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IMMUNOASSAY METHOD FOR DETECTION OF HISTAMINE IN FOODS

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A novel screening immunoassay for histamine was used for detection of histamine in different foodstuffs. The detection limit of this assay was 20 μg kg^{-1}. The concentration of histamine varied between 182–982 μg kg^{-1} in sauerkraut, cheese and fish samples and 26–18433 μg l^{-1} in milk, sparkling wine and wines.

The applied competitive enzyme immunoassay (ELISA) seemed a reliable technique for simple and rapid determination of histamine in food.

Keywords: histamine, food, ELISA

The study of histamine content in foods is of interest for its possible toxicological risk. Histamine in food and beverages has been shown to induce intolerance reactions mimicking food allergy. Ingestion of excessive amounts of this amine can cause headache, nausea and rashes to sensitive individuals (BEUTLING, 1996). Histamine is formed by the enzymatic decarboxilation of histidine, and therefore has to be considered as a biogenic amine. Biogenic amines can be found in various foods and beverages. For formation of biogenic amines free amino acids occur as such in foods. Decarboxylase positive microorganisms may constitute part of the associated population of the food or may be introduced by contamination before, during or after processing of the food (HALÁSZ et al., 1994).

Dietary biogenic amines do not represent any health hazard to individuals unless large amounts are ingested, or the natural mechanisms for the catabolism of the amines are inhibited or genetically deficient.

Dairy products, especially cheeses, are one of the foods with the highest amine content. Several toxic and non-toxic amines are produced during the fermentation of milk. Many plant products (e.g. sauerkraut) can also be fermented. Usually, the mixed microbial community of the cabbage is used for the process. Protein-rich foods (fish, meat) and beverages (wine, beer) can contain high amount of biogenic amines. The most hazardous amines are histamine and tyramine. The seriousness of the effects may be influenced by the presence of other polyamines.

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Some countries have proposed maximum tolerance levels for biogenic amines in food. Upper limits of 100 mg histamine kg^{-1} in foods, 2 mg histamine l^{-1} in alcoholic beverages and 100–800 mg tyramine kg^{-1} in foods have been suggested (BRINK et al., 1990).

Various analytical methods have been developed for the determination of histamine in foods. These techniques include spectrophotometry, fluorimetry, High Performance Liquid Chromatography (SLOCUM & FLORES, 1991), Ion Exchange Chromatography (SIMON-SARKADI & HOLZAPFEL, 1994), Automated Overpressured-layer Chromatography (KOVÁCS et al., 1998) and Capillary Electrophoresis (KOVÁCS et al., 1999). The methods differ in sensitivity, selectivity, ease of sample preparation, speed of separation and cost of analysis. Each method for biogenic amine analysis has its own particular advantages and disadvantages. Although many methods are available, the development of new and more useful approaches for the analysis of biogenic amines continues today.

Immunoassay is a new method for histamine detection in food. The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with histamine. Free histamine in standards or sample solution and immobilized histamine compete for the antibody binding sites to form an antigen–antibody complex. Secondary antibodies labelled with enzyme bind to these antibody histamine complexes. Enzyme substrate and chromogen is used for identification. The measurement is done photometrically. The resulting absorbance is inversely proportional to the histamine concentration of the sample.

The objective of this work was to determine the histamine content in different foods using competitive enzyme immunoassay (ELISA) method.

1. Materials and methods

1.1. Reagents

Enzyme immunoassay diagnostic kit for quantitative analysis of histamine (RIDASCREEN ®HISTAMINE, 1997) was purchased from R-Biopharm (GmbH, Germany). Reagents required but not provided with the kit were purchased from REANAL and prepared freshly as follows: 0.01 M phosphate buffered saline (PBS washing buffer and sample diluents, pH 7.2, consisted of 0.55 g $NaH_2PO_4 \times H_2O$; 2.85 g $Na_2HPO_4 \times 2\ H_2O$ and 9 g NaCl) containing 0.1% Tween 20 solution and gelatine solution (consisted of 40% (v/v) ethanol, 5% (w/v) gelatine 2% (w/v) and polyvinyl-pyrrolidone, Sigma).

1.2. Samples

Samples (red and white wine, sparkling wine, milk, cheese, natural and fruit yoghurt, kefir, sour cream, sauerkraut, fish) were purchased from the commercial line.

1.2.1. Preparation of the samples. Red wine. One cm^3 of sample was mixed with 1 cm^3 of gelatine solution, shaked for 30 min then diluted to 1:10 in sample diluent. An aliquot (50 μl) was used for the ELISA.

Sparkling wine, white wines (Tokaji Aszú and Badacsonyi). One cm^3 sample was diluted to 1:10 in sample diluent. An aliquot (50 μl) was used for the ELISA.

Milk. Five cm^3 of sample was centrifuged for 10 min at 3000 g, 4 °C. Fat layer was aspirated and the aqueous supernatant was diluted to 1:10 in sample diluent. An aliquot (50 μl) was used for the ELISA.

Natural and fruit yoghurt, sour cream, kefir, cheese, fish. Two g of sample was homogenized in a centrifuge vial and filled up to 10 cm^3 with sample diluent then shaked for 30 min. The mixture was centrifuged for 10 min at 3000 g, 4 °C. Fat layer was aspirated and the aqueous supernatant was diluted to 1:10 in sample diluent. An aliquot (50 μl) was used for the ELISA.

1.3. Competitive indirect ELISA for qualitative analysis of histamine in food

1.3.1. Test principle. The basis of the test is the antigen-antibody reaction. The microtiter wells are coated with histamine. Anti-histamine antibodies and standards sample solution are added, respectively. Free and immobilized histamine compete for the antibody binding sites. After washing, secondary antibodies labelled with peroxidase are added. These bind to antibody histamine complexes. Any unbound enzyme conjugate is then removed by washing. Enzyme substrate (urea peroxide) and chromogen (tetramethylbenzidine) are added to the wells and incubated. Bound enzyme conjugate causes a colour change from blue to yellow. The measurement is done photometrically at 450 nm (reference wavelength 630 nm). The resulting absorbance is inversely proportional to the histamine concentration of the sample.

1.3.2. Test procedure. The microtiter wells were coated with histamine. Fifty μl of anti-histamine antibodies and 50 μl of standards (histamine solution containing 0 mg kg^{-1}-zero standard-, 0.05 mg kg^{-1}, 0.15 mg kg^{-1}, 0.45 mg kg^{-1}, 1.35 mg kg^{-1} and 4.05 mg kg^{-1}, respectively), and 50 μl of sample solution were added in triplicate. The plate was incubated at room temperature for 1 h. After a washing step (3×250 μl of washing buffer) 100 μl of anti-histamine rabbit antibody enzyme conjugate was added to the wells. After incubating the plate for 30 min at room temperature, the washing step was repeated. Fifty μl of substrate and chromogen solution, respectively, were added to the wells. Following a 30 min incubation at room temperature in the dark, 100 μl of stop solution was added to the wells. The absorbance was measured by ELISA reader (MR 7000, Dynatech) at 450 nm (reference wavelength 630 nm) against an air blank. Results were read within 30 min and calculated on the basis of the standard curve.

2. Results

The histamine contents of food samples were determined by competitive indirect ELISA. The calibration curve is shown in Fig. 1. The results were evaluated by fitting a logarithmic regression curve (y=–0.2688Ln(x)+2.1397; R^2=0.986).

The mean lower detection limit of this assay was 20 μg kg^{-1}. The precision within a series was determined from the results of triplicate determination. The SD values varied between 1.16–138.03 depending on the samples.

The histamine contents of the samples are presented in Table 1.

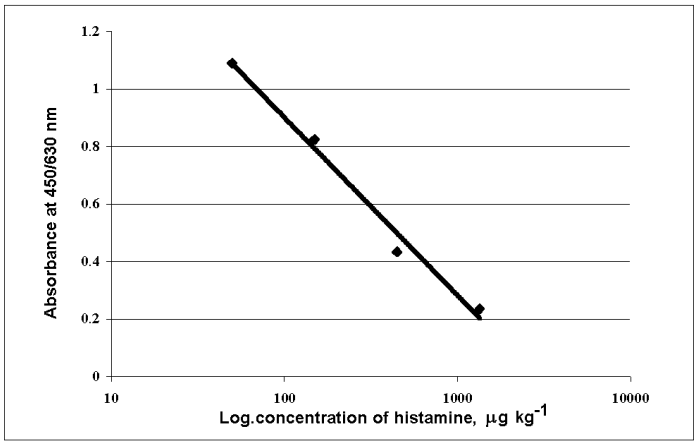

Fig. 1. Calibration curve for the determination of histamine content using competitive indirect ELISA (y=–0.2688 Ln(x) + 2.1397; R^2 = 0.986)

Table 1. Histamine content of different foods

Samples	Histamine (μg kg^{-1} or μg l^{-1})	SD
Milk	26.11	1.52
Sour cream	577.91	5.50
Yoghurt	549.76	1.97
Fruit yoghurt	1477.96	30.96
Kefir	593.23	6.76
Cheese	910.18	11.43
Fish	982.48	27.62
Sauerkraut	181.62	2.23
Sparkling wine	27.34	1.16
White wine	365.10	19.84
Red wine	18433.48	41.34
Aszú wine	6162.66	138.03

The histamine content of milk was 0.03 mg l^{-1} and that of the dairy products varied between 0.6–1.5 mg kg^{-1}. Sauerkraut had a low level of histamine (0.18 mg kg^{-1}). Sparkling wine and wines contained 0.03–18 mg l^{-1} histamine. The histamine level in red (18 mg l^{-1}) and *Tokaji Aszú* (6 mg l^{-1}) wines only exceeded the maximum tolerated dose accepted in Hungary for wine (5–10 mg l^{-1}).

3. Conclusions

ELISA based determination is a novel screening method to quantify histamine in food. To investigate the usefulness of this method, wide varieties of food samples were selected for the measurements. Literature data and our previous experience have shown that dairy products and beverages contained the highest level of histamine among foodstuffs. The present results confirmed those findings.

The new screening method can be applied successfully to histamine determination in different foods. The histamine ELISA test provides a new tool to set up a national database for the histamine levels in commercially available foods.

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