The Histamine Content in Some Samples of Food Products

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Abstract

Leszczyńska J., Więdłocha M., Pytasz U. (2004): **The histamine content in some samples of food products**. Czech J. Food Sci., **22**: 81–86.

Histamine level determination in some food products was performed by two known methods: spectrofluorimetric method and ELISA method. The immunoenzymic method enables a rapid and precise determination of histamine in many samples. In spite of that, it does not require any complicated apparatus, is very simple and easy to use. Coefficient of correlation as stablished for these methods equals 0.9539. Samples were investigated of different products, such as: kefir, fruit and natural yoghurt, a kind of sour soup, and tinned fish (tuna and herring). No histamine content exceeding the acceptable level was found.

Keywords: histamine; spectrofluorimetric methods; ELISA method; food

Histamine is one of the biogenic amines that belong among the naturally occurring substances. Biogenic amines such as histamine, tyramine, putrescine, and others can be formed in food as a result of metabolic processes of microorganisms. If the concentrations of amines is above the normal level, possibly due to the bacterial contamination of food, harmful effects may occur (Veciana-Nogues *et al*. 1997). Poisoning with histamine can cause the so called pseudoallergic reactions, in other words, it can produce symptoms such as: urticaria, eczema, diarrorea, or spasm of bronchi. The content of histamine is regarded as a criterion of the quality of food. Even if bacteria have been killed, the enzyme activity may continue to produce histamine. Histamine is produced by decarboxylation of histidine. Foods rich in proteins (fish, meet, cheese – rich in free histidine) as well as sparkling wine and beer are regarded as histamine containing products because lactic acid fermentation also increases the histamine production (Fletcher *et al*. 1998). The major sources of dietary biogenic amines include several types of fish species.

Several physicochemical methods for the histamine determination, in particular fluorimetry and liquid chromatography (LC), have been described, and these are so far the most commonly used routine assays for biogenic amines in food in general (Straton *et al*. 1991).

Spectrofluorimetric method (in agreement with AOAC) may be used as a basic method for the histamine determination in tinned fish (Fonberg-Broczek *et al*. 1988).

Among the most important chromatographic methods can be mentioned: HPLC method with reversed phases, in which histamine is determined as fluorescent derivative (Aygun *et al*. 1999; Lange *et al*. 2002); HPLC with a spectofluorimetric detection (Hwang *et al*. 1997); electrokinetic capillar chromatography (Krizek & Pelikanova 1998; Kovacs *et al*. 1999; Su *et al*. 2000); ionic chromatography (Draisci *et al*. 1998); and thin-layer or gas chromatography (Su *et al*. 2000).

These methods are usually costly, require extensive sample cleanup, and have a low sample throughput. To imgrove the food control measure-

ments, rapid and easy analytical methods for this compound would be useful.

Most immunochemical methods for the detection of histamine in human serum, biological fluids, and food are based on antibodies against N-aminoderivates of histamine synthesised by the reaction with, for example, p-benzoquinone or propionic acid esters (Chevier *et al*. 1986; Hammar *et al*. 1990; Rauch *et al*. 1992; Serrar *et al*. 1995). However, because the antibodies used in these tests do not interact with the parent compound, chemical derivatisation of histamine is necessary before analysis. Such procedure is either time-consuming or requires toxic reagents. Several commercial tests based on such anibodies are commercially available (IBL GmbH, Hamburg, Germany; Transia Diagnostics, Ober-Morlen, Germany). Recently, the first polyclonal anti-histamine antibodies recognising the intact histamine were prepared (Schneider 1996) and incorporated in commercial ELISA test kit (R-Biopharm GmbH, Darmstadt, Germany).

MATERIAL AND METHODS

Investigated material

1–5 yoghurts; 6–8 kefirs; 9–13 traditional Polish sour soup; Salad tuna in their own sauce; Herring in tomato sauce; Common sprat in tomato sauce; Zander fillet in tomato sauce; Herring fillet in oil; Smoked mackerel I; Smoked mackerel II; Investigated samples were commonly available foods.

Fluorimetric method for histamine determination

Test principle. Histamine and orthophtalate aldehyde form a stable fluorescent compound which is the basis for its determination.

The histamine determination by fluorimetric method (in agreement with PN-90 A-86786) is based on the isolation of histamine from the methanol extract by means of a column filled by ion-exchanger Dowex 1 × 8 followed by the spectrofluorimetric determination of the condensation product of histamine and orthophtalic aldehydes. Histamine forms a stable fluorescent compound with orthophtalic aldehydes is shown in Figure 1.

Aparatus. F-2000 Hitachi spectrofluorometer is used for the determination of histamine level.

Reagents

All chemicals were of analytical reagent grade. Deionised water was used throughout. The following compounds were used: 0.1 mol/l HCl; 1.0 mol/l NaOH; 1.2 mol/l H_3PO_4 (dilute 12.2 ml of 85% acid in a 100ml flask); 1% solution of orthophtalate aldehyde; 1 mg/ml histamine solution (dissolve 167.4 mg of histamine hydrochloride and add 0.1 mol/l HCl in 100ml flask).

Sample preparation

The content of the tinned fish preserve was transferred into the mortar and minced until homogenous mass was obtained. Two 10 g samples of the mass prepared were quantitatively transferred into the homogeniser vessel, 50 ml of methanol was added, and the samples were homogenised for 2 min at 2000 g. The suspension obtained was then transferred into metric bulb. The vessel and the homogeniser knives were carefully washed up. The bulb was put into a water bath at 6°C for about 15 min, then it was cooled to room temperature and methanol was added up to 100 ml. Methanol extract was filtered through a paper filter into a dry cone bulb with a glass stopper. The filtrate was kept in the freezer. 1 ml of methanol extract was applied on the ion exchange column, which was then eluted with a few portions of 4–5 ml distilled water into a 50ml metric bulb containing 5 ml of 0.1 mol/l HCl until

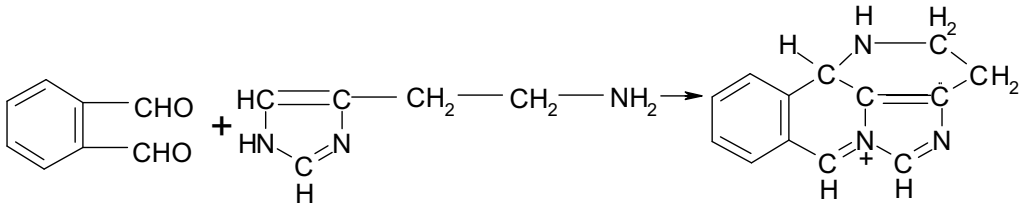

Figure 1. Histamine forms a stable fluorescent compound with orthophtalic aldehydes

35 ml of eluate was obtained. The volume was made up to 50 ml with distilled water, corked and cooled in refrigerator. 0.5 ml aliquots of the eluate were measured into test-tubes after which 1 ml of 0.1 mol/l HCl was added to every sample. The subsequent procedure was the same as that used during the preparation phase.

Determination procedure

Using the stock histamine solution, diluted solutions are prepared in the concentration range of 0.1 to 10 μg/l. To 0.5 ml of each of the diluted solutions, 1.0 ml of 0.1 mol/l HCl, 0.3 of 1 mol/l NaOH solution, and 0.1 ml of orthophtalate aldehyde are added. After 4 min, 0.3 ml of phosphoric acid is added, and not later than after 30 min radiation emission is measured at EX wavelength of excitation λ = 350 nm, and EM wavelength of emission λ = 444 nm.

Enzyme immunoassay for the quantitative analysis of histamine

Test principle. The basis of the test resides in the antigen-antibody interaction. The microtiter wells are coated with histamine. Anti histamine antibodies and standards, respectively sample solutions, are added. The free and the immobilised histamines compete for the antibody binding sites. After washing, secondary antibodies labelled with peroxidase are added. These bind to the antibody histamine complexes. Any unbound enzyme conjugate is then removed by the washing step. Enzyme substrate (urea peroxide) and chromogen (tetramethylbenzidine) are added to the wells and incubated. Bound enzyme conjugate converts the colourless chromogen into a blue product. The addition of the stop reagent causes the colour change from blue to yellow. The measurement is done photometrically at 450 nm. The resulting absorbance values are inversely proportional to the histamine concentration in the sample.

Aparatus. We use microtiter plate spectrophotometer Multiscan EX and automatic Wellwash 4.

Reagents. We use test Ridascreen Histamin (R-Biopharm GmbH, Darmstadt, Germany).

Each test kit contains: a microtiter plate (48 wells coated with histamine); histamine standard solution: 0 ng/ml, 50 ng/ml, 150 ng/ml, 450 ng/ml, 1350 ng/ml, 4050 ng/ml; peroxidase-conjugated anti rabbit IgG; rabbit anti histamine; substrate (urea peroxide); chromogen (tetramethylbenzidine); stop reagent (1 mol/l sulfuric acid); PBS-Tween buffer (washing buffer and sample diluent), pH 7.2 (0.55 g $NaH_2PO_4 \times H_2O$, 2.85 g $Na_2HPO_4 \times 2H_2O$, 9 g NaCl and 1 ml Tween 20, fill up to 1000 ml with deionised water).

Preparation of samples. 2 g of the homogenised sample is weighed into a centrifuge vial which is subsequently filled up to 10 ml with the sample diluent and shaken for 30 min. Defatting is then carried out by centrifugation for 10 min at 3000 g and 4°C. The fat layer is aspirated, an aliquot of the aqueous supernatant is diluted in the ration 1:10 with the sample diluent, and 50 μl per well is applied for the assay.

Test procedure

1. Add 50 μl of each standard solution or the prepared sample to separate duplicate wells.
2. Add 50 μl of the histamine antibody to each well. Mix thoroughly and incubate for 1 h at room temperature.
3. Wash the wells with precisely 250 μl of the washing buffer. Repeat 4 times.
4. Add 100 μl of the enzyme conjugate to each well. Mix thoroughly and incubate for 30 min at room temperature.
5. Wash the wells with precisely 250 μl of the washing buffer. Repeat 4 times.
6. Add 50 μl of the substrate and 50 μl of chromogen to each well. Mix thoroughly and incubate for 30 min at room temperature in the dark.
7. Add 100 μl of stop reagent to each well. Mix thoroughly and measure the absorbance at 450 nm against air blank. Read within 60 min after the addition of the stop solution

RESULTS AND DISCUSSION

A standard curve for the histamine determination by fluorimetric method based on the reaction with orthophtalic aldehyde is a linear relation in the whole investigated range, e.g. from 0.1 to 2 μg/l (y = $4676x$ – 90.92, R^2 = 0.9974). However, the standard ELISA method curve is not a straight line over the whole range of concentrations, which is undoubtedly a disadvantage of the immunoenzymic method. In our case, the calculation was done in two ranges: from 50 to 450 ng/ml on the basis of the curve described by equation $y = -0.0005x + 0.4963$; and from 450 to 4050 ng/ml according to the curve $y = 3.4612x^{-0.4204}$.

Table 1. Comparison of spectrofluorimetric and ELISA methods

Parameter	ELISA method	Spectrofluorimetric method
Recommended range of determination (μg/g)	0.25–20.25	2.5–100
Limit of detection (mg/100 g)	0.125	1.75
Number of detections	6	6
Coefficient of variation (%)	4.0–9.32	1.5–7.7
Time of analysis (10 determinations) (h)	3	14

In Table 1, statistic parameters are shown for the two methods used for the histamine determination. Statistic parameters analysis for both methods enables us to conclude that the immunoenzymic method allows to determine lower histamine levels.

The detection limit for ELISA method equals 0.125 mg per 100 g of product, whereas with the spectrofluorimetric method it is 1.75 mg per 100 g of product. The scatter of the results estimated with the help of the most reliable parameters such as variation coefficient is a little higher than in ELISA method, which means that spectrofluorimetric method gives more precise results. However, the possibility of the determination of much lower concentrations, the simple preparation of the sample and the short time of analysis prove the advantage of the immunoenzymic method. A higher probability of a mistake made by the human factor is in the case of the spectrofluorimetric method, which also requires a more complicated sample preparation and a longer time for the analysis.

The results obtained by these two methods do not exceed the values described by the norm. Both results are comparable, their magnitude is of the same order, but the content of histamine only was determinable in two samples (Nos. 7 and 8). In the remaining samples its level was too low.

The results given in Table 2 show that the results obtained by the spectrofluorimetric method are a some wheat higher than in the case of ELISA method. This may be the result of several factors. Namely, the curve in ELISA method had to be estimated for the concentrations covering the range of our interest. It was not linear, as in the case of the spectrofluorimetric method.

The higher results obtained with the fluorimetric method may also be due to the non-specific character of the reaction of histamine with orthophtalic aldehydes. In the case of antibody-antigen interaction, which is characteristic for histamine, the content of cross reaction is only 0.005% for histidine, 1.0% for spermidine, and 0.2% for putrescine. The values of the correlation coefficient, R^2, are, respectively: for diary products 0.8332; for sour soup 0.7385; for tinned fish 0.9954; and for all the other products

Table 2. Histamine determination in dairy products, sour soup and tinned fish (μg/100 g)

	No.	ELISA method	Spectrofluorimetric method
Dairy products	1	0.50 ± 0.03	Below 1.75
	2	0.78 ± 0.04	1.76 ± 0.03
	3	0.29 ± 0.03	Below 1.75
	4	0.39 ± 0.02	Below 1.75
	5	0.63 ± 0.03	Below 1.75
	6	0.67 ± 0.03	Below 1.75
	7	1.32 ± 0.04	3.08 ± 0.06
	8	0.41 ± 0.02	Below 1.75
Sour soup	9	2.37 ± 0.16	5.55 ± 0.13
	10	2.91 ± 0.09	7.35 ± 0.07
	11	1.24 ± 0.12	3.51 ± 0.09
	12	1.14 ± 0.08	3.46 ± 0.11
	13	2.07 ± 0.17	3.34 ± 0.09
Tinned fish	14	0.63 ± 0.06	Below 1.75
	15	0.50 ± 0.05	Below 1.75
	16	2.54 ± 0.08	6.60 ± 0.11
	17	3.34 ± 0.08	8.02 ± 0.16
	18	1.50 ± 0.09	3.75 ± 0.14
	19	2.66 ± 0.11	6.65 ± 0.15
	20	1.31 ± 0.11	3.14 ± 0.09

1–5 yoghurts; 6–8 kefirs; 14 – salad tuna in their own sauce; 15 – herring in tomato sauce; 16 – common sprat in tomato sauce; 17 – Zander fillet in tomato sauce; 18 – Herring fillet in oil; 19 – smoked mackerel I; 20 – smoked mackerel II

investigated 0.9539. The best correlation was found in the case of fish products. This may reflect the fact that the fluorimetric method was conceived for fish and tinned fish, so these products provide the most reliable results. The spectrofluorimetric method is more precise but more labourious and adequate for higher concentrations. ELISA method is much quicker and more reliable, but, unfortunately, more expensive.

According to the Polish norm the histamine content must not exceed 20 mg per 100 g of the product for tuna – kind fish and herring – kind fish. Similar limits are valid in the EU countries, whereas in the USA Food and DRUG Administration suggests a lower content of histamine, i.e. 5 mg per 100 g of product. This level (determined, however, only by fluorimetric method) was been exceeded in 5 samples. The results obtained by ELISA method are in all cases lower than 5 mg. FDA recommendations were worked out on the basis of HPLC method in which a good correlation between the immunoenzymic and chromatographic methods was found (Aygun *et al*. 1999). We can claim consequently, that no contamination exceeding the permissible histamine levels was found in the samples investigated.

There may be even up to few hundred mg of histamine (Gallardo & Sotelo 1997; Dufour *et al*. 2003) in 100 g of mackerel, tuna, or sardine closely to the spoiled state. There is only 1 mg of histamine in 100 mg of cod at the date of expiration while 5 times more is contained in spoiled fish. In conclusion, the norms should be more precise, especially if it is known that the fluorometric method overestimates the results.

Conclusions

The detection limit for histamine in ELISA method is 0.125 mg per 100 g of product, whereas with the spectrofluorimetric method it is 1.75 mg per 100 g of product. The spectrofluorimetric method gives more precise results. The immunoenzymic method enables a rapid and precise determination of the histamine content in many samples. In spite of that, it does not require any complicated apparatus, it is easy and simple. The possibility of the determination of lower concentrations, the simplicity of the sample preparation, as well as the short time of analysis provide evidence for the advantage of the immunoenzymic method. The results obtained by both methods are in agreement with Polish norms. The results obtained by the fluorimetric method are higher than those obtained by the ELISA method. This may be caused by the lower specificity of the fluorimetric method.

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Received for publication February 24, 2004
Accepted after corrections April 23, 2004

Souhrn

Leszczyńska J., Więdłocha M., Pytasz U. (2004): **Obsah histaminu ve vybraných vzorcích potravinářských výrobků**. Czech J. Food Sci., **22**: 81–86.

Stanovení obsahu histaminu bylo provedeno ve vybraných potravinářských výrobcích dvěma známými metodami, a to spektrofluorimetricky a metodou ELISA. Imunoenzymatická metoda umožňuje rychlé a přesné stanovení histaminu v řadě vzorků bez potřeby složité aparatury, je jednoduchá a snadno proveditelná. Korelační koeficient je u těchto metod 0,9539. Sledování byly podrobeny vzorky různých výrobků jako kefír, ovocný a bílý jogurt, druh kyselé polévky a konzervované ryby (tuňák a sleď). Obsah histaminu převyšující povolený limit nebyl zjištěn.

Klíčová slova: histamin; spektrofluorimetrické metody; metoda ELISA; potraviny

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