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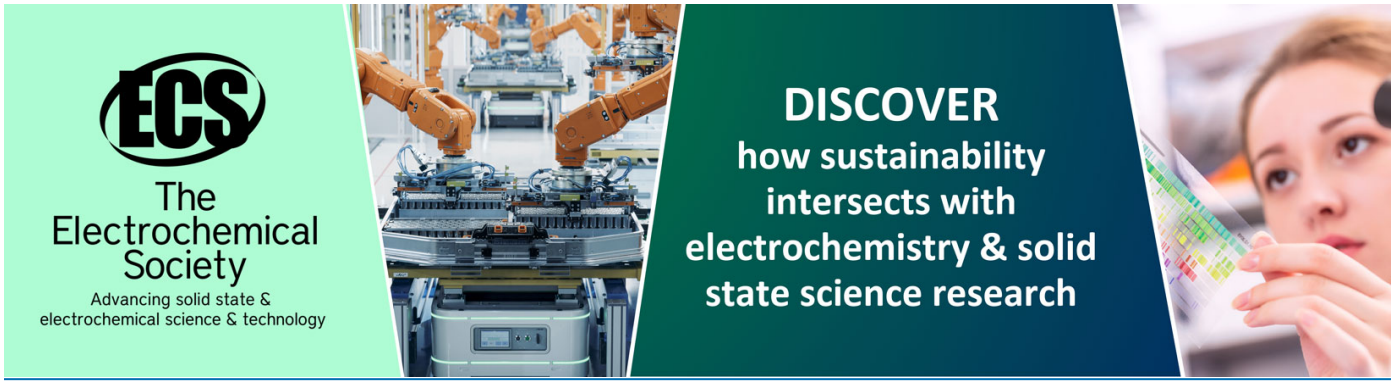

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Comparison of analytical methods for the determination of histamine in reference canned fish samples

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Abstract. Two screening methods for histamine in canned fish, an enzymatic test and a competitive direct enzyme-linked immunosorbent assay (CD-ELISA), were compared with the reversed-phase liquid chromatography (RP-HPLC) standard method. For enzymatic and CD-ELISA methods, determination was conducted according to producers' manuals. For RP-HPLC, histamine was derivatized with dansyl-chloride, followed by RP-HPLC and diode array detection. Results of analysis of canned fish, supplied as reference samples for proficiency testing, showed good agreement when histamine was present at higher concentrations (above 100 mg kg^{-1}). At a lower level (16.95 mg kg^{-1}), the enzymatic test produced some higher results. Generally, analysis of four reference samples according to CD-ELISA and RP-HPLC showed good agreement for histamine determination (r=0.977 in concentration range 16.95–216 mg kg^{-1}) The results show that the applied enzymatic test and CD-ELISA appeared to be suitable screening methods for the determination of histamine in canned fish.

1. Introduction

Histamine is a product of the decarboxylation of histidine by microbiological histidine decarboxylase, caused by the growth of certain bacteria in protein-rich food like fish, cheese and wine [1]. The amount of histamine formed depends on the bacterial species, the temperature and the time of exposure [2,3]. Histamine is a causative agent of scombroid poisoning or histamine fish poisoning. The consumption of food containing significant concentration of histamine can cause symptoms similar to those associated to seafood allergies [4]. Histamine can be present mainly in *Scombridae* (Tuna, Mackerel) and *Clupeidae* (Herring, Sardine), in species which contain a high level of free histidine [5].

The critical dose of oral histamine has been estimated to be in the range of 100–200 mg kg^{-1}, and the EC regulation [6] stated for histamine that nine samples must be taken from each batch. These samples must fulfil the following requirements:

- the mean value must not exceed 100 mg kg^{-1};
- two samples may have a value of more than 100 mg kg^{-1} but less than 200 mg kg^{-1};
- no sample may have a value exceeding 200 mg kg^{-1} [6].

A wide variety of procedures for the determination of histamine and biogenic amines have been published: colorimetric methods, thin layer chromatography (TLC) methods, enzymatic methods, immuno-enzymatic methods, and flow injection analysis with fluorimetric detection [7]. However,

precise and reliable quantitative analysis for histamine and biogenic amines are chromatographic techniques, such as gas chromatography (GC), high performance liquid chromatography (HPLC), and high performance thin layer chromatography (HPTLC), as well as capillary electrophoresis [8,9]. Those allow the simultaneous analysis of histamine and other biogenic amines in fish and fishery products. These methods usually required extensive sample cleanup, and expensive equipment as well as trained staff. To enhance food control measurements, rapid, easy, and simple analytical methods for this compound are usually used as screening methods. In the case of contaminated samples, the use of confirmatory methods is necessary. In the EU regulation, it is specified that examinations must be carried out in accordance with reliable, scientifically recognized reference methods, such as HPLC. The reference method for histamine in Europe is the International Standard Organisation (ISO) method [10].

The aim of this paper was evaluation and comparison of histamine determination in four reference materials by the standard HPLC method, and by screening methods, an enzymatic test and competitive direct enzyme-linked immunosorbent assay (CD-ELISA).

2. Material and Methods

Studies were performed using canned fish samples intended for proficiency testing (PT). Proficiency testing provider, FAPAS, processes these samples under strict quality control procedures to ensure a homogenous reference material. Since numerous laboratories participate in those schemes, samples are considered as reference materials and are used for evaluation and comparison of screening and confirmatory methods for histamine determination. Four samples were used, with different histamine concentrations (table 1). All determinations in the study were performed in triplicate.

2.1. Screening methods

Two screening procedures were used in our study: Ridascreen® Histamine enzymatic test and Veratox® for Histamine CD-ELISA.

Ridascreen® Histamine (enzymatic) (Art. No. 1605, R-Biopharm, Germany) is an enzymatic test in microtiter plate format for the quantitative determination of histamine in fresh fish, canned fish, fish meal, wine, cheese and milk. The test was performed according to the manufacturer's manual. The basis of this test is an enzymatic reaction. The microtiterplate is coated with a reagent (electron carrier) and a dye. Histamine-dehydrogenase catalyzes the oxidation of histamine to imidacetaldehyde in presence of an electron carrier and a dye. The formation of dye is measured at 450 nm and is proportional to the histamine concentration. Calculation of histamine concentrations used linear regression; the dilution factor of the samples was taken into account. Since the quantitation range of the test is 2–100 mg kg^{-1}, samples with histamine contents higher than 100 mg kg^{-1} were further diluted with water and re-tested.

Veratox® for Histamine (AOAC-RI #070703 approved method, Product No. 9505, Neogen, USA) is used for the quantitative analysis of histamine in scombroid species of fish, such as tuna, bluefish and mahi-mahi. The test is a competitive direct ELISA. Free histamine in the sample and controls competes with enzyme-labeled histamine (conjugate) for the antibody-binding sites. After a wash step, substrate reacts with the bound enzyme conjugate to produce blue color that is measured at 630 nm. Since the range of quantitation was from 2.5 mg kg^{-1} to 50 mg kg^{-1}, samples with histamine contents higher than 50 mg kg^{-1} were further diluted with water and re-tested.

The color intensity in the microtiter wells was measured photometrically using a Thermo Multiskan FC photometer (Thermo Scientific). Special software, the Rida®Soft Win (Art. No. Z9999, R-Biopharm, Germany), was used to evaluate the results.

2.2. RP-HPLC method

Determination of histamine by HPLC was done according to the standard method [10]. Extraction was performed by mixing the sample with perchloric acid. Precolumn derivatization was performed using dansyl-chloride. Histamine was separated from other biogenic amines by HPLC Dionex UltiMate

3000 Series (Thermo Scientific, Germany) on SupelCosil™ LC-18-DB column (250 x 4.6 mm id, particle size 5 μm), by gradient elution, using acetonitrile and water as components of mobile phase, and UV detection (254 nm). The system was controlled by Chromeleon® 7 software (Thermo Scientific). Histamine concentration (mg kg^{-1}) was calculated from the area ratio of histamine and internal standard (1,7-diaminoheptane), using a matrix matched calibration curve covering the concentration range from 0 to 500 mg kg^{-1}.

2.3. Statistical Analysis

The difference between the PT declared histamine content in samples and the values obtained using different methods was analyzed using the *t* test. The difference between the histamine content obtained by the screening methods and the HPLC method for tested contaminated samples of canned fish was analyzed using linear regression analysis (PAST, Version 2.12, Oslo, Norway).

3. Results and Discussion

Results of histamine determination in all analyzed reference samples are presented in table 1. As can be seen from table 1, results for all samples except for FAPAS Proficiency test 27161 obtained by both screening methods were in the acceptance range. However, the result for sample with low histamine concentration (16.96 mg kg^{-1}), obtained by enzymatic method (21.33 mg kg^{-1}) was some higher than the acceptable value (13.4–20.5 mg kg^{-1}). We noted, on analyzing data from all participants in the proficiency scheme report for FAPAS 27161, that laboratories with screening methods obtained a higher average value (19.16 mg kg^{-1}) than the declared value. Contrary to this, statistical analysis (*t* test) showed that there was no significant difference between the contents of histamine obtained by the enzymatic test and declared content at the 0.05 level (p=0.074) in our study. Also, there was no significant difference in either case between the determined and declared content at the 0.05 level for all analyzed samples.

Generally, the screening methods gave some higher recovery (98–126%) compared to the HPLC method (99–111%), while intermediate precision (day-to-day variability) was in a similar range (3.8-15.5 and 5.5-14.7%).

Linear regression analysis (figure 1) showed good agreement between screening methods and the standard method in all analyzed samples (r=0.977).

The results obtained are similar to literature data. CD-ELISA and HPLC methods showed good agreement (R=0.969; concentration range 0.7–420 mg/kg) for histamine analysis in commercial soybean paste, suggesting that the CD-ELISA can be used as a rapid indicator for biogenic amines, including histamine [11]. Analysis of 50 commercial cheeses according to CD-ELISA and RP-HPLC also showed good agreement for histamine (r=0.979; concentration range 2–1800 mg kg^{-1}) [12].

Although commercial test kits are generally used for determining histamine in fresh and canned fish and fish meal, information on their performance and application to traditional fish products, which differ in product properties, showed different correlations with HPLC [13]. Therefore, new commercial test kits should be evaluated against the approved analytical method before being applied to new types of products.

Table 1. Results of histamine determination in reference samples using different methods (*N*=3).

Sample (Histamine in canned fish)	PT declared value (acceptance range) mg kg^{-1}	Method	
FAPAS Proficiency test 27149	137 (116–158)	Enzymatic test	HPLC
Average (mg kg^{-1})		148.2	138.2
SD (mg kg^{-1})		5.6	16.7
RSD (%)		3.8	12.1
Recovery (%)		108	101
p [a]		0.074	0.915
FAPAS Proficiency test 27161	16.95 (13.4–20.5)	Enzymatic test	HPLC
Average (mg kg^{-1})		21.33	16.84
SD (mg kg^{-1})		2.10	2.47
RSD (%)		9.8	14.7
Recovery (%)		126	99
p [a]		0.068	0.944
FAPAS Proficiency test 27176	216 (186–247)	Enzymatic test	HPLC
Average (mg kg^{-1})		200.1	237.0
SD (mg kg^{-1})		11.6	13.3
RSD (%)		5.5	5.5
Recovery (%)		98	111
p [a]		0.659	0.089
FAPAS Proficiency test 27197	174 (148–199)	ELISA	HPLC
Average (mg kg^{-1})		183.5	165.3
SD (mg kg^{-1})		28.4	9.0
RSD (%)		15.5	5.4
Recovery (%)		105	95
p [a]		0.620	0.235

t test value (*p* values) comparison of data obtained using different methods and declared content.

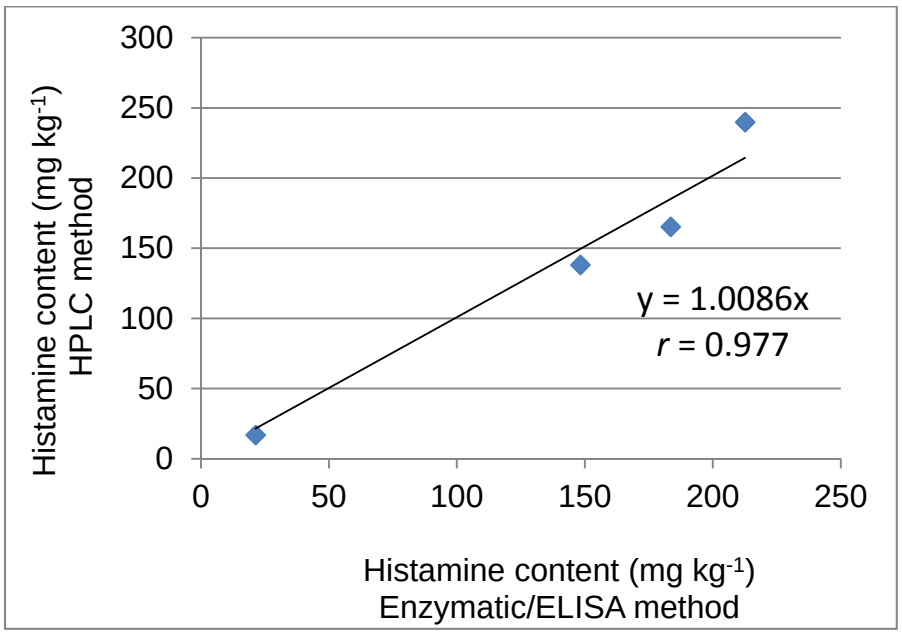

Figure 1. Correlation of results obtained by screening and standard method.

4. Conclusion

To ensure food safety with respect to histamine, it is preferable to use a rapid, simple and cheap method for screening. Results of histamine determination in reference samples showed that the enzymatic test and CD-ELISA give reliable and accurate values, especially for determination of critical high histamine concentrations (100–200 mg kg^{-1}). However it is necessary to validate any screening method and its reliability. According to the EC [6], it is important to confirm any high concentration of histamine in potentially positive samples by the reference method. Since quality control and consumer safety is of great importance from the aspect of histamine contamination, there is a constant challenge to develop new, fast, and reliable methods for different types of samples.

Acknowledgments

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