated, cut with a dirty knife, and mixed for a long time in the same tank with the brine during and after sun drying. Additionally, the fish are usually kept at room temperature, which is a condition under which flies can easily pollute the fish. These may be the causes why histamine-forming bacterial strains are often present in dry-salted fish products (Simora and Peralta, 2018).

Conclusions and Recommendations

It can be concluded that the action of bacteria which can decarboxylate histidine is the main factor that controls the production and accumulation of histamine in the fish tissues. In winter, the sample fish species had significantly fewer biogenic amines than in summer. In addition, the level of histamine decreased as the preservation salt concentration was increased. The results of this study suggest that the histamine content of fish should be investigated under various preservation conditions. In addition, research on other biogenic amines such as tyramine, putrescine, cadaverine, and phenylethylamine in other fish species is recommended.

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تأثير التغير المناخي والحفظ بالتمليح الجاف في تركيز (هستامين) في ثلاثة أنواع أسماك بحرية

أشرف عمر خشروم*

*قسم الانتاج النباتي, كلية الزراعة, جامعة جرش, الاردن

تاريخ استلام البحث: 2021/3/20 وتاريخ قبوله: 2021/9/24.

الملخص

أجريت هذه الدراسة بهدف تقدير تراكيز (هستامين) في ثلاثة أنواع أسماك بحرية طازجة ومحفوظة بالتمليح الجاف، هي ((Xiphias gladius) و (Scomberoides commersoniamus) و (Chirocenterus dorab)). لم يوجد فروق ذوات دلالة إحصائية ($p\geq0.05$) في تراكيز (هستامين) بين أنواع السمك الثلاثة خلال فصل الشتاء أو خلال فصل الصيف. ومن ناحية اخرى, وجد فروقا ذوات دلالة إحصائية ($p<0.05$) في تراكيز (هستامين) في كل نوع من أنواع السمك الثلاثة المدروسة بين فصلي الشتاء والصيف. وفي حالة الأسماك المملحة, لم توجد فروق ذوات دلالة إحصائية في تراكيز (هستامين) في سمك (Chirocenterus dorab) بين تراكيز الأملاح المختلفة المستخدمة في التجفيف، وهي (5.0%) و (10.0%) و (15.0%) و (20.0%).وفي المقابل وجد فروقا ذوات دلالة إحصائية في تراكيز (هستامين) في أسماك (Scomberoides commersoniamus) بين تركيزي الأملاح (5.0%) و (20.0%).

الكلمات الدالة: لحم السمك، هستامين، الأمينات الحيوية، السمك المجفف، التمليح، التغير المناخي.