chen Schinkens, Entwicklung einer Technologie für die Zuwendungsschweine, Standardisation einer Technologie für die Bearbeitung von Schweineschinken, Definierung – auf Grund der Forschung – der physikalisch-chemischen und organoleptischen Eigenschaften des slawonischen Schinkens, Organisation eines nationalen Herstellungverbandes für slawonische Schinkensorten, der das Verfahren für die Registration des slawonischen Schinkens als ursprünglich kroatisches Erzeugnisses in Wege leiten würde, gesetzlichen Schutz für den Artikelnamen auf der RH und EU Ebene (Schutz der Ursprünglichkeit und/oder des geographischen Ursprungs).

Schlüsselwörter: *slawonischer Schinken, Qualität*

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IDENTIFICATION OF HISTAMINE CONTENT IN FISH SAMPLES

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SUMMARY

Histamine is biogenic amine, which is developed in food rich in proteins as a result of histidine decomposition. The decomposition is caused by growth of certain types of bacteria.

Samples of imported fish from B&H market were analysed for the presence and quantity of histamine by ELISA (RIDASCREEN® Histamine). The results should repre-

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sent the basis for introducing permanent control of the histamine presence in food rich in proteins (fish, cheese, milk, and meat) and in wine. This control should ensure the prevention of human poisoning and it must be legally regulated.

Key words: *histamine, fish, ELISA*

INTRODUCTION

Histamine is a biologically active amine of broad spectrum of physiological and pathological activity in the body. The histamine activity is manifested through its specific receptors after local release in some tissues and organs (Adams, 2001; Hardman and Limbird, 2001). Together with some other substances, such as serotonin, endogenic peptides, leukotrienes, prostaglandins and cytokinins, histamine is also classified as autacoids, tissue or local hormones respectively, the action of which is manifested at the site of release or closely to it (Hardman and Limbird, 2001). In mammals, histamine is an important mediator of immediate allergic hypersensitivity, it is involved in inflammatory reactions, influences the gastric secretion of hydrochloric acid (Adams, 2001; Hardman and Limbird, 2001), and has also been postulated to be a neurotransmitter in the hypothalamus of mammals (Rang et al., 2007). Histamine is also an important factor in the development of atherosclerosis and coronary diseases (Liao et al., 1997), etc.

Exogenous histamine is a degradation product of the amino acid histidine, caused by a growth of certain bacteria species in food rich in proteins, such as fish, particularly blue fish (fresh and in cans), cheese, milk, meat, different types of wine (white, red and sparkling wine) and beer. The quantity of developed histamine depends on the bacteria species, temperature and duration of exposure to the bacteria effect, and it can be as high as 1000 mg/kg (ppm).

Good quality fish should not contain more than 10 ppm of histamine (Instructions for RIDASCREEN® Histamine ELISA).

Nausea, vomiting, headache and other symptoms are induced by consumption (ingestion) of foods containing high levels of histamine. The same symptoms can occur also after the consumption of red wine, especially in individuals with naturally reduced ability of histamine decomposition (Hardman and Limbird, 2001.)

Consequently, human histamine poisoning is mostly, and rightly, associated with canned fish containing high levels of this amine.

The aim of this Study was to determine possible presence and content of histamine in larger number of commercial fish samples of different origin, which were intended for human consumption. The reason for investigation was the occurrence of fish poisoning among the population of the city of Mostar. In June 2007, several cases of human poisoning with canned fish were reported, and human lives were saved thanks to prompt medical intervention. Histamine was suspected as the cause of poisoning. However, the accurate identification of the cause of poisoning was impossible because of unavailability of both adequate equipment for qualitative and quantitative determination of histamine concentration and food remnants.

ELISA is commonly used as a “screening” method for quantitative determination of histamine in the above-mentioned types of food. According to literature data, this method is reliable, available, rapid and cheap.

The widely used analytical method for the histamine determination in fish in the EU countries is HPLC (Anon., 2005).

MATERIAL AND METHODS

Fish samples for analysis were obtained from the Sanitary Inspection of the Ministry of Health of the Canton Sarajevo. Samples of imported fish were randomly collected from various shops in Bosnia and Herzegovina. The analysis was carried out at the Toxicological laboratory of the Department for pharmacology and toxicology of the Veterinary Faculty in Sarajevo.

In total 35 samples were submitted of which 12 were samples of frozen fish (hake, whole - 2, pickerel - 3, mackerel, whole - 2 samples, and 1 sample of each hake fillet, mackerel fillet, sprat, Dory fillet and shark cutlet). Twenty-three samples were canned fish (tuna in oil - 13, tuna - 2, pilchard in oil - 3, and 1 sample of each hake in oil, mackerel in oil, blue fin tuna, Russian salad with tuna and salad with tuna in partridge sauce). After the completed preparation, all samples were analysed for the presence and content of histamine using a commercial kit for competitive enzymatic immunoanalysis RIDASCREEN® Histamine (Art. No.: R1604), manufacturer R-Biopharm Group, Germany. A limit of detection of this test for cheese, fresh fish and canned fish is 2.5 ppm.

Test kit contains all the reagents required for analysis, including detailed instructions for the analytical process.

One test kit sufficed for 48 samples, including standards.

Spectrophotometer for micro-titration plate is required for the quantification of results.

Time required for analysis:

- Preparation of samples (10) about 15 minutes
- Test duration (regardless of the number of samples) about 90 minutes.

After the sample preparation, histamine was quantitatively converted to N-acylhistamine, using an acylation

reagent. Free acylated histamine and bound histamine compete for the antibody-binding sites in the competitive ELISA.

After washing, the secondary peroxidase-conjugated antibodies (enzyme conjugate) are added. These antibodies bind to the antibody-histamine complex. Unbound antigen is ten removed by washing. Substrate (urea peroxide) and chromogen (tetramethyl-benzidine) are added into wells of the micro-titration plate and then incubated. During incubation, the bound enzyme conjugate converts a colourless chromogen into blue product, and blue colour changes into yellow by the after addition of stop solution.

After the substrate reaction, the optical density is measured at 450 nm on the ELISA plate reader. The amount of complexes bound to the plate and the optical density are inversely proportional to the histamine concentration of the sample.

RESULTS AND DISCUSSION

All the analysed samples contained less than 100 ppm of histamine.

According to the Regulation (EC) No 2073/2005 (Anon., 2005), histamine limit values for fish in the countries of the European Union are 100 mg/kg (m) and 200 mg/kg (M).

Although, according to this Regulation, the histamine levels in the analysed samples were within limits and thus, adequate for human consumption, it should be pointed out that small oscillations in temperature values during transportation and storage of fish could lead to rapid multiplication of bacteria, which can cause the formation of harmful products in meat, meat spoilage respectively. Histamine is one of those products.

Rapid cooling of fish immediately after fishing is a crucial element in the strategy of prevention of spoiled fish food, and consequently, of the formation of biogenic amines in fish. This refers particularly to fish exposed to high temperature of water or air, and also to big tuna fish known for retaining the warmth in their tissues even after death (Anon, 2001).

It is recommended as follows:

- In general, fish should be put on ice, in cooled seawater or brine at the temperature of 4.4ºC or lower for 12 hours after death or at 10ºC or lower for 9 hours after death.
- Fish exposed to air or water temperature exceeding 28.3ºC, should be put on ice, in cooled sea water or brine at the temperature of 4.4ºC or lower for 6 hours after death (FDA, 2001.).

This will prevent rapid development of the enzyme histidine decarboxylase, because the hazard control is no more possible after the formation of this enzyme (Anon, 2001).

CONCLUSION

Permanent control of the histamine presence in food rich in proteins and in wine should be introduced, because of the possibility of histamine development in such foodstuffs, detrimental to human health. Since the “screening” method for quantitative determination of histamine is easy to perform, the control of histamine presence should be legally regulated for the protection of human health.

ZUSSAMENFASSUNG

BESTIMMUNG VON HISTAMIN IN FISCHMUSTERN

Das Histamin ist biogenes Amin, das in proteinreichen Nahrungsmitteln durch die Zerlegung von Aminosäuren der Histidine entsteht. Diese Zerlegung verursacht das Wachstum einer bestimmten Bakteriensorte.

Kommerzielle Fischsmuster aus der Einfuhr wurden auf Anwesenheit und Menge von Histamin ELISA mit Hilfe von Test (RIDASCREEN® Histamin) analysiert. Die bekommenen Resultate sollten die Grundlage für die Einführung einer ständige Kontrolle der Anwesenheit von Histamin in proteinreichen Nahrungsmitteln (Fisch, Käse, Milch, Fleisch) und Wein darstellen, und damit die Vorbeugung einer Vergiftung bei Menschen sichern, was gesetzlich reguliert werden muss.

Schlüsselwörter: *Histamin, Fisch, ELISA*

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