PROFESSIONAL PAPER

Histamine in fish products on Croatian market

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Abstract

It is recommended to eat blue fish at least twice a week, due to its high nutritional value and ingredients, primarily healthy fats and proteins. In addition to its advantages, the consumption of fish and fish products can bring potential hazards. Histamine poisoning has been reported as one of the most common issues related to seafood consummation. It is considered as relatively mild illness with symptoms like rash, urticaria, nausea, vomiting, but in some cases, due to high sensitivity, it can lead to severe symptoms and even to death. In order to insure safety of fish products, histamine determination is one of the safety requirements of European and Croatian legislation (Law on food hygiene and microbiological criteria for food, NN 83/2022). Despite that, there has been several recalls of fish products by Ministry of Agriculture from Croatian market in recent years.

The aim of this paper was to determine histamine levels in 20 different products from Croatian retail stores by HPLC method. All tested samples have been in accordance with legally determined limits for histamine levels.

Keywords: histamine, bluefish, histamine poisoning, high performance liquid chromatography

Introduction

It is recommended to consume blue fish twice per week as a prevention of cardiovascular diseases. Small fish like sardines, anchovy, mackerel and big fish like tuna are considered as important source of biologically valuable proteins, iodine, selenium and polyunsaturated fatty acids (Nosić and Krešić, 2015). Fish consumption has been related to reducing stroke, cardiovascular diseases, inflammatory diseases, hypertension, the risk of Alzheimer's disease etc. However, seafood consumption is also related to many potential hazards like parasites, poisonings of didderent ethiology, heavy metals, allergens, histamine and viruses (Mol and Coşansu, 2022). Histamine fish poisoning (HFP), also known as scombroid poisoning, represents 37 % of all poisonings related to seafood consumption (Nosić and Krešić, 2015). Freshly caught fish does not contain histamine (Yesudhason et al., 2013). Histamine and other biogenic amines like tyramine or putrescine, can be formed during storage or processing due to release of specific amino acids by the action of microorgani-

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sms' decarboxylases (Abu-Almaaly, 2017; Al Bulushi et al., 2009). Histamine or scombrotoxin is produced by decarboxylation of the amino acid histidine. The amount of produced histamine depends on the type of bacteria, the length of incubation and the temperature (Kriještorac et al., 2018). Although the optimal temperature for enzyme and histamine production is 25 °C, it has been noticed that it can also be formed at lower, refrigerated temperatures because enzyme histidine-decarboxylase continues with histamine production even if bacteria are not active (Nosić, 2010; Vusilović et al., 2008). Bacteria associated with the formation of histamine in salt water naturally inhabit the gizzards and intestines, while this activity does not endangering the fish in any way. However, after death, the defence mechanisms in the fish organism can no longer inhibit the growth of bacteria (Nosić, 2010). Some species of Enterobacteriaceae, Clostridium and Lactobacillus produce the enzyme histidine decarboxylase during growth. Entric bacteria have been found to be most important histamine producing bacteria (Köse, 2010).

Once synthesized, histamine in food can no longer be destroyed even at high temperatures (Kriještorac et al., 2018). Histamine intake of 70-1000 mg per single meal can cause chemical intoxication, usually mild illness with variety of symptoms such as rash, urticaria, nausea, vomiting and diarrhoea, but in people with histamine intolerance, in patients suffering from asthma or heart disease it can be lethal (Muscarella et al., 2013; Nosić, 2010). Therefore, HFP is significant public health and safety concern as well as trade issue (Lehane and Olley, 2000). It must be highlighted that toxic histamine levels can be observed in fish despite their acceptable sensory quality, so it can not be detected organoleptically (Abu-ALmaaly, 2017; Köse, 2010).

Therefore, prevention of histamine formation through preventing or delaying the growth of histamine forming bacteria (HFB) and also slowing down the enzymes' activity is crucial (Köse, 2010). The key measure is rapid cooling of fish after catching and the maintenance of adequate refrigeration during handling and storage (Lehane and Olley, 2000). Fish should be put on ice, in cooled seawater or brine at temperatures 4.4 °C (or 10 °C), for several hours depending on the temperature of the sea and water (Smajlović et al., 2008). Unfortunately, climate change through global warming and consequently increasing the water and air temperatures makes this task even harder (Mol and Coşansu, 2022). Beside of standard methods, a number of new methods of preventing the development of bacteria (storage in a modified atmosphere, application of preservatives, high pressure, radioactive iradiation, as well as combinations of these method) or application of enzymes that break down histamine have been developed (Šarkanj et al., 2010). However, despite obligatory preventing measures, HFP outbreaks have been reported all over the world (Lehane and Olley, 2000), even in developed countries such as Japan, United States, Canada, France, Germany and many others (Yesudhason et al., 2013). Croatia is no exception in this context. Law on food hygiene and microbiological criteria for food (NN 83/2022) follows European Commission Regulations regarding food safety criteria, and determines necessary steps in quality assurance and control of fish and fish products. European Commission regulation (EC 2073/2005) sets microbiological criteria which should form an integral part of the implementation of HACCP-based procedures and other hygiene control measures. Food business operators determine frequencies of the necessary sampling and testing as part of their procedures based on HACCP principles and other hygiene control procedures. Despite that, products with high histamine levels could reach the consumers. Croatian agency for Agriculture and Food announced recall of fish products from Croatian retail sales twice in last three years due to high histamine levels (Hrvatska agencija za poljoprivredu i hranu, 2022.). As presumption, due to mild symptoms, true statistics are missing since many of individual cases are not reported to doctors (Bogdanović et al., 2009), The objective of this research is to determine histamine content in fishery products from Croatian retail sales in order to aquire useful data for surveillance study which should give large perspective on histamine control measures and possible outbreaks prevention.

Materials and methods

Samples of 20 different canned fishery products (Table 1.) were bought in retail stores and markets in the first quarter of 2021.

Sample preparation

From the fish samples in cans all sauces,

Table 1 Analyzed samples of fishery products

PRODUCT	EXPIRATION DATE	COUNTRY OF ORIGIN
1. Adriatic sardine in own juice	10.11.2022	Croatia
2. Adriatic sardines in olive oil	12.11.2024	Croatia
3. Adriatic sardines in vegetable oil with a slice of lemon	13.11.2024	Croatia
4. Tuna pieces in sunflower oil	31.12.2022	Spain
5. Adriatic sardines in sunflower oil	07.05.2025	Croatia
6. Cleaned salted sardines	13.07.2021	Croatia
7. Anchovies salted fillets	14.07.2021	Croatia
8. Marinated anchovies	14.11.2021	Croatia
9. Marinated anchovy fillets in sunflower oil	20.05.2021	Not known
10. Marinated anchovy fillets in sunflower oil	Not known	Not known
11. Adriatic sardine with vegetables in tomato sauce	11.11.2023	Croatia
12. Adriatic sardine with vegetables in tomato sauce	11.11.2023	Croatia
13. Octopus salad	15.02.2022	Croatia
14. Smoked mussels	15.02.2022	Croatia
15. Chunks of tuna in tomato sauce	31.12.2023	Spain
16. Tuna salad cuscus	31.12.2023	Spain
17. Tuna salad mexicofrinsa	31.12.2023	Spain
18. Adriatic sardine in lemon marinade	28.11.2022	Croatia
19. Adriatic sardine with Mediterranean herbs	29.07.2024	Croatia
20. Sardines in tomato sauce	02.04.2022	Croatia

vegetable pieces and liquids have been removed by filter paper, than 2 g of homogenized samples and 8 mL of perchloric acid were mixed on vortex for 60 s and centrifuged for 15 min at 4600 rpm. Quantity of 200 µL of supernatant was mixed with 400 µL of saturated sodium carbonate solution (Na_2CO_3) and 800 µL of dansyl chloride, and than mixture was incubated at 60 °C/5 min. Proline was added in quantity of 200 µL and stored in dark for 15 min. After that, 1 mL of toluene was added and vortexed to separate the layers. Upper layer was separated into test tubes and evaporated to dryness in a stream of liquid nitrogen. At the end, 500 µL of acetonitrile (ACN) is added to the test tubes, and the samples are filtered by using a membrane filter into glass vials for HPLC.

Table 2 Gradient elution of mobile phase

Time (min)	Mobile phase A (%)
1	40
6	25
8	25
13	5
20	5
20	40
30	40

Liquid chromatography

Analyses of fish products were carried out on an Agilent 1200 device (USA) for high performance liquid analysis. Ammonium acetate (0.1M) was used as the mobile phase A, with gradient elution (Table 2.). Column temperature was 40 °C, mobile phase flow rate was 0.8 ml/min, whilst used detector was UV-254 nm with the volume of injected sample of 50 µL (In house method, Teaching Institute for Public Health "Dr. Andrija Štampar").

Each sample consisted of 9 units and every unit was analysed in 9 repetitions. Results were expressed as mg of histamine per 1 kg of sample.

Results and discussion

American Food and Drug Administration (FDA) has set 5 mg/100 g fish as the maximum level allowable, while European community limited histamin amount to a maximum of 20 mg/100 g for fish belonging to Scombridae, Clupeidae, Engraulidae, Coryphaennidae, Pomatomidae and Scomberesocidae families (Yesudhason et al., 2013; Özoğul and Özoğul, 2019). It is very important to highlight that toxicity of histamine alone is lower than in

cases when histamine is ingested in food matrix. Al Bulushi et al. (2009) explained that detoxification system in human body eliminates histamine by specific intestinal enzymes like diamine oxidase. However, in cases when histamine is taken in food matrix like fish, together with other biogenic amines like cadaverine and putrescine, histamine metabolizing enzymes are inhibited. Tables 3. to 5. represents histamine concentrations in analysed samples of different fish products. According to sampling plan (Table 6.), each sample consisted of 9 units. Besides, this, each unit was analysed in 9 repetitions because of the fact that level of histamine may vary in different parts of the fish (Nosić and Krešić, 2015). However, parallel measurements did not exhibit high variations in standard deviations. An amount of less than 5 mg/kg is considered to be below determination limit of the method used in the experiment. Regarding the sampling criteria and limit parameters shown in Table 6, the results

Table 3 Histamine in fish products in their own juice or oil*

Sample	Histamin (mg/kg) in sample units								
	1.	2.	3.	4.	5.	6.	7.	8.	9
1.	< 5	< 5	< 5	16,1±2,2	16,0±2,2	< 5	< 5	< 5	< 5
2.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
3.	< 5	< 5	8,72±1,2	< 5	16,2±2,3	< 5	< 5	< 5	8,68±1,2
4.	< 5	< 5	< 5	6,11±0,86	< 5	< 5	< 5	< 5	< 5
5.	10,7±1,5	10,4±1,5	14,2±2,0	11,7±1,6	17,6±2,5	10,3±1,4	9,45±1,32	16,3±2,3	8,45±1,18
6.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
7.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
8.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
9.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
10.	22,2±3,1	16,8±2,4	10,9±1,5	14,6±2,0	9,11±1,28	13,9±2,0	8,58±1,20	8,15±1,14	5,73±0,80

**Results were expressed as the mean of nine repetitions ± standard deviation*

Table 4 Histamine in fish products in sauce with additives*

Sample	Histamin (mg/kg) in sample units								
	1.	2.	3.	4.	5.	6.	7.	8.	9
11.	9,40±1,32	11,0±1,5	18,3±2,6	14,9±2,1	9,97±1,40	18,1±2,5	11,7±1,6	11,8±1,7	13,3±1,9
12.	< 5	-	-	-	-	-	-	-	-
13.	< 5	-	-	-	-	-	-	-	-
14.	12,3±1,7	9,57±1,34	10,6±1,5	13,6±1,9	15,7±2,2	12,2±1,7	10,2±1,4	10,0±1,4	12,5±1,8
15.	5,53±0,77	< 5	5,80±0,81	6,95±0,97	< 5	6,80±0,95	5,89±0,83	< 5	5,48±0,77
16.	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
17.	7,33±1,03	7,56±1,06	8,73±1,22	8,46±1,18	7,98±1,12	7,42±1,04	7,88±1,10	8,21±1,15	7,54±1,06
18.	7,12±1,0	19,0±2,7	12,1±1,7	12,4±1,7	8,87±1,2	11,7±1,6	18,2±2,6	10,9±1,5	16,0±2,2
19.	12,1±1,7	12,0±1,7	19,0±2,7	7,77±1,09	< 5	8,70±1,22	10,7±1,6	11,1±1,6	16,6±2,3

**Results were expressed as the mean of nine repetitions ± standard deviation*

Table 5 Histamine content in fish products matured in brine*

Sample	Histamin (mg/kg) in sample units								
	1.	2.	3.	4.	5.	6.	7.	8.	9
20.	192±27	191±27	193±27	192±27	197±28	186±26	186±26	188±26	194±27

**Results were expressed as the mean of nine repetitions ± standard deviation*

Table 4 Food safety criteria (EC 2073/2005)*

Product category	Sampling plan		Limit
	n	c	
Fishery products which have undergone enzyme maturation treatment in brine, manufactured from fish species associated with a high amount of histidine	9	2	m = 200 mg/kg M = 400 mg/kg
Fishery products from fish species asso- ciated with a high amount of histidine	9	2	m = 100 mg/kg M = 200 mg/kg

n - the number of elementary sample units that make up the sample, c - the number of sample units, where the number of bacteria can be between "m" and "M", m - the limit value below which all results are considered satisfactory, M - the limit value above which the results are considered unsatisfactory

of the histamine analysis of all samples shown in Tables 3 and 4 are considered satisfactory, since none of the samples had histamine level above “m” value of 100 mg/kg. Sample number 20, presented in Table 5. is sample maturated in brine and histamine levels are much higher than in samples presented in Tables 3. and 4. However, results of all analysed units are not higher than “m” value of 200 mg/kg (Table 6.). Kriještorac et al. (2018) in their survey analysed total number of 78 different fish products and 86 samples of fresh fish in Bosnia and Hercegovina. Only two samples of fresh tuna had histamine concentrations above the maximum residue limit, but they highlighted the importance of continuous monitoring of histamine in fish and fish products.

Conclusions

Preliminary random analysis of different fish products from retail stores and markets in Croatian market showed satisfactory results, despite occasional findings of products with unsatisfactory histamine level. These reults should be confirmed by a study including larger number of samples throughout a longer period of time.

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Dostavljeno/Received: 06.10.2022. **Prihvaćeno/Accepted: 24.10.2022.**

Histamin u ribljim proizvodima na hrvatskom tržištu

Sažetak

Plavu ribu preporuča se jesti najmanje dva puta tjedno zbog visoke nutritivne vrijednosti, prvenstveno zdravih masnoća i bjelančevina. No, osim svojih prednosti, konzumacija ribe i ribljih proizvoda može donijeti potencijalne opasnosti. Trovanje histaminom je jedan od najčešćih problema povezanih s konzumacijom plodova mora. Smatra se relativno blagom bolešću sa simptomima poput osipa, urtikarije, mučnine, povraćanja, ali u nekim slučajevima, zbog visoke osjetljivosti, može dovesti do teških simptoma, čak i smrti. Kako bi se osigurala sigurnost ribljih proizvoda, određivanje histamina jedan je od sigurnosnih zahtjeva europskog i hrvatskog zakonodavstva (Zakon o higijeni hrane i mikrobiološkim mjerilima za hranu, NN 83/2022). Unatoč tome, proteklih je godina bilo više povlačenja ribljih proizvoda od strane Ministarstva poljoprivrede s hrvatskog tržišta zbog utvrđenih vrijednosti histamina koje su prelazile maksimalnu dozvoljenu količinu (MRL).
Cilj ovog rada bio je High Performance Liquid Chromatography (HPLC) metodom odrediti razinu histamina u 20 različitih proizvoda iz hrvatskih maloprodajnih trgovina. Svi ispitani uzorci bili su u skladu sa zakonski određenim granicama razine histamina.

Ključne riječi: histamin, plava riba, trovanje histaminom, HPLC

Histamin in Fischprodukten auf dem kroatischen Markt

Zusammenfassung

Mindestens zweimal pro Woche sollte man blauen Fisch essen, da er einen hohen Nährwert hat und viele Inhaltsstoffe enthält, vor allem gesunde Fette und Proteine. Neben seinen Vorteilen kann der Verzehr von Fisch und Fischerzeugnissen aber auch potenzielle Gefahren mit sich bringen. Eine Histaminvergiftung ist eines der häufigsten Probleme im Zusammenhang mit dem Verzehr von Meeresfrüchten. Sie gilt als eine relativ milde Erkrankung mit Symptomen wie Hautausschlag, Urtikaria, Übelkeit und Erbrechen, kann aber in einigen Fällen aufgrund der hohen Empfindlichkeit zu schweren Symptomen und sogar zum Tod führen. Um die Sicherheit von Fischprodukten zu gewährleisten, ist die Bestimmung von Histamin eine der Sicherheitsanforderungen der europäischen und kroatischen Gesetzgebung (Gesetz über Lebensmittelhygiene und mikrobiologische Kriterien für Lebensmittel, Amtsblatt NN 83/2022).

Trotzdem hat das Landwirtschaftsministerium in den letzten Jahren mehrere Fischprodukte vom kroatischen Markt zurückgerufen.
Ziel dieser Arbeit war es, den Histamingehalt in 20 verschiedenen Produkten aus kroatischen Einzelhandelsgeschäften mittels der HPLC-Methode (High Performance Liquid Chromatography) zu bestimmen. Alle untersuchten Proben entsprachen den gesetzlich festgelegten Grenzwerten für Histamingehalte.

Schlüsselwörter: Histamin, blauer Fisch, Histaminvergiftung, Hochleistungsflüssigkeitschromatographie (HPLC)

Histamina en productos pesqueros en el mercado croata

Resumen

Se recomienda comer pescado azul al menos dos veces por semana debido a su alto valor nutricional, principalmente grasas saludables y proteínas. Sin embargo, además de sus beneficios, el consumo de pescado y productos pesqueros tiene peligros potenciales. La intoxicación por histamina es uno de los problemas más comunes asociados con el consumo de los mariscos. Se considera una enfermedad relativamente leve con síntomas como el sarpullido, urticaria, náuseas y vómitos, pero en algunos casos, debido a la alta sensibilidad, puede provocar síntomas graves, incluso la muerte. Para garantizar la seguridad de los productos pesqueros, la determinación de histamina es uno de los requisitos de seguridad de la legislación europea y croata (Ley de Higiene de los Alimentos y Criterios Microbiológicos para los Alimentos, NN 83/2022). No obstante, en los últimos años ha habido varios retiros de productos pesqueros por parte del Ministerio de Agricultura del mercado croata, debido a valores determinados de histamina que superaban la Cantidad Máxima Permitida (MAQ). El fin de este trabajo fue determinar el nivel de histamina en 20 productos diferentes de tiendas minoristas croatas, utilizando el método de cromatografía líquida de alta eficacia (HPLC). Todas las muestras analizadas estaban de acuerdo con los límites legalmente definidos para los niveles de histamina.

Palabras claves: histamina, pezcado azul,intoxicación por histamina, HPLC

L'istamina nei prodotti ittici sul mercato croato

Riassunto

Si consiglia di mangiare pesce azzurro almeno due volte a settimana per il suo alto valore nutritivo, principalmente grassi “buoni” e proteine . Tuttavia, a parte i loro benefici, consumare pesce e prodotti ittici può comportare potenziali pericoli. La intossicazione da istamina è uno dei problemi più comuni associati al consumo di frutti di mare. Considerata una malattia relativamente lieve, con sintomi come eruzioni cutanee, orticaria, nausea e vomito, in alcuni casi, a causa dell'elevata sensibilità dell’individuo interessato, può portare a sintomi gravi e, persino, alla morte. Al fine di garantire la sicurezza dei prodotti ittici, la determinazione del livello di istamina è uno dei requisiti di sicurezza della legislazione europea e croata (Legge sull'igiene alimentare e norme microbiologiche per gli alimenti, G.U. croata “Narodne novine” n. 83/2022). Nonostante ciò, negli ultimi anni si è assistito al ritiro dal mercato croato di diversi prodotti ittici da parte del Ministero dell'Agricoltura a causa dei valori di istamina accertati, che superavano i limiti massimi di residui consentiti (MRL).
Lo scopo di questo lavoro consisteva nel determinare il livello di istamina in 20 diversi prodotti prelevati dai punti vendita al dettaglio croati utilizzando il metodo della cromatografia liquida ad alta prestazione (High Performance Liquid Chromatography o HPLC). Tutti i campioni testati sono risultati conformi ai limiti del livello di istamina definiti dalla legge.

Parole chiave: istamina, pesce azzurro, intossicazione da istamina, HPLC