SIGNIFICANCE OF HISTAMINE CONTENT IN FISH FROM THE ASPECT OF HYGIENIC QUALITY

Vusilović[1], R., Ž. Cvrtila Fleck[2], N. Zdolec[2], I. Filipović[2], L. Kozačinski[2], B. Njari[2], M. Hadžiosmanović[2]

SUMMARY

Significance of histamine content in fish is presented in this paper. After 3 days of storage at room temperature, the analysed samples of mackerels (n=36) were found to be of unacceptable hygienic quality because of increased histamine level (winter 2007). On day 4 of storage, histamine level exceeding 100 mg/kg was found also in samples kept at refrigerator temperature. In samples analysed in summer of the same year, increased histamine content was found already on day 2 (room temperature), day 3 respectively, in case of samples stored at refrigerator temperature, and their hygienic quality was evaluated as unacceptable. Systemic measures of fish storage at lower temperatures could prevent histamine poisoning in men. Established standards, critical histamine levels, as well as measures for prevention of histamine poisoning within the framework of veterinary-sanitary control in the Republic of Croatia are in compliance with the Book of rules on hygienic quality of food of animals origin (Official Journal of Croatia, 99/07).

Key words: *histamine, fish, storage temperature*

INTRODUCTION

Post-mortem changes are of special significance in the evaluation of freshness and hygienic quality of fish. As pointed out by Šoša (1989), characteristic tissue structure, unstable protein composition and unstable fat are largely responsible for both rapid deterioration and instability of fish during storage. One of main causes of rapid deterioration is increased activity of digestive and other enzymes. Psychrophilic bacteria that multiply optimally at temperatures of 10-15°C are prevailing in bacterial degradation of fish meat. The absence of connective-tissue fasciae facilitates the penetration of bacteria, and another contributing factor is a greater quantity of water in fish meat than in meat of warm-blooded animals. Muscles of fresh and healthy fish are practically sterile, but during catch, reloading, storage and processing, the present microorganisms penetrate the meat through skin and peritoneum or through the blood-vascular system. As a result, an accelerated growth of microorganisms occurs after termination of *rigor mortis* (Schalaby, 1997).

Autolysis and bacterial breakdown are interwoven. Aldehydes, ketones, lower fatty acids, amino groups, cyclic amines (histamine, phenylethylamine), diamines (putrescine and cadaverine) are degradation products of bacteria. Further degradation into most simple components follows. Oxidation of fats can also cause decomposition of fish meat. Such changes are not caused by bacteria, but are equally rapid and tumultuous. Two phases of development are possible: invisible and visible. Under the influence of fish tissue enzymes and microorganisms in invisible phase are formed biogenic amines, among which prevails histamine. This phase is of special health hazard due to the fact that it is not accompanied by organoleptic changes i.e. it is invisible. Organoleptic changes are manifested in visible phase as alterations of colour, smell, consistency and other fish traits (ŠOŠA, 1989). Cases of intoxication with biogenic amines in consumers of fish and fish products have been reported. This refers to histamine poisoning occurring in the process of histidine decarboxylation. Of special practical importance is the fact that toxicity of biogenic amines is possible in early stages of deterioration of fish meat when the advanced organoleptic changes are not yet manifested (Živković and Hadžiosmanović, 1989).

Histamine, one of the most important biogenic amines in fish, was discovered in nature as late as 1910. Already in 1907, histamine was synthesised but, what is interesting, it was found in nature later. Histamine, beta-imidazolethylamine ($C_5H_9N_3$) is a solid substance in the form of colourless crystals, and freely soluble in water. It is a

[1] **Renata Vusilović, DVM, Vukovarska 36, 43280 Garešnica**

[2] **Željka Cvrtila Fleck, DSc, assistant professor; Nevijo Zdolec, DSc, junior researcher- senior assistant; Ivana Filipović, DVM, junior research assistant, Lidija Kozačinski, DSc, associate professor; Bela Njari, DSc, full professor, Mirza Hadžiosmanović, DSc, full professor, Department of Hygiene and Technology of Foodstuffs of Animal Origin, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb**

Table 1. Results of organoleptic examination of fish samples in winter of 2007 (n= 18)

		SKIN and SMELL	GILLS	EYES	ANUS	MEAT
Day 1	AT (2A, 2B)	Intact, tense, bluish-green, without mucus, smell to fresh fish	Moist, clear and red	Clear and convex	Closed	Firm, elastic – finger indentation not retained
	RT (1A, 1B)					
Day 2	AT (2A, 2B)	Intact, reduced glow, some scales falling off, sharp	Less red, mucous with bloodspots	Concave, turbid	Opened	Reduced consistency, finger indentation still not retained
	RT (1A, 1B)	Shining, transparent mucus, intensive to fish	Paler, mucous	Slightly concave, clouded	Opened	Reduced consistency, finger indentation still not retained
Day 3	AT (2A, 2B)	Sticky, brown-grey without glow, unpleasant	Brown, opaque mucus, brown	Completely sunken, opaque	Opened, liquid coming out under pressure	Pinkish, finger indentation retained
	RT (1A, 1B)	Damages, scales falling off, loss of glow	Less red, mucous with bloodspots	Sunken, opaque	Opened	Pinkish, finger indentation on fish surface retained
Day 4	AT (2A, 2B)	/	/	/	/	/
	RT (1A, 1B)	Sticky, brown-grey, without glow, unpleasant like rotten	Brown, opaque mucus, brown	Completely sunken, opaque	Opened, liquid coming out under pressure	Grey-brown, soggy, finger indentation on fish surface retained

AT – ambient (room) temperature; RT – refrigerator temperature

normal constituent of every organism and belongs to tissue hormones. Free histamine has a very short life, since other enzymes cause its disintegration in tissues (Garai et al., 2006).

As known from literature data, the dynamics of histamine formation depends primarily on the amount of free histidine in the muscles of fish, and also on the species and number of histidine-decarboxylase-producing bacteria (Moeller and Peter, 2000). The initial histidine level in fish proteins is about 6.5%, in contrast to only 1 to 3% in proteins of slaughter animals. This is the main reason why the formation of a greater quantity of histamine occurs more rapidly in fish. Furthermore, the environment temperature is one of important factors for histamine formation. Already the earliest performed studies have confirmed the formation of histamine in mackerels kept at the temperature of 17°C (Kimata and Kawai, 1953, quotation Pfeifer et al., 1985). Other researchers have mentioned the temperatures between 20° and 25°C as optimal (Smith et al., 1980, quotation Pfeifer et al., 1985). According to findings of Toković and Slavić (1986), individual species of fish stored at +4°C for 3-7 days can contain up to 100 mg% of histamine. Investigation of histamine content in frozen fish (mackerel, tuna, seabass and pilchard) before canning showed an increased histamine content in fish in summer period compared to spring months (Šimičević, 1995).

Garlini et al. (1996) have performed an 8-year-study of histamine content in different fish cans. Histamine content exceeding the permissible level was found only in 4.7% of analysed samples. The authors pointed out the need of monitoring other production parameters and concluded that contamination was most frequently found in canned anchovy and pilchard. The highest histamine level was found in canned mackerel and tuna. veciana-Nogues et al. (1997) have investigated the effects of storage of semi-durable and not thermally processed canned anchovies immersed in oil. Histamine, tyramine, trypthamine and beta-phenylethylamine levels were increased during storage under cooling conditions and at room temperature. Cooling reduces but does not prevent the formation of

Table 2. Histamine level (winter 2007) (n=18)

	NC	PC	1A	1B	2A	2B
Day I	2.602	0.901	1.527	1.524	1.789	1.801
Day II	1.643	0.741	1.154	1.125	1.189	1.107
Day III	1.329	1.045	1.136	1.093	0.545	0.559
Day IV	1.256	1.001	0.368	0.288	-	-

NC - negative control; PC - positive control; 1A. 1B. 2A. 2B – tested samples

amines in canned anchovies immersed in oil.

Formation of histamine during storage of tuna at temperatures of 0°C, 8°C and 20°C was also investigated (Lopez-Sabater et al., 1996). Histamine content bellow the level of health hazard was found also in organoleptically changed fish, although toxic histamine levels can usually be identified after the evaluation of tuna as hygienically unacceptable due to organoleptic changes. Histamine content in fish stored at +8°C amounted to 100-200 mg%, when the appearance of fish has already been disgusting. In addition, the conclusion of the authors was that organoleptic properties are not a good criterion for the assessment of either stability of fish or toxic levels of histamine. Visciano et al. (2007) have investigated the effect of storage temperature (4°C and 25°C) on histamine formation in fish after catch and over a period of 24, 72 hours respectively. Significantly higher histamine concentration was found in samples stored at higher temperature, regardless of the time of analysis.

Histamine poisoning in men

Toxic doses of histamine for men have been a topic of numerous scientific discussions. The only conclusion reached was that excessive doses of histamine could present high risk to human health. Three fundamental impacts of histamine on the body of mammals are: dilatation and increased permeability of capillaries, dilatation of arterioles, increased tonus and strong long-lasting contractions of smooth muscles, in particular those of the alimentary system and bronchi, and intensified secretion

Table 3. Results of organoleptic examination of fish samples in summer of 2007 (n=18)

		SKIN and SMELL	GILLS	EYES	ANUS	MEAT
Day 1	AT (2A. 2B)	Smooth, shining, moist, bluish-green, no mucus, like fresh fish	Reddish-brown, mucus opaque, brownish, like fresh fish	Slightly concave, opaque	Closed	Greyish-brown, soft, with limited haemorrhages along spine – finger indentation retained for 3-4 seconds
	RT (1A. 1B)					
Day 2	AT (2A. 2B)	Sticky, brownish-grey, dull, unpleasant like decayed	Brown, mucus opaque, brown, unpleasant like rotten	Sunken, opaque	Opened	Greyish-brown, soggy, finger indentation on fish surface retained
	RT (1A. 1B)	Shining, bluish-green, transparent mucus, intensive to fresh fish	Dark reddish-brown, opaque, brown mucus, very intensive	Slightly concave, opaque	Opened, content coming out, belly burst	Greyish-brown, soft, finger indentation on fish surface retained
Day 3	AT (2A. 2B)	/	/	/	/	/
	RT (1A. 1B)	Dull, greyish-green, mucus opaque, yellow-green, smell like stale fish	Pale red-brown, opaque mucus, yellow-brown, unpleasant like rotten	Sunken, opaque	Opened, surrounding tissue decomposed, content coming out, belly burst	Brown-red, soggy, finger indentation on fish surface retained

AT – ambient (room) temperature; RT – refrigerator temperature

Table 4. Histamine level (summer 2007) (n=18)

	NC	PC	1A	1B	2A	2B
Day I	2.275	0.997	1.331	1.471	1.439	1.706
Day II	1.178	0.860	0.952	1.021	0.312	0.208
Day III	1.429	1.078	0.436	0.393	-	-

NC - negative control; PC - positive control; 1A. 1B. 2A. 2B – tested samples

of alimentary system glands, especially of the gastric fundus. In humans, a histamine dose of 2.5 mg is clinically manifested by blush, warmth, elevated skin temperature, headache, accelerated pulse, drop in blood pressure, contraction of bronchioles, and intensified secretion of saliva, tears and gastric juice. According to literature data, human sensitivity to histamine significantly varies among individuals (Hercig et al., 1982).

Assessment of health safety of sea-fish for human consumption includes also the actual problem of detection of histamine. Consequently, the aim of this study was to determine histamine levels in blue sea-fish during storage at room and refrigerator temperature.

MATERIAL AND METHODS

Organoleptic examination and determination of histamine content was carried out in samples of freshly caught mackerels (n=36). The analyses of mackerel samples was done in winter (January) and in summer (July). Fish samples were divided into two groups, of which one (Group I) was kept at refrigerator temperature and the other (Group II) at room temperature. Analyses were performed on the day of delivery of fish (one day after catch, defined as day I) and were continued daily until the finding of histamine exceeding the maximum permissible levels. Transia Tube Histamine-Immuno-enzyme test for the detection of histamine in fish (Diffchamb SA) was used. This is a quantitative analytical method for the detection of histamine in fish, with detection limit of 100 ppm.

RESULTS AND DISCUSSION

Determination of histamine content in samples of mackerels was performed in winter (January; n=18) and summer (July; n=18). Each time, the samples were divided into two groups and stored at either refrigerator temperature (Group I, samples 1A and 1B) or ambient temperature (Group II, samples 2A and 2B). All fish samples were subjected to organoleptic examination and analysed for histamine content. Analyses of histamine content were carried out until a positive result was recorded (histamine level exceeding 100 mg/kg) for each group of samples.

Study results are presented in Tables 1 to 4.

Histamine was not detected in any of fish samples analysed on first and second day of storage. Results of the third day analysis showed histamine content identical to or exceeding 100 mg/kg in samples kept at room temperature. According to the study plan, those samples were excluded from further testing. On day 4 of analysis, histamine level exceeding 100mg/kg was found also in samples kept at refrigerator temperature. In samples analysed in summer of the same year, histamine was not detected in fish on day 1, regardless of storage conditions. However, histamine (>100 mg/kg) was found on day 2 of analysis in samples kept at room temperature that were then excluded from further testing. Histamine content (>100 mg/kg) was found on day 3 also in samples kept in refrigerator. According to the Regulations on permissible levels of toxins, metals, metalloids and other noxious substances in food (Official Journal of Croatia, 51/05), the maximum permissible histamine level in fish and fish products should not exceed 100 mg/kg (for 9 tested samples). Two samples, however, could contain more than 100 but less than 200 mg/kg, and none of samples should contain more than 200 mg/kg. Cured fish subjected to enzymatic ripening procedure could contain a higher histamine level but not two-fold higher than recommended.

Taking into consideration the above-mentioned regulations, fish samples (winter 2007) kept at room temperature and with increased histamine content on day 3 of analysis, day 4 respectively, in case of samples kept at refrigerator temperature, were evaluated as unacceptable with regard to health quality. The same applies to samples analysed in July after two days of storage at room temperature, 3 days respectively, at refrigerator temperature.

Considering the fact that the external temperature during summer period is significantly higher than in winter period, it is certainly one of reasons of earlier histamine formation in fish. This refers primarily to fish samples kept at room temperature, but also includes those kept in refrigerator. It is impossible to exclude the period of fish handling at room temperature during organoleptic examination and the period of fish meat sampling for histamine analysis. During the period of fish storage at room temperature, all the processes occurring in fish are accelerated (autolysis and deterioration), and the consequence is more rapid formation of histamine. Results recorded in our study are consistent with those of other studies, and show significant increase in histamine content in fish in summer

period compared to winter period. The general conclusion reached is that storage temperature significantly influences the formation of increased content of histamine in fish (Edmunds and Eitenmiller, 1975; Pfeifer et al., 1985; Toković and Slavić, 1986; Šimičević, 1995; Visciano et al., 2007). Also, in fish analysed in summer period, increased histamine level (100 mg%) was recorded much earlier. Due to the fact that histamine level in fish influences organoleptic characteristics of fish, only fresh and optimally health safe fish can be distributed and sold on the market in order to prevent histamine poisoning. Hygienic quality of fish and fish products with respect to histamine content becomes questionable with every deviation from the established critical levels, and consequently, presents health hazard, since the potential risk of histamine poisoning is increased. Fish, as biologically highly valuable food, could really become a part of healthy diet, provided modern principles and concepts of safety are strictly implemented. Only systemic control, education of both manufacturers and consumers can ensure that fish and fish products will be characterised as "safe food".

* The paper is an extract from the graduation thesis of Ms. Renata Vusilović, DVM (mentor Mrs Željka Cvrtila Fleck, DSc, assistant professor).

* The authors would like to thank the Ministry of Science, Education and Sports for its support of scientific projects: Veterinarsko javno zdravstvo u proizvodnji zdrave hrane (Veterinary public health in the production of healthy food (053-0531854-1851).

ZUSAMMENFASSUNG

HYGIENISCHE BEDEUTSAMKEIT VON HISTAMIN IN FISCH

In der Arbeit ist die Bedeutsamkeit von Histamin in Fisch dargestellt. Die untersuchten Makrelenmuster (n=36), bewahrt auf Zimmertemperatur, waren am dritten Tag wegen des erhöhten Histaminquantums gesundheitlich unannehmbar (schädlich) (Winter 2007). Am vierten Tag wurde auch an den im Kühlschrank gehaltenen Mustern ein Histamingehalt höher als 100 mg/kg festgestellt. An den noch im Sommer desselben Jahres analysierten Mustern wurde schon am zweiten Tag ein höheres Histamingehalt festgestellt (Zimmertemperatur), bzw. am dritten Tag an den im Kühlschrank gehaltenen Mustern, so dass diese Muster als gesundheitlich schädlich betrachtet werden. Systematische Maßnahmen für Fischaufbewahrung auf niedrigeren Temperaturen können Histaminvergiftungen bei Menschen verhindern. Die vorgeschriebenen Normen, kritische Grenzen des Histamingehalts angeführt gemäß Verordnungen der Vorschrift (NN RH 51/05), sowie die Präventionsmaßnahmen gegen Histaminvergiftung im Rahmen der Veterinär/sanitätskontrolle in der Republik Kroatien sind im Einklang mit der Vorschrift für Hygiene der Nahrung tierischen Ursprungs (NN RH 99/07).

Schlüsselwörter: *Histamin, Fisch, Temperatur der Aufbewahrung*

REFERENCES

Edmunds, W.J., R.R. Eitenmiller (1975): Effect of storage time and temperature on histamine content and histidin decarboxylase activity of aquatic species. J. Food Sci. 40, 16-519.

Galrini, R., N.N. Haouet, E. Manuali (1996): Heavy metals and histamine content of fish products. 3. Histamine content during the 1988-1995 period. Ind.alimentari 35, 1194-1198.

Garai, G., M.T. Duenas, A. Irastorza, P.J. Martin-Alvarez, M.V. Moreno-Arribas (2006): Biogenic amines in natural ciders. Journal of Food Protection 69, 12, 3006-3012.

Hercig, V., Manda Knežević, J. Lisak (1982): Histamin u ribi i ribljim proizvodima. Prehrambeno-tehnološka revija 20, 183-188.

Lopez-Sabater,E.I., J.J. Rodrigez-Jerez., M.Hernandez-Herrero, A.X.Roig-Sagues, M.T. Mora-Ventura(1996): Sensory quality and histamine formation during controlled decomposition of tuna (Thunnus thynnus). J.Food Protection, 59, 167-174.

Moeller, D.R.Peter (2000): Finfish Toxins. In: Marine & Freshwater Products Handbook. Editors: Martin, R.E., E.P. Carter, G.J. Flick, Jr, L. M. Davis. Technomic Publishing Co. Inc., Lancaster, Basel. Str. 717-725.

Pfeifer Klara, J. Živković, Smiljana Gamulin, M. Hadžiosmanović (1985): Dokaz histamina kao kriterij za ocjenu upotrebljivosti ribe. Makedonski veterinaren pregled 14, 33-43.

Schalaby,A.R. (1997): Significance of biogenic amines to food safety and human health. Food Res.Intern. 29, 675-690.

Šimičević,M. (1995): Dokaz histamina u konzervama plave ribe. Diplomski rad. Veterinarski fakultet u Zagrebu. Rukopis (strojem), 32.

Šoša,B. (1989): Higijena i tehnologija prerade morske ribe. "Školska knjiga", Zagreb.

Toković,B., M. Slavić (1986): Određivanje histamina u ribama metodom hromatografije na tankom sloju. Vojnosanit. Pregled. 43, 34-37.

Vasciano, P., G. Campana, L. Annunziata, A. Veraga, A. Ianeri (2007): Effect of storage temperature on histamine formation in Sardina pilachardus and Engraulis Encrasiocolus after catch. Journal of Food Biochemistry 31, 577-588.

Veciana-Nogues, M.T., A. Marinefond, M.C. Vidal-Carou (1997): Changes in biogenic amines durig storage of mediterranean anchivies immersad in oil. J.Agric:Food Chem. 45, 1385-1389.

Živković,J., M. Hadžiosmanović (1989): Veterinarski priručnik. 4. dopunjeno izdanje. Uredili Vjekoslav Srebočan i Hrvoje Gomerčić. Jumena.

Prispjelo / Received: 14.1.2008.
Prihvaćeno / Accepted: 21.1.2008.