Reagents and materials for biogenic amines determination and quantification

All chemicals were of a HPLC grade. Ultrapure water was produced by a Millipore Direct-Q 3 UV system (Millipore, Molsheim, France). The mixed standard solution of BAs was prepared from the following reagents: cadaverine dihydrochloride 99% (Fluka, Buchs, Switzerland), histamine dihydrochloride 99% (Fluka, Buchs, Switzerland), phenyletilamine hydrochloride 99 %, putrescine dihydrochloride 99% (Sigma Aldrich, St. Louis, MO, USA) spermidine trihydrochloride 99,5 % (Sigma Aldrich, St. Louis, MO, USA), spermine tetrahydrochloride 99,5 % (Sigma Aldrich, St. Louis, MO, USA), tryptamine hydrochloride 98% (Fluka, Buchs, Switzerland) and tyramine hydrochloride 97% (Sigma Aldrich, St. Louis, MO, USA), 1,7-heptane diamine – HEP (Sigma Aldrich, St. Louis, MO, USA), dansyl-chloride 95% - Dns-Cl (Sigma Aldrich, St. Louis, MO, USA). Other chemicals in use were perchloric acid sodium bicarbonate ($NaHCO_3$), acetone, ammonia solution, acetonitrile and ammonium acetate, all produced by Merck (Darmstadt, Germany). The standard stock solution of each of the eight biogenic amines was prepared in ultrapure water in the concentration of 1,000 mg/L. The working mix standard solution (100 mg/L) containing the eight biogenic amines was prepared by virtue of diluting the stock solution with 0.4 mol/L of perchloric acid and was used for the addition of biogenic amines to sausage samples.

HPLC-DAD analysis

The HPLC analysis of BAs was performed using high-performance liquid chromatograph (Agilent 1200 Series HPLC, Santa Clara, CA) equipped with a binary gradient pump (G1312A), an auto-sampler with a thermostatted sample compartment (G1329A), a thermostatted column compartment (G1316A) and a DAD detector (G1315D). The separation of the compounds was done in a LiChrospher C18 analytical column (ID 250 mm x 4.0 mm, particle size 5 μm) using a C18 Security Guard Cartridge (ID 4 mm x 3 mm) supplied by Phenomenex (Agilent, Santa Clara, CA), which was maintained at 40 °C and processed the injection volume of 20 μL. The mobile phase was a mixture of acetonitrile and 0.1 mol/L ammonium acetate processed in a gradient mode, flowing at the rate of 1 mL/min [20]. The initial 50 %-acetonitrile content was increased to 90% in 19 minutes. The initial conditions were reached in a minute and maintained for 9 min before the next run. The total run time was 29 min. The compounds were quantified using internal calibration curves plotted for each BA and covering for eight concentration levels ranging from 0.25 mg/kg to 500 mg/kg.

Table S1. Selected performance indicators of the method in use: linearity, limit of detection (LOD), limit of quantification (LOQ), recovery and precision (RSD_r)

BA	Linearity (r^2)	Cheese			
		LOD (mg/kg)	LOQ (mg/kg)	Recovery (%)	RSD_r
TRP	1.00	0.77	2.79	101.0	13.05
β-PHE	1.00	0.63	2.39	99.6	6.87
PUT	1.00	0.59	1.99	88.5	7.98
CAD	1.00	0.61	1.79	102.1	5.48
HIS	1.00	0.59	1.77	99.6	9.44
TYR	1.00	0.89	3.19	102.9	13.88
SPD	1.00	0.39	1.23	98.6	5.97
SPM	1.00	1.01	3.59	94.1	8.88

BA, biogenic amines; TRP- tryptamine; β-PHE- β-phenylethylamine, PUT – putrescine; CAD-cadaverine; HIS- histamine; TYR- tyramine; SPD-spermidine; SPM- spermine; LOD, limit of detection; LOQ, limit of quantification; r^2, determination coefficients of the analytical seven-point curves plotted for standard solutions (0.25 – 500 mg/kg); Recovery, mean recovery at three concentrations used in the precision assessment (cheese samples were spiked in concentrations of 50, 100 and 250 mg/kg); RSDr for each BA at three concentration levels (cheese samples were spiked at the concentrations of 50, 100, 250 mg/kg) under repeatability (r) conditions used for the method precision assessment.

The results concerning linearity, LOD, LOQ, recovery and RSDr are presented in Table S1. The LOD established for the cheese samples ranged from 0.39 to 1.01 mg/kg, while the LOQ varied from 1.23 to 3.59 mg/kg.

The LOD was calculated from the average of ten BA-negative cheese samples (mold ripened, semi hard and hard cheeses), earlier analysed for BA presence and used for validation as the blank material; to the above average, the tripled standard deviation was added (LOD = mean ± 3SD) [36]. In order to determine the LOQ, the mean concentration determined in ten BA-negative cheese samples was summed up with the six-fold standard deviation (LOQ = mean ± 6SD). For each BA, the mean recovery of a 36-sample set was calculated and used for the accuracy assessment, evaluated based on the intra-laboratory coefficient of variation. The specificity was checked by analysing the 8 BAs in each of the ten blank cheese samples and verifying the presence of interferences in the region of interest where single BAs were expected to elute. The linearity was checked through the regression coefficients of determination (r^2) of the analytical curves using the standard mix working solution containing the 8 BAs at seven concentration levels ranging from 2.5 to 500 mg/kg. In the first step, a three-point calibration curve (50, 100 and 250 mg/kg for cheese and meat products; 100, 250 and 500 mg/kg for fish and fishery products) was plotted using linear regression with the calibration standards in the solvent solution.

The matrix effect experiment includes 2 steps. In the first step, a three-point calibration curve (50, 100 and 250 mg/kg) was plotted using linear regression with the calibration standards in the solvent solution. In the next step, another three-point calibration curve using the same concentrations per cheese blank samples as detailed above and a fixed amount of internal standard (50 mg/kg) was plotted based

on the measurement data of the matrix-matched calibration standards. The slopes of the regression curves representative of the two sets of calibration solutions were evaluated statistically.

During this study, the HIS content in the control sample of canned fish sample T27137QC, FAPAS, was measured nine times, resulting in the mean value of 221 ± 9.2 mg/kg (mean recovery value, 104 %). The recoveries obtained within the frame of the internal quality control spanned from 80.1 to 109.8 %, which is in accordance with the criteria established under the EC Regulation No. 333/2007 [36]. The mean slopes of the regression curves for the standard and matrix sets of calibration solutions were not significantly different. Therefore, matrix-matched standard calibrations were not used. The applied analytical method fulfils all methodological requirements set out under the EC Regulation No. 333/2007 [1] and can therefore be considered as suitable for the determination of 8 BAs in food groups under this study.

Reference

1. European Commission. Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. *Off. J Eur. Union* **2007**, *88*, 29.