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Histamine Level in Freshwater and Marine Fish Sold in Bulgarian Markets

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HIGHLIGHTS

- Histamine was detected in 26 out of 40 (65%) fish samples, and none of them exceeded the regulatory limit.
- The average histamine content in marine fish was insignificantly higher than that in freshwater fish.
- Histamine range in positive freshwater and marine fish samples were 2.500-9.590 and 2.500-41.009 mg/kg, respectively.

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Acronyms and abbreviations
ELISA=Enzyme-Linked Immunosorbent Assay

ABSTRACT

Background: Histamine is an essential biogenic amine produced as a result of microbial decomposition of histidine during seafood processing and storage. The objective of this study was to evaluate histamine concentration in freshwater and marine fish marketed in Stara Zagora region, Bulgaria.

Methods: Forty fish samples were purchased from local fish farms and retail stores in Stara Zagora, Bulgaria. Enzyme-Linked Immunosorbent Assay was used to determine histamine levels. The data were processed using GraphPad Software InStat 3.

Results: Histamine was detected in 26 out of 40 (65%) samples, and none of them exceeded the regulatory limit of 200 mg/kg. The average histamine content in marine fish (6.965±3.187 mg/kg) was insignificantly (p>0.05) higher than that in freshwater fish (4.503±1.133 mg/kg).

Conclusion: The results reveal low levels of histamine in freshwater and marine fish indicating their good quality. However, its presence in seafoods remains a major food safety problem that requires permanent regulation of histamine concentration in fish.

Introduction

Histamine is a biogenic amine that occurs in different types of food including fish, meat, cheese, and wine (Papageorgiou et al., 2018; Visciano et al., 2012, 2014). The formation of histamine in foods can result in a human allergic reaction (histamine fish poisoning) with a variety of symptoms such as facial flushing, rash, facial edema, nausea, vomiting, diarrhea, tingling, and itching of the skin (Hungerford, 2010; Yesudhason et al., 2013). Histamine production in food is a result of the amino acid histidine being microbially decarboxylated. Histidine decarboxylase is the enzyme responsible for histidine decomposition. Many bacterial species can produce the enzyme such as Enterobacteriaceae, *Clostridium*, *Lactobacillus*, *Vibrio*, *Pseudomonas*, and *Photobacterium*. Most of these bacteria are naturally present on the gills and in the gut of fish. *Scombridae*, *Engraulidae*, and *Clupeidae* are fish species that naturally contain elevated amounts of histidine (Duflos et al., 2019).

Free histidine content in fish muscles is known to differ between fish species, feeding, season, sex, and stage of

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maturity. Histamine production is related to exposure over a prolonged period to high temperatures. The enzyme slowly starts formation of histamine at refrigeration temperatures and stays stable if it is frozen. While histidine decarboxylase can be killed by high temperatures, histamine is very stable in heat, and once formed, normal cooking does not kill it and its toxicity remains (Cicero et al., 2020; Yesudhason et al., 2013).

European Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs lays down specific criteria for histamine in fishery products. Legislation provides for a maximum level of up to 200 mg/kg for histamine in fresh fish and up to 400 mg/kg for fish products which have undertaken enzyme maturation (Visciano et al., 2014).

Hence, the objective of this study was to evaluate histamine concentration in freshwater and marine fish collected from local fish farms and retail stores in Stara Zagora region, Bulgaria using Enzyme-Linked Immunosorbent Assay (ELISA) kits.

Materials and methods

Samples collection

Between May and September 2018, a total of 40 samples consisting of freshwater and marine fish were collected from fish farms and retail stores in the area of Stara Zagora, Bulgaria. The fish samples included Common carp (*Cyprinus carpio*; n=5), Rainbow trout (*Oncorhynchus mykiss*; n=9), Striped catfish (*Pangasianodon hypophthalmus*; n=3), Atlantic mackerel (*Scomber scombrus*; n=11), Southern blue whiting (*Micromesistius australis*; n=5), Argentine hake (*Merluccius hubbsi*; n=5), and Bluefish (*Pomatomus saltatrix*; n=2). The samples were kept in ice during transportation and stored at -20 °C until further analysis.

Samples preparation

Quantitative determination of histamine was performed using RIDASCREEN® Histamine (enzymatic) Kit (R-Biopharm AG, Darmstadt, Germany). All the samples were prepared according to the manufacturer's instructions. A piece of back muscle was collected and 10 g were homogenized using homogenizer T18 digital ULTRA-TURRAX (IKA-Werke, Germany). Nine ml of distilled water were added to 1 g of homogenized sample weighed in a centrifuge tube. The tubes were centrifuged for 5 min at 2 500 g at room temperature. One ml of supernatant was mixed with 9 ml of water. Two hundred µl of the mixture were mixed with 9.8 ml of distilled water in a test tube. Finally, 100 µl of the final solution were applied per well in the acylation plate.

Acylation procedure

A volume of 100 µl of each standard solution, controls, and prepared samples were added to separate wells of the acylation plate. Twenty five µl of the acylation reagent and 200 µl of the acylation buffer were consistently added to each acylation well, mixed gently by shaking the plate manually and incubated for 15 min at room temperature.

ELISA test procedure

Twenty-five µl of each acylated standard solution, controls and prepared samples were added in separate microplate wells. Histamine standards were used at concentrations of 0, 0.5, 1.5, 5, 15, and 50 µg/kg. ELISA procedure was carried out following manufacturer's recommendations. Microplate Reader (Rayto RT-2100C, China) was used for reading the ELISA plate at 450 nm. The detection limit was 2.5 mg/kg. The RIDA®SOFT Win (version 1.07) was used for calculation of histamine concentration.

Statistical analysis

The data were processed using GraphPad InStat 3 (GraphPad Software, San Diego, CA) and presented as means and standard deviations. One-way ANOVA with Tukey post hoc test was applied to detect differences between the histamine concentrations in freshwater and marine fish. The statistical significance was determined at $p<0.05$.

Results and discussion

Histamine was detected in 26 out of 40 (65%) of the samples, and none of them exceeded the regulatory limit of 200 mg/kg. The average histamine content in marine fish (6.965±3.187 mg/kg) was insignificantly ($p>0.05$) higher than that in freshwater fish (4.503±1.133 mg/kg). The histamine range in positive freshwater and marine fish samples were 2.500-9.590 and 2.500-41.009 mg/kg, respectively (Table 1).

Our findings showed that the overall level of histamine in samples did not exceed the European Union (EU) acceptable level and should be considered safe for consumption with regard to histamine. Good storage conditions for fish products are indicated by the low levels registered in our survey. We measured the highest histamine concentrations in Atlantic mackerel, which is a fish species associated with a high amount of histidine according to Commission Regulation (EC) No 2073/2005. Mackerel is a frequently consumed scombroid fish in Bulgaria and health services should provide prevention and vendor screening. Although histamine levels in our

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Table 1: Histamine concentrations in freshwater and marine fish sold in Bulgarian market

Fish species	Sample size	Histamine level (mg/kg)	
		Range	Mean±SD
Freshwater			
Common carp (*Cyprinus carpio*)	5	2.500-4.452	3.218±0.987
Rainbow trout (*Oncorhynchus mykiss*)	9	2.500-9.590	5.358±3.223
Striped catfish (*Pangasianodon hypophthalmus*)	3	3.509-5.906	4.934±1.261
Marine			
Atlantic mackerel (*Scomber scombrus*)	11	2.500-41.009	11.011±13.581
Southern blue whiting (*Micromesistius australis*)	5	2.500-9.819	4.266±3.155
Argentine hake (*Merluccius hubbsi*)	5	2.500-10.127	4.573±3.195
Bluefish (*Pomatomus saltatrix*)	2	2.506-13.511	8.009±7.782

study do not represent a health hazard according to EU requirements, they may become hazardous due to several factors such as improper handling, processing, and storage. Chilling of fish is critical but not sufficient to prevent spoilage and formation of histamine caused by bacterial pathogens (Hungerford, 2010).

Lower unacceptable histamine levels obtained in freshwater fish were in line with most of the data having previously been reported. According Kordiovská et al. (2006), histamine is highly problematic in saltwater fish, but it is posed no health risk to consumers in freshwater fish because of its low amount. The same findings are supported by another research studies about the production of biogenic amines in fishes (Křížek et al., 2004; Ruiz-Capillas and Moral, 2001). Low levels of histamine in the range from 1.0 to 39.3 mg/kg were reported in the survey of Park et al. (2010). Histamine was detected in 48.7% of samples of fish and fish products in Korea and the maximum detection levels were less than EU acceptable level. According Yesudhason et al. (2013), 41% of fresh fish samples in the Sultanate of Oman contained histamine from 1 to 229 mg/kg, with mean value of 2.6 mg/kg. Bilgin and Genccelep (2015) found histamine in fish products from Turkish markets in maximum concentration up to 110.33 mg/kg. Histamine levels greater than 100 mg/kg were detected in seven tuna samples, which exceed the acceptable safe level of the European Union. According to Muscarella et al. (2013), 70% of fresh fish from Italy had a histamine concentration lower than 10 mg/kg, while 6% of them had a histamine level greater than 100 mg/kg. Also, canned tuna and canned mackerel had a content of histamine lower than 10 mg/kg. The authors suggested that the higher histamine level is due to poor hygienic conditions and uncontrolled temperature often found in street vendors. This is in line with the findings of several researchers, who stated that the combination of time and temperature influenced the formation of histamine (Comas-Basté et al., 2019; García-Tapia et al., 2013).

Contrary to our findings, Cicero et al. (2020) found histamine with a maximum of 4 110 mg/kg in 7.6% of all fresh fish samples examined in Southern Italy. In consistence with other reports, most of the positive samples were found in street vendors and stored at improper conditions. In most cases, fresh fish is displayed on ice providing a partial covering that lead to an exposure to ambient temperature (Muscarella et al., 2013). It is known that histamine production is most often affected by the combined influence of time and temperature. Numerous studies reported higher histamine levels in fresh fish samples due to bad storage and hygienic conditions. Moreover, fresh fish samples with high histamine concentrations above 1000 mg/kg are found in some street vendors and retail markets where fish is often kept in poor hygienic condition (Mejrhit et al., 2018).

By applying good hygiene practices and proper handling, distribution and storage procedures, high levels of histamine and other biogenic amines can be prevented. Rapid cooling and freezing immediately after the death of the fish is an effective way of preventing histamine formation. Despite low levels of histamine, it should be noted that variations in temperature during fish transport and storage could lead to bacterial growth and multiplication. They either cause spoilage or produce harmful products such as histamine making the meat unsafe for consumption (Park et al., 2010; Visciano et al., 2012).

Conclusion

Histamine levels did not exceed the maximum limits set by Commission Regulation (EC) No 2073/2005. The results reveal low levels of histamine in freshwater and marine fish indicating their good quality. However, its presence in seafoods remains a major food safety

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problem that requires permanent regulation of histamine concentration in fish.

Author contributions

All authors contributed equally to study designing, experimental work, data analysis, and also manuscript writing. All authors read and approved the final manuscript.

Conflicts of interest

There is no conflict of interest to declare.

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