FISH PRODUCT SAFETY IN LITHUANIA: ASSESSMENT OF HISTAMINE LEVELS

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Abstract
The paper provides research data on histamine content in fish and fish products purchased in a supermarket in Lithuania. The prevalence of histamine in the analyzed fish ranged from 5.00 mg kg^{-1} to 240.78 mg kg^{-1}, with mean value of 36.97±12 mg kg^{-1}. Histamine levels in 16% analyzed salmon samples exceeded the limits defined in Regulation (EC) 2073/2005 (7). Such increase was influenced by seasonality: significantly higher histamine levels were found in fish after spawning than in those caught in the autumn and winter seasons.
Histamine levels in salmon varied from 5.00 mg kg^{-1}up to 240.78 mg kg^{-1}, In herring, the levels of histamine ranged from from 5.00 mg kg^{-1}up to 177.45 mg kg^{-1}. Smaller quantities - in a range of 5.00-102.50 mg kg^{-1} were found in mackerel, although this fish belongs to the species with an abundant prevalence of histidine. The levels of determined histamine in fish products were significantly higher than in fresh fish, at $p < 0.05$. The highest levels of histamine were detected in herring products, yet they varied from 6.09 mg kg^{-} to 177.45 mg kg^{-1}although the mean value was 59.89±25 mg kg^{-1}. Technological processing had impact on the accumulation of histamine in fish products. The smallest quantities of histamine were found in fermented fish products; slightly higher amounts, in salted dried fish, and the highest levels of histamine were determined in cold and hot smoked fish. In cold smoked escolar the level of histamine was 176.00 mg kg^{-1}, as well as in hot-smoked mackerel – up to 128.01 mg kg^{-1}. Considering the safety of fish products in relation to histamine content, herring products are the least safe as there were significantly higher levels of histamine found ($p<0.05$) in these products than in other analyzed products.

Keywords: fresh fish, smoked fish, histamine

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1. INTRODUCTION

Fish is an important source of proteins and minerals. When compared to animal meat, fish meat contains less connective tissue - about 3% of fish weight (in animal meat, up to 15% of body weight). Muscle structure is also different. Fish muscle fibers are short, separated by a thin layer of connective tissue. This provides to the fish meat a layered and porous structure. However, the fish are perishable, protein degrade under the influence of bacterial 0enzymes forming biogenic amines. Therefore, the content of biogenic amines in fish is attributed as the quality index and can be an important indicator of hygiene (Praškevičius *et al.*, 2003, Zhai *et al.*, 2012).
Histamine is a biogenic amine formed naturally in fish after the post-mortem and certain fish products due to improper handling, technology deviations, i.e. improper storage and temperature fluctuations. Free histidine is usually determined in the muscles of scombroidea fish under the influence of histidine decarboxylase, converted to histamine, which can cause scombroid fish poisoning (Hungerford and Wu, 2012, Taylor, 1986).
Recently conducted scientific investigations revealed that other non-scombroid fish species, such as mahi-mahi (*Coryphaena spp.*), Sardinellas (*Sardinella spp.*), Sardines (*Sardinapilchardus*), anchovy (*Engraulis spp.*), herring (*Clupea spp.*), marlin (*Makaira spp.*) bluefish (*Pomatomus spp.*) (Hwang *et al.*, 1997, Taylor, 1986) as well as Western Australian salmon (*Arripis truttaceus*), red salmon (*Oncorhynchus nerka*), Amberjacks (*Seriola spp.*) (Gessner *et al.*, 1996) and swordfish (Xiphias gladius) (Chang *et al*, 2008) are also potentially dangerous and can cause scombroid poisoning. Most of these fish are rich in free histidine (Antoine *et al*, 1999),

except salmon and swordfish (Suzuki *et al.,* 1987).

Scombroid poisoning can cause a variety of symptoms including rash, urticaria, nausea, vomiting, diarrhea, skin redness, itching (Taylor, 1986). Severity of symptoms can vary considerably from the consumption of histamine in the body and individual sensitivity to histamine (Hungerford, 2010).

The histamine forming bacteria isolated from fish are those of the Enterobacteriaceae family - *Morganella morganii* – the most productive bacteria, *Klebsiella pneumoniae, Hafnia alvei* and naturally occuring marine bacteria (*Photobacterium spp., Pseudomonas spp. Vibrio spp. Aeromonas spp.),* also some Gram-positive bacteria (Cinquina *et al.*, 2004, Shakila and Vasundhara, 2002). There exist two different histidine decarboxylase enzyme classes: gram positive bacteria which produce heteromeric histidine decarboxylase containing an active threatening pyruvic group (Konagaya *et al.*, 2002) and gram-negative bacteria, which produces histidine decarboxylase, belonging to Pyridoxal 5-phosphate group (Kamath *et al,* 1991). It is also possible that some fermented products, such as fish sauce, can contain both types of histidine dekarboxylases which are produced by gram-negative and gram-positive bacteria during the fermentation process.

Although histamine formation can be controlled by properly selecting temperature regime and avoiding downtime during the manufacturing process, it is now known that bacteria that produce histidine decarboxylase can develop at a temperature below the 0-5^{o}C (Emborg and Dalgaard, 2006, Kanki *et al.*, 2004). Recent studies in Denmark and Japan show that the presence of ***psychrotolerant*** bacteria *Morganella psychrotolerans* and *Photobacterium phosphoreum* is the cause of toxic levels of histamine in fish product because these psychrotolerant bacteria may cause toxic concentration of histamine in seafish, especially when these products are stored refrigerated. The studies confirmed the toxic histamine formation in chilled seafood during storage. More than 500 ppm of histamine was found in 18 cases out of 59 analytical studies (31%) carried out in naturally deteriorated seafood when warehousing under the -1 to +5 ^{0}C storage conditions (Emborg and Dalgaard, 2008).

Histamine is not evenly distributed across the defective fish or its parts, therefore not all consumers who consumed the fish could be histamine poisoned. This means that the 50 ppm can be found in one part of the fish, and more than 500 ppm in the other part of the fish (Lehane and Olley 2000).

Fish, which under normal conditions are usually very active, e.g. tuna and mackerel fish, can suffer stress or even die during catching. It has been found that an increase in bacterial contamination is especially noticeable under fish stress conditions. Any physical damage to fish accelerates its deterioration, reduces the commercial value and suitability for further processing. In addition, marine equipment and fishermen themselves can be a source of fish contamination (Masteikienė, 2007).

Histamine is heat stable and remains in the subsequent stages of technological process, e.g. when preserving fish with high histamine levels (more than 1000 ppm), and can cause adverse health effects. Bacterial breakdown and histamine formation is possible at any stage of the food manufacturing chain (i.e. during fish loading, processing, product distribution, processing at catering facilities, or at home) (McLauchlin *et al.,* 2005). The best way to prevent the formation of histamine is to control the temperature which should not exceed 5 °C (Cinquina *et al.,* 2004, Križek *et al.*, 2002).

Austrian scientists (Rauscher-Gabernig *et al.*, 2009) conducted a comprehensive analysis of scientific references on histamine effect to human health and summarized the results of analytical investigations in the period of 2011-2006 on histamine contents in foods. The authors claim that unharmful concentration of histamine in a quality fish should contain histamine levels less than 50 ppm, and the levels between 50-200 ppm may cause intoxication, while the levels from 200 – 1000 mg kg are severely dangerous. Histamine concentration of 500 ppm in tuna fish is

established as dangerous. Regulation (EC) 2073/2005 establishes maximum histamine levels for species especially rich in histidine (EU, 2005).
Aim of the work: to estimate the safety of fish and fish products in relation to histamine.

2. MATERIAL AND METHODS

The research was conducted within the period of 2010 - 2012, at the Chemistry Laboratory of Food Institute of the Kaunas University of Technology. Total 146 fish product samples, including herring, salmon, cod, carp, and trout were bought to a supermarket from different manufacturers. The samples were stored under the manufacturer recommendations before the biogenic amine analysis.
Histamine levels were determined by high performance liquid chromatography. 5 g of homogenized fish or fish product sample were weighted. Biogenic amine histamine isolated by extraction with 0.4 mol / l perchloric acid. The extracted part was derivatized with dansylchlorid solution (5-dimethylaminonaphtalene -1 -sulfonyl chloride) for 45 minutes at 40°C. Residual dansylchlorid was removed with 25% ammonia after derivatization when cooled down to room temperature. The samples were filtered through a 0.45 mm membrane filter, 10μl injected into the chromatographic system. Analysis was performed using LiChroCART ® 125-4 column filled with LiChrospher ® 100 RP-18-e (5 mm), carrier-phase - Eluents: B - acetonitrile, A - ammonium acetate 0.1 mol / l. The analysis took 28 min: the first 19 minutes changing the eluent composition from 50 % B to 90 % B (from 50 % A to 10 % A), then 1 min, leaving a constant eluent composition - 90 % B (10 % A). The refilling of the column was performed with eluent of 50 % B and 50 % A composition for 8 min to ensure substances separation during the next analysis. The flow rate of the entire analysis remained constant - 0.9 ml / min, UV detection was at 254 nm. Histamine was identified by comparing its retention time with the retention time of the reference material. Quantitative analysis was performed by the internal standard method calculating the peak area for the defined quantities of the reference material.
Histamine levels were assessed in a series of three consecutive tests. Statistical evaluation of the data was done using SPSS 13.0 for Windows software. The results of the experiment are presented as mean values (x) and standard deviations (±SD). Significance of the differences was defined at the 5% level ($p<0.05$).

3. RESULTS AND DISCUSSION

Fish and fish product abundant in histidine were selected for the analysis. Samples of fish and fish products were analyzed for histamine content. Histamine concentrations in mg kg^{-1} of the analyzed Atlantic salmon samples are presented in Table 1.
Histamine content significantly varied in cold smoked salmon samples: 5.00-240.78 mg kg^{-1}. Marinated salmon samples contained 48 times less histamine compared to those of cold-smoked salmon products and 23 times less, compared to those of chilled salmon products. Besides, significant differences were observed between the chilled salmon and chilled Atlantic salmon, as well as between smoked salmon and cold smoked Atlantic salmon. 4.6 times lower histamine levels were detected in Atlantic salmon products compared to those of salmon products. Less than 5.00 mg kg^{-1} of histamine level was revealed in marinated salmon fillet samples.
Data on histamine content analysis in herring samples are presented in Table 2.
The studies revealed histamine prevalence in a range of 6.09-177.45 mg kg^{-1}. Histamine level was 5.3 times lower in salted herring fillet in oil among all herring fillet samples and 3.5 times lower than in slightly salted herring fillet in oil samples. According to the content of NaCl, salted fish are divided into slightly salted - from 3 to 10%, medium salted - from 10 to 14% and salted heavily - more than 14% NaCl. Many fish depleting microorganisms multiply rapidly before NaCl content in fish has not yet reached 3%.

Table 1. Histamine content in Atlantic salmon samples, mg kg^{-1}

Samples	Histamin, mg kg^{-1}		
	Min value	Max value	Mean ± SD
Chilled salmon	13.05	117.28	65.00±73.54
Chilled Atlantic salmon	10.13	18.00	14.00±4.00
Salmon frozen bellies	85.27	159.29	122.00±28.00
Sligthly salted Atlantic salmon fillet	8.56	48.78	28.00±28.28
Marinated salmon fillet	<5.00	5.30	<5.00
Smoked salmon fillet	5.00	240.78	44.82±82.61
Smoked Atlantic salmon fillet	5.00	78.81	17.67±22.91

* Method‘s lowest determination limit – 5.00 mg/kg.

Table 2. Histamine content in herring samples, mg kg^{-1}

Samples	Histamine, mg kg^{-1}		
	min value	max value	mean ± SD
Chilled herring fillet	6.09	158.76	34.42±48.86
Herring fillet	8.56	124.02	66.00±82.02
Salted herring fillet in oil	6.16	60.75	12.50±18.72
Slightly salted herring fillet in oil	6.45	177.45	43.47±50.45
Slightly salted Atlantic herring	14.34	47.34	30.50±23.33
Herring fillet in oil	8.67	121.81	59.75±44.75
Atlantic herring fillet in oil	5.00	68.53	36.50±44.55
Smoky flavor herring fillet in oil	10.00	142.54	65.33±68.54
Marinated herring fillet in oil	67.02	93.38	80.00±18.38
Marinated herring without head in mustard sauce	6.55	8.16	7.00±2.16
Fermented herring fillet	6.04	10.00	8.00±2.00

* Method‘s lowest determination limit – 5.00 mg/kg.

Salting of poor quality fish at higher temperatures, can cause deterioration of fish before NaCl penetrates into the tissues. Data on histamine content analysis in herring delicatessen samples are presented in Table 3.

It was found that herring delicatessen products contained 5.00-124.20 mg kg^{-1} levels of histamine. Lower contents of histamine (by 5.7 times) were detected in herring fillet with stewed vegetables and beans, compared to those of herring fillets in tomato sauce samples, and on the average by 6.8 times lower than in the samples of herring fillet with stewed vegetables. Among the herring samples, significantly higher histamine prevalence was detected in herring with stewed vegetables - up to 124.20 mg kg^{-1}, in herring with tomato sauce – 108.50 mg kg^{-1} and in herring with sliced carrots – 107.03 mg kg^{-1}. Smaller amounts of histamine were detected in the samples of herring fillet with stewed vegetables and beans – 10.00 ± 2.99 mg kg^{-1}. Lower histamine levels (100 mg kg$^{-1)}$ were determined in 5 samples and in 3 samples the content of histamine varied within the limits of 100 mg kg^{-1}. There were no samples where the prevalence of histamine exceeded 200 mg kg^{-1} concentration. Histamine levels in mackerel samples in mg kg^{-1} are presented in Table 4.

The investigated mackerel products showed histamine prevalence within a range of 5.00-102.50 mg kg^{-1}.

Table 3. Histamine content in herring delicatessen samples

Samples	Histamine, mg kg^{-1} min value	max value	mean ± SD
Herring stewed with vegetable	12.09	124.20	68.00±79.20
Herring stewed with vegetable and beens	7.01	13.98	10.00±2.99
Herring in mayonise and shampinion sauce	7.12	19.56	13.00±5.56
Skinless herring in sunflower seeds sauce	<5.00*	67.06	33.00±34.06
Herring in tomato sauce	<5.00*	108.50	56.50±51.50
Herring in beetroot and mayonise sauce	<5.00*	100.52	52.50±47.52
Skinless herring in sunflower seeds sauce	<5.00*	107.03	56.00±51.03

* Method‘s lowest determination limit – 5.00 mg/kg.

Table 4. Histamine content in mackerel samples

Samples	Histamine, mg kg^{-1} min value	max value	mean ± SD
Frozen mackerel	12.65	28.03	11.25±12.03
Frozen Atlantic mackerel, deheaded, gutted	10.04	18.00	14.00±14.02
Atlantic mackerel	<5.00*	7.00	6.00±2.00
Mackerel	<5.00*	11.00	8.03±3.00
Hot smoked, Atlantic mackerel, deheaded, gutted	17.02	18.73	17.50±0.71
Hot smoked mackerel, dehead, gutted	78.00	128.01	103.00±25.01
Cold smoked, Atlantic mackerel, deheaded, gutted	6.10	26.45	13.67±7.50
Cold smoked mackerel, deheaded, gutted	23.19	102.50	56.00±37.48

* Method‘s lowest determination limit – 5.00 mg/kg.

Lower (by 17.2 times) histamine levels were detected in Atlantic mackerel samples when compared with those of hot smoked deheaded and gutted mackerel and by 9.3 times lower than in the samples of cold-smoked deheaded and gutted mackerel .

When comparing mackerel samples, higher amounts of histamine were detected in hot-smoked mackerel and in the samples of deheaded and gutted cold-smoked mackerel: 103.00±25.01 mg kg^{-1} and 56.00±37.48 mg kg^{-1}, while smaller quantities of histamine were found in Atlantic mackerel and mackerel, 6.00±2.00 mg kg^{-1} and 8.03±3.00 mg kg^{-1} respectively. Histamine levels lower than 100 mg kg^{-1} were detected in 7 samples and two samples had histamine level higher than 100 mg kg^{-1}, but not exceeded 200 mg kg^{-1}. The analysis revealed the impact of technological process on the formation of histamine: more than 4-time higher levels of histamine were detected in cold smoked mackerel compared to those of Atlantic cold smoked deheaded and gutted samples.

The same pattern was noticed in deheaded and gutted hot smoked mackerel and in Atlantic hot-smoked mackerel samples: 5.9 times higher histamine levels in mackerel samples than in Atlantic mackerel samples were detected. Salmon is more dangerous product against histamine, where the levels exceeded in 16% of tested salmon samples. It was mainly influenced by seasonality: significantly higher histamine levels were found in fish after spawning than in fish caught in autumn and winter period.

Table 5. Histamine level in fermented fish products of different species

Samples	Histamine level, mg kg^{-1} min value	max value	mean ± SD
Fermented bream	9.66	-	-
Fermented flatfish	63.04	-	-
Fermented herring fillet	8.87	-	-
Fermented icefish	7.54	-	-
Fermented hake	62.11	-	-
Fermented roach	<5.00*	-	-

* Method‘s lowest determination limit – 5.00 mg/kg.

Histamine levels in the samples of different fish species in mg kg^{-1} are presented in Table 5. The investigation revealed higher histamine levels in fish products compared to those of fresh fish samples ($p<0,05$). It was found that histamine prevalence in various species of fish products was within the limits of 5.00 – 176.00 mg kg^{-1}. A lower histamine level (by 22 times) was found in herring fillet with olive oil with a sweetener than in cold-smoked escolar samples and by 1.7 times lower histamine levels compared to cold-smoked halibut samples.

Histamine levels in different species of fermented fish samples in mg kg^{-1} are presented in Table 5.

The analysis revealed histamine prevalence in fermented fish products, where the concentrations ranged 7.54-63.04 mg kg^{-1}. Lower histamine levels (by 9 times) were detected in fermented icefish compared with gal dried flat fish. Histamine levels lower than 5 mg kg^{-1} were detected in fermented roach samples. The highest histamine levels were found in fermented flat fish and hake, respectively 63.04 mg kg^{-1} and 62.11 mg kg^{-1}, while smaller quantities were found in fermented icefish and herring fillet, respectively 7.54 mg kg^{-1} and 8.87 mg kg^{-1}. The level of histamine in products is in line with the requirements of Regulation (EC) 2073/2005 (100 mg kg^{-1}).

The analysis of the scientific references revealed that the accumulation of histamine in fish and fish products may be influenced by the chemical composition of raw materials, bacterial contamination, species of the fish, seasonality, additives type of processing, deviations from the technology processes, storage.

Investigation of freshwater fish products revealed low levels of histamine 2.50 - 63.04 mg kg^{-1}. That is in line with (Çoban and Patır, 2008) research data: where it was also found small amounts of histamine in carp *Cyprinus carpio* 7.39 - 15.31 kg^{-1}. Auerswald *et al.* (2006) found 3.80 - 48.40 mg kg^{-1} histamine levels in trout *Salmo trutta*, and only 2.80 kg^{-1} of histamine in marine salmon *Salmo salar*. The data presented in the reference is in line with our investigations, where chilled Atlantic salmon and frozen Atlantic mackerel were found to contain small amounts of histamine, respectively, 14.00 ± 4.00 mg kg^{-1} and 6.00 ± 2.00 mg kg^{-1}.

Authors (Križek *et al.*, 2011) had investigated the formation of biogenic amines in freshwater fish. Carp, rainbow trout and perch fillet samples were kept at 3^0C and 15^0C for 7 days. Fish stored at low temperatures (3^0C) showed small levels of histamine, but in those kept at 15 ^{0}C, histamine was detected already on the second day of storage, and on the seventh day the average levels of histamine were 38.6 mg kg^{-1} in carp, 79.7 mg kg^{-1} in rainbow trout and 110.0 mg kg^{-1} in carp meat.

Other authors claim that temperature is not the only factor influencing histamine accumulation. Histamine accumulation due to *M. psychrotolerans* and *P. phosphoreum* cannot be avoided simply by storing seafood at 0-5 ^{0}C. In order to keep histamine levels under control, the shelf life period has to be restricted, depending on the characteristics of the product (Emborg and Dalgaard, 2008).

4. CONCLUSIONS

The results of the study indicate that the levels of histamine in fish vary within a wide range:

from 5.00-240.78 mg kg-1 in salmon and from 5.00-177.45 mg kg-1 in herring. It was influenced by seasonality, significantly higher histamine levels were found in fish after spawning, than in fish caught in autumn and winter period. Technological processing affects the accumulation of histamine in fish as well: small quantities of histamine were found in fermented fish products, slightly higher in salted ($p<0.05$), the highest levels of histamine identified in cold ($p<0.01$) and hot smoked fish ($p<0.01$). When consider fish products safety in relation to histamine content, less safety demonstrated herring products, there were significantly higher levels of histamine found than in other analyzed products ($p<0.01$). It should be noted that it is permissible quantities and product safety is acceptable. There were no samples where the prevalence of histamine exceeded 200 mg kg^{-1}.

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