

Histamine in canned and smoked fishery products sold in Serbia

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Abstract: An increase in seafood-related illnesses as a consequence of an increase in seafood consumption has been documented recently. The United States Food and Drug Administration (FDA) has identified histamine as the major chemical hazard of seafood. The aim of this study was to determine histamine levels in 227 production lots (2043 subsamples) of canned tuna, canned sardines, canned mackerel and smoked salmon obtained from Serbian retail stores in 2018. Levels of histamine were determined using high performance liquid chromatography tandem mass-spectrometry. In addition, we compared histamine levels found in analysed products with levels established by the European Commission (EC) and FDA. Mean levels of histamine in compliant lots, according to EC Regulation 2073/2005, were 9.21, 3.16, 3.34 and 5.22 mg/kg in canned tuna, canned sardines, canned mackerel and smoked salmon, respectively. The highest histamine levels were found in canned tuna and canned mackerel, 1112 mg/kg and 412 mg/kg, respectively. Histamine was detected in 468 (22.91%) of all analysed subsamples. Levels above 100 mg/kg, which is the maximum histamine level allowed in canned fish in the EU, were found in 38 (1.86%) of analysed subsamples. According to EC Regulation 2073/2005, six (2.64%) of all analysed lots were found non-compliant, while 21 (9.25%) of analysed lots should be rejected according to the FDA limit (50 mg/kg).

Keywords: histamine, canned tuna, mackerel, sardine, food safety.

Introduction

Globally, more than 63.5 million tons of seafood is caught and eaten each year, representing a steady global increase in seafood consumption (Silva *et al.*, 2011). Some authors have indicated the health benefits of marine fish consumption, mostly due to the role of n-3 fatty acids in alleviating cardiovascular diseases, lowering blood pressure and in preventing the development of hypertension (Narayan *et al.*, 2006). This is probably the reason why consumer awareness of the health benefits of incorporating fish in diets is constantly growing. However, an increase in seafood-related illnesses as a consequence of this increase in seafood consumption has been documented (Butt *et al.*, 2004). The United States Food and Drug Administration (FDA) has identified histamine as the major chemical hazard of seafood. Recently, greater interest can be observed in the area of seafood safety, especially when it comes to histamine, both in Serbia and worldwide (Karmi, 2014; Dimitrijevic *et al.*, 2016; Petrovic *et al.*, 2016; Ryok Kang *et al.*, 2018; Zhang *et al.*, 2018).

Histamine fish poisoning (HFP), also known as scombrototoxin fish poisoning (SFP), is a food-borne chemical intoxication mostly caused by the ingestion of spoiled or bacterially contaminated scombroid fish that are characterised by high levels of the amino acid histidine in muscle tissue. Scombroid fish belong to the families Scombridae (mackerel, tuna, bonito) and Scomberesocidae (saury). High levels of histidine are also found in other fish families such as Clupeidae (sardine, herring), Coryphaenidae (mahi-mahi), Gempylidae (escolar), Istiophoridae (marlin, sailfish) and Carangidae (amberjack or yellowtail), which were implicated in histamine intoxication outbreaks (Schulze *et al.*, 1979; Muller *et al.*, 1992; Feldman *et al.*, 2005; Chen *et al.*, 2010; Chen *et al.*, 2011).

Histamine is a biogenic amine with a heterocyclic structure and it can be found in foods such as meat, cheese, wine and other fermented foods as well as in fish. Histamine is derived from the decarboxylation of histidine by the enzyme histidine decarboxylase (HDC). HDC is produced by bacteria which are part of natural microbiota of fish.

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According to the European Food Safety Authority (EFSA, 2011), bacteria species that are the strongest histamine producers are *Hafnia alvei*, *Morganella morgani*, *Klebsiella pneumonia*, *Morganella psychrotolerans*, *Photobacterium phosphoreum* and *Photobacterium psychrotolerans*. Some bacteria that belong to the family *Enterobacteriaceae*, such as *Enterobacter asburiae*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Citrobacter amalonaticus* and *Cronobacter sakazakii*, are also capable of producing histamine (Zhang *et al.*, 2018). Although the main means to prevent the occurrence of histamine is maintenance of low temperatures during fish processing, Emborg *et al.* (2005) found that psychrotolerant bacteria are capable of producing toxic concentrations of histamine in tuna at 2°C.

In contrast to bacterial pathogens, histamine is heat stable, and it cannot be eliminated or reduced by heat treatment; therefore, maintenance of the cold chain along all stages of fish processing is essential. Heating processes can only be used to eliminate histamine-producing bacteria and their HDC enzymes from the product (FAO/WHO, 2013). However, some authors reported different methods that can degrade histamine and other biogenic amines such as gamma irradiation and application of diamine oxidase bacteria (Dapkevicius *et al.*, 2000; Kim *et al.*, 2004).

The symptoms associated with HFP are similar to those of an allergic reaction and include hypertension, flushing, headache, urticaria, nausea, vomiting, diarrhoea and abdominal cramps (Maintz and Novak, 2007). The presence of certain histamine potentiators in fish, such as cadaverine and putrescine, could decrease the dose of histamine needed to provoke an adverse reaction in humans (FAO/WHO, 2013). The symptoms typically occur rapidly, from 5 mins to 2 h after ingestion, with a usual duration of 8 h. Many incidents go unreported because of the mildness of the disease, lack of mandatory reporting and misdiagnosis, because symptoms can be confused with *Salmonella* infection and food allergy (Silva *et al.*, 2011).

According to reports from the Rapid Alert System for Food and Feed, in 2018, 29 products were reported in which histamine levels were above the European Commission (EC) regulated maximum limit (European Commission, 2015). Well designed good hygienic practice (GHP), good manufacturing practice (GMP) and hazard analysis (and) critical control point (HACCP) plans are essential steps in preventing histamine formation above the safety limits. Rapid chilling of the fish soon as

possible after harvesting and maintenance of the cold chain through all stages of fish processing are the most important factors in preventing histamine production above the safety limit. EFSA indicate that the food categories showing the highest mean histamine levels are dried anchovies, fish sauce, fermented vegetables, cheese, other fish and fish products and fermented sausages (EFSA, 2011). According to Zhai *et al.* (2012), the main fish species used in canned products are scombroid fish (Spanish mackerel, tuna) and non-scombroid fish (anchovy, sardine) that are commonly associated with HFP. Sensory evaluation of the fish is not sufficient to detect the absence or presence of histamine, and it cannot be taken as final assurance of low histamine level; therefore chemical testing is required.

The aim of this study was to provide information regarding the presence of histamine in canned tuna, canned sardines, canned mackerel and smoked salmon present in Serbian retail stores in 2018 and to evaluate the safety of these products in regard to histamine levels established by the European Commission (European Commission, 2015) and FDA (FDA, 1995).

Materials and Methods

Standards and reagents

Histamine standard was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and water of HPLC grade were from Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate and formic acid were purchased from Merck (Darmstadt, Germany). Trichloroacetic acid (TCA) was from Fisher Chemical (Hampton, NH, USA).

Fish samples and extraction

A total of 227 lots of canned fish and smoked salmon samples were analysed. Every lot consisted of 9 subsamples, making a total of 2043 subsamples. All subsamples were collected from Serbian retail stores during 2018 and they were all imported from other countries. The fish samples analysed included canned tuna (91), canned sardines (54), canned mackerel (13) and smoked salmon (69). All samples were homogenised using a bowl chopper (Blixer 2, Robot Coupe, France). A 1 g amount of each sample was transferred into a polypropylene tube, followed by addition of 10 mL of 6% (w/v) TCA. The mixture was vortexed for 20 min and then centrifuged at 4000 rpm for 5 min.

The supernatants were filtered through 0.45 µm pore-size nylon syringe filters (Amtast, Lakeland, FL, USA) into HPLC vials.

LC-MS/MS

The quantification of histamine was carried out using a Shimadzu (Shimadzu Corporation, Kyoto, Japan) system consisting of two LC-30AD UPLC pumps connected in binary gradient mode, DGU-20A degassing unit, SIL-30AC autosampler, CTO-20AC column oven, CBM-20A system controller and LCMS 8040 triple quadrupole MS detector. Heat block, interface and desolvation line temperatures were 400°C, 350°C and 250°C respectively. Instrument control, data acquisition and evaluation were accomplished with the LabSolutions software (Shimadzu Corporation, Kyoto, Japan). Three transitions of histamine were monitored (m/z 112>54, m/z 112>68 and 112>95). Chromatographic separation of histamine was carried out on a Kinetex® (Phenomenex, Torrance, CA, USA) column (100×2.1 mm, 2 µm) at 50°C with an isocratic flow rate of 0.35 mL/min. The mobile phase consisted of 10 mM ammonium acetate in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) in the ratio A:B = 20:80.

Method validation

The analytical method was validated according to *Thompson et al.*, (2002). The experimentally determined limit of detection (LoD) was 2 mg/kg, while the limit of quantification (LoQ) was 5 mg/kg. Linearity was acceptable within the concentration range of 5–250 mg/kg, and the relative standard deviation of reproducibility was between 1.69% (at 250 mg/kg) and 13.93% (at 5 mg/kg). Measurement uncertainty of the method was calculated according to the NORDTEST concept (*Magnusson et al.*, 2012) and was 4.42% at 100 mg/kg.

Statistical analysis

The percentage of samples containing histamine among groups based on different histamine level, mean histamine levels and standard deviation were determined using SPSS package (SPSS 23.0, Chicago, IL, USA). To distinguish statistical differences between the data, T-tests were performed with statistical significance being set at $p < 0.001$, using the above-mentioned software package.

Results and Discussion

Histamine levels in 2043 subsamples of canned tuna, canned sardines, canned mackerel and smoked salmon are presented in Table 1. Large variations in histamine level in different cans with same lot number were previously reported by *Petrovic et al.* (2016) while investigating a histamine intoxication outbreak in Serbia. When considering product safety, it is essential to present results of all analysed subsamples, rather than mean levels. According to EFSA, fish containing less than 50 mg/kg of histamine seems to be safe for human consumption, whereas concentrations between 50 and 200 mg/kg may cause adverse health effects and levels above 200 mg/kg histamine are reported to cause toxic effects in humans (*EFSA*, 2011). Based on these limits, 78 (3.82%) of 2043 analysed subsamples could have caused adverse health effects when eaten. In the EU, for simple fish products, the critical concentration of histamine is 100 mg/kg, and for the enzyme matured products, the critical histamine concentration is 200 mg/kg. One sample consists of nine units, two of which can contain levels between 100 and 200 mg/kg, i.e. 200–400 mg/kg for enzyme-treated products (*Commission Regulation (EC) No. 2073/2005*). In Serbia, the regulation that governs histamine in fish (*Official Gazette RS, 2010*) is in accordance with EC Regulation 2073/2005. In 78 (3.82%) of all our analysed subsamples, histamine levels were above the *FDA* (1995) action level (50 mg/kg), making a total of 21 rejected lots, while 6 lots were non-compliant according to EC Regulation 2073/2005. Figure 1 shows a chromatogram of three transitional products of histamine at 50 mg/kg in one fish sample.

Histamine levels above 200 mg/kg, which is the upper limit in the EU, were found in 12 subsamples of canned tuna and in 8 subsamples of canned mackerel. *Suyama and Yoshizawa* (1973) investigated the free amino acid composition of some fish species and found histidine levels above 12000 mg/kg and 7400 mg/kg in *Thunnus albacares* (yellowfin) and *Thunnus obesus* (big-eye tuna), respectively. High levels of histidine (8020 mg/kg) were also found in *Scomber japonicus* (mackerel) (*Hibiki and Simidu*, 1959). According to the FAO, the highest histidine level reported for *Sardina pilchardus* (sardines) was 2888 mg/kg, which is much lower than those found in tuna and mackerel (*FAO/WHO*, 2013). In the current study, the mean histamine level found in canned sardines was lower than those of canned tuna and canned mackerel, which

Table 1. Histamine content in all subsamples (n=2043) analysed in this study and number of non-compliant lots according to EC and FDA regulations

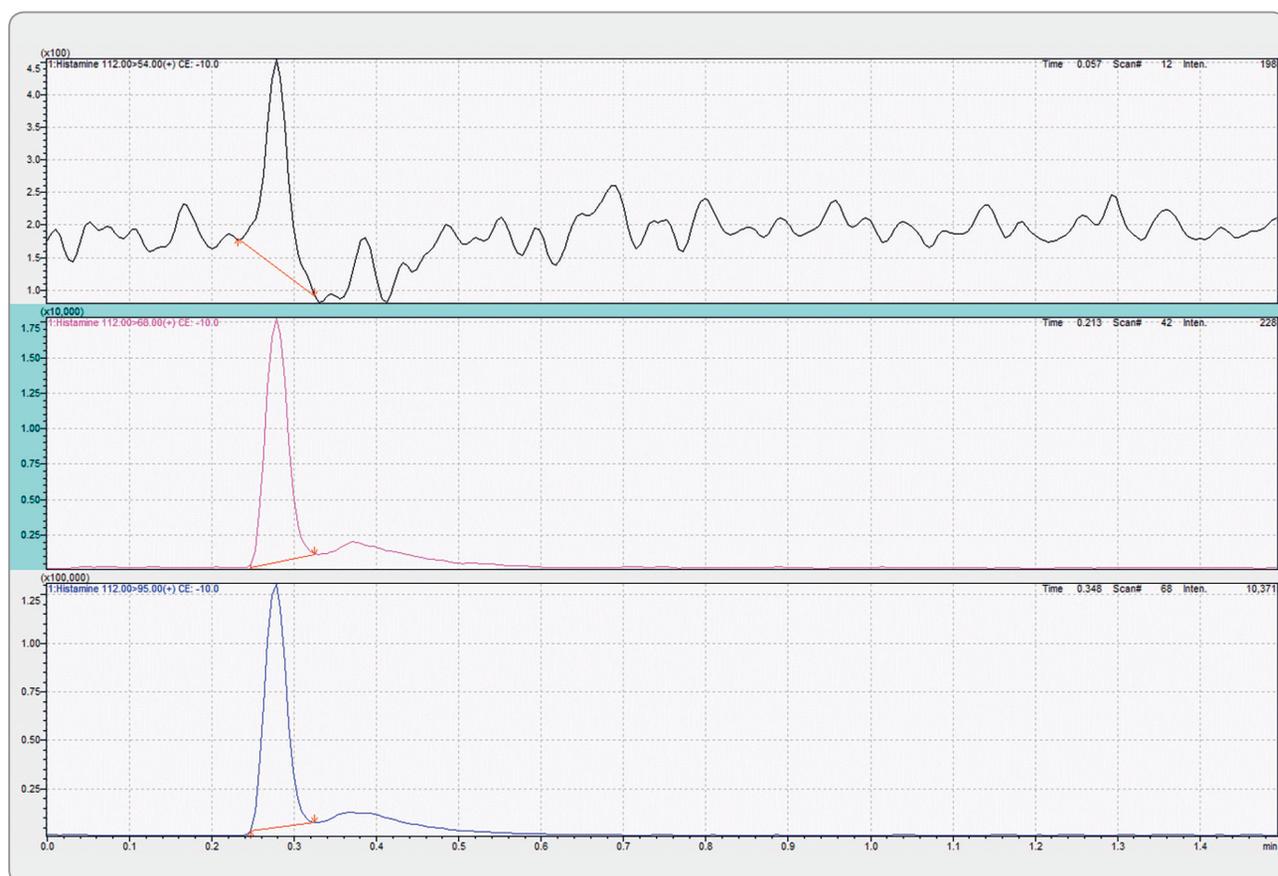
Product	No. of subsamples (lots)	No. (%) of subsamples with a histamine content (mg/kg) of:						No. (%) of non-compliant lots (EC) ^c	No. (%) of non-compliant lots (FDA) ^d
		<LoQ ^a	5–50	50–100	100–150	150–200	>200 ^b		
Canned tuna	819 (91)	480 (58.91)	286 (34.92)	27 (3.30)	3 (0.37)	11 (1.34)	12 (1.46)	4 (4.40)	13 (14.29)
Canned sardines	486 (54)	440 (90.53)	44 (9.05)	2 (0.41)	0	0	0	0	2 (3.70)
Canned mackerel	117 (13)	92 (78.63)	14 (11.97)	2 (1.71)	1 (0.85)	0	8 (6.84)	2 (15.38)	2 (15.38)
Smoked salmon	621 (69)	563 (90.66)	45 (7.25)	10 (1.61)	2 (0.32)	1 (0.16)	0	0	4 (5.80)

^a Subsamples in which histamine levels were below limit of quantification (LoQ) (5 mg/kg).

^b Subsamples in which histamine levels were above 200 mg/kg.

^c Non-compliant lots according to EC Regulations

^d Non-compliant lots according to FDA Regulations

**Figure 1.** MRM chromatograms of histamine at a concentration of 50 mg/kg. Chromatograms depict transitional products 112>54 Da, 112>68 Da, 112>95.

indicates the strong relationship between histidine level in muscle tissue and high histamine content in the product.

The quality of the analytical process was confirmed by the analysis of the certified reference material T27229QC (Fera Science Ltd., York, United Kingdom) within each batch of analysed samples. The certified histamine content was 45 mg/kg. Obtained histamine contents for this certified product were within $\pm 15\%$ of the certified content. Chromatograms of three transitional products of histamine in the certified reference material is presented in Figure 2.

According to Commission Regulation 2073/2005, all subsamples of our canned sardines and smoked salmon showed histamine levels below the safe level of 100 mg/kg, with the exception of three subsamples of smoked salmon from three different production lots. Histamine levels below the laboratory LoQ were found in 1601 (76.68%) of all analysed samples, while histamine levels in 58.91% samples of canned tuna were below the LoQ. This percentage was slightly higher than that reported by *Dimitrijevic et al.* (2016) who found histamine levels in 50.37% of canned tuna samples to be below the LoQ. In a similar study conducted in Serbia, histamine levels in canned tuna samples were found to be below the LoQ in 54.40%, between 10 and 50 mg/kg in 34.40% and between 50 and 100 mg/kg in 4% of analysed lots (*Petrovic et al.*, 2016), which is in accordance with results found in this study. Levels above 100 mg/kg were found in 25 (3.04%) of our canned tuna samples. In contrast to this, *Karmi* (2014) found histamine levels in 33.3% of canned tuna samples were above 100 mg/kg. High histamine levels in these canned tuna indicate poor temperature treatment and a lack of hygiene during processing. Canned tuna is often used in the preparation of different types of salads, where recontamination and time-temperature abuse can lead to formation of histamine in levels that can cause HFP (*Predy et al.*, 2003; *McCarthy et al.*, 2015).

The highest histamine level found in our canned sardines was 78 mg/kg, which is in accordance with the results reported by *Karmi* (2014) and *Pacheco-Aguilar et al.* (1998), who found maximum levels of 62 and 70.3 mg/kg, respectively. These results differ from data presented by *Petrovic et al.* (2016), who reported a maximum histamine level of 19.1 mg/kg in canned sardines.

Kim et al. (2002) investigated histamine production by *Morganella morganii* in four different fish families at 37, 25, 15 and 4°C for 36, 36, 60 and

14 h, respectively. They found levels below 50 mg/kg in all salmon samples, while levels in mackerel, albacore and mahi-mahi exceeded 50 mg/kg in a few hours at different temperatures, with the exception of fish held at 4°C. Similar results were reported by *Crapo and Himelbloom* (1999), who found no histamine production in pink salmon during 14 days of storage at 10°C. These results are comparable with results found in present study, in which we found 97.91% of smoked salmon samples had histamine levels below 50 mg/kg, while 90.66% were below the LoQ. This is attributed to low histidine contents in muscle tissue of salmon and probably due to good hygiene and low storage temperatures during fish processing. Although salmon is not histidine rich, *Bartholomew et al.* (1987) reported 12 incidents of salmon being involved in HFP from 1976 to 1986 in Britain.

Mean histamine contents were calculated for all analysed subsamples of each product and for all subsamples of compliant production lots according to the Commission Regulation 2073/2005 in order to avoid outliers. Histamine levels varied significantly ($p < 0.001$) among the analysed fish products (Table 2). These results are in accordance with those reported by *Yesudhason et al.* (2013), who found a significant difference ($p < 0.05$) between mean histamine levels in canned tuna and canned sardines. Our mean histamine level (9.21 mg/kg) in canned tuna calculated for compliant production lots was similar to those found by *Er et al.* (2014), lower than that reported by *Tsai et al.* (2005) but higher than those found by *Silva et al.* (2011), who reported very low levels of histamine for different canned tuna products in Brazil. In the current study, the mean histamine level in canned mackerel samples for compliant lots (3.34 mg/kg) was in accordance with that reported by *Tsai et al.* (2005) but much lower than that reported by *Karmi* (2014), who found histamine at 68 mg/kg in canned mackerel, with a maximum level of 82 mg/kg.

In several histamine intoxication outbreak studies, reported levels of histamine in leftovers of different fish and fish products ranged from 293 mg/kg to 5200 mg/kg (*Feldman et al.*, 2005; *Tsai et al.*, 2005; *Chen et al.*, 2008; *Chen et al.*, 2010; *Ryok Kang et al.*, 2018). Reported symptoms were flushing, headache, rash, nausea, diarrhoea, sweating, peppery taste and dizziness but no cases of death. In the current study, maximum levels of histamine found in canned tuna, canned mackerel and smoked salmon were 1112, 412 and 122 mg/kg, respectively. Based on histamine intoxication studies,

we can conclude that levels of histamine we found in tuna and mackerel could have caused HFP in healthy individuals. However, the usual portion size of canned tuna per consumer is about 130 g, which corresponds to a total of 144.5 mg of histamine in a portion of canned fish containing 1112 mg/kg of histamine. The high levels of histamine we measured in these canned fish products leads to the conclusion that poor quality raw fish has been used for their production. *Motil and Scrimshaw*

(1979) reported that ingestion of 50, 100, 150 and 180 mg of histamine *via* tuna caused typical symptoms such as mild headache and flush in 1, 3, 4 and 7 of 8 healthy individuals, respectively. However, this dosage levels will not apply to individuals with a specific sensitivity to histamine and would not apply to children, particularly because they consume more food per unit body weight than adults (*FAO/WHO*, 2013). Storage temperature is the most important factor contributing to biogenic amine

Table 2. Histamine levels (mg/kg) for different fish products analysed in this study and levels for compliant lots according to EC Regulations (mean±SD)

	Compliant lots ^a	Median	Max	All analysed lots ^b	Median	Max
Canned tuna	9.21±14.8 ^c	2.5	1112	21.70±89.1 ^c	2.5	169
Canned sardines	3.16±3.8 ^d	2.5	78	3.16±3.8 ^d	2.5	78
Canned mackerel	3.34±3.5 ^d	2.5	26	26.45±77.2 ^c	2.5	412
Smoked salmon	5.22±13.7 ^c	2.5	171	5.22±13.7 ^c	2.5	171

^a Subsamples of compliant lots. For levels below LoQ, the level used was 2.5 mg/kg.

^b Subsamples of all analysed lots. For levels below LoQ, the level used was 2.5 mg/kg.

^{c-c} Values in the same column followed by different letters are significantly different ($p < 0.001$).



Figure 2. MRM chromatograms of histamine in certified reference material used for quality control of analytical process. Chromatograms depict transitional products 112>54 Da, 112>68 Da, 112>95.

formation, although a variety of techniques can be combined together to control the microbial growth and enzyme activity during processing and storage for better shelf life extension and food safety (Chong et al., 2011).

Conclusion

In this study, four (4.40%) production lots of canned tuna and two (15.38%) lots of canned mackerel were non-compliant for histamine levels according to Commission Regulation (EC) No. 2073/2005. In contrast, in 13 (14.29%) lots of canned tuna and two (15.38%) lots of canned mackerel, histamine

levels were above the FDA action limit of 50 mg/kg (FDA, 1995). Although the histamine content in all our canned sardines and smoked salmon samples were compliant according to EC Regulation 2073/2005, two (3.70%) production lots of canned sardines and four (5.80%) production lots of smoked salmon would be non-compliant if we had applied the FDA action limit, due to the much stringent requirements. Canned tuna had the highest mean histamine levels, followed by smoked salmon and canned mackerel, while canned sardines contained the lowest mean histamine level. Histamine was detected in 468 (22.91%) subsamples of all analysed fish products.

Histamin u konzerviranim i dimljenim proizvodima od ribe koji se prodaju na tržištu u Srbiji

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Apstrakt: Posljednjih godina primećeno je povećanje broja bolesti povezanih sa konzumacijom plodova mora kao posledica povećanja konzumacije ovih proizvoda. Uprava za hranu i lekove Sjedinjenih Američkih Država (eng. FDA) označila je histamin kao glavnu hemijsku opasnost plodova mora. Cilj rada bio je da se odredi sadržaj histamina u 227 proizvodnih lotova konzervirane tune, konzervirane sardele, konzervirane skuše i dimljenog lososa. Svi uzorci su prikupljeni iz maloprodajnih objekata u Republici Srbiji tokom 2018. godine. Za ispitivanje histamina korišćena je tačna hromatografija visokih performansi sa masenom detekcijom. Dobijene rezultate smo uporedili sa nivoima koje su uspostavili Evropska komisija i FDA. Histamin je detektovan u 468 (22.91%) jedinica analiziranih proizvoda. U skladu sa propisom Evropske komisije 2073/2005, šest (2.64%) ispitivanih proizvodnih lotova su ocenjeni kao nezadovoljavajući, dok su čak 21 proizvodnih lotova ocenjeni kao nezadovoljavajući u skladu sa propisom FDA.

Cljučne reči: histamin, tuna u konzervi, skuša, sardela, bezbednost hrane.

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