INTERCOMPARISON STUDY: BIOGENIC AMINES IN DRY SAUSAGES

Susanna Eerola[1] and Koen Molly[2]

[1]National Veterinary and Food Research Institute, Department of Chemistry, PL 368 (Hämeentie 57), 00231 HELSINKI, Finland
[2]University of Gent, Department of Animal Production, Proefhoevestraat 10-12, B-9090 MELLE, Belgium

Key words: biogenic amines, analysing methods, intercomparison study

Background

Varying concentrations of histamine, tyramine and phenylethylamine are regurarly found in dry sausages sold in the retail markets (e.g. Rice et al., 1975, Vandekerckhove, 1977, Wortberg and Woller, 1982, Tschabrun et al., 1990). The study on the factors affecting their formation has been a topic in many research groups and when comparing reports from different laboratories is important to know whether the analysing methods of biogenic amines are comparable.

Objective

In order to get an impression of the performance of the quantitative determination of biogenic amines in dry sausages 5 laboratories were invited to join this study.

Methods

5 different dry sausages were homogenized in laboratory A, freezed at -80°C and delivered to other laboratories. Samples were analysed in the same week in every laboratory. Laboratories A, B, C, E and F were asked to analyse samples as duplicate.

As an extraction solvent 0.4 M perchloric acid was used in laboratories B, E and F, acetone and 5% trichloroacetic acid (TCA) in laboratory A and 0.2 M TCA and water in laboratory C. Analyses were performed with RP-LC-technique and all laboratories except laboratory C detected amines as their dansyl derivatives (254 nm UV). Laboratory C used direct UV-detection of amines at a wavelength of 215 nm. The determination levels changed between 0.5-10 mg/kg.

Results and discussion

The results of the laboratories are given in Table 1. All determinations were taken to the calculations of means and to the deviation counts. Summary of these statistical values are presented in Table 2. No results were considered as outliers because of the low number of participants. The Horwitz acceptable values (RSD) were calculated to the concentrations of amines and they were used as a fixed target values in determinating the Z-scores of histamine and tyramine (Table 3). In general, the absolute value of Z greater than three suggests poor performance in terms of accuracy.

The precision of the determination of histamine, tyramine and putrescine are considered satisfactory (between-laboratory SD≤3x RSD). The largest variation were in the results of tryptamine, cadaverine and spermine. Reason for differences can be caused by interfering peaks (free amino acids, derivation reagent). Possible co-elution of interfering compounds with amines has to be controlled in analysing methods. The accurate concentrations of biogenic amines in sausage samples were not known and instead of real samples (amines from metabolic origin) it would be more easier to perform intercomparison study by using standard solutions or spiked matrix.

Acknowledgement

Authors thank Christina Montel, Tuija Sinervo and Anna-Maria Thim for co-operation in this study.

Literature

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Table 1. The levels of biogenic amines in five different samples (mg/kg).

Laboratories	Samples	His	Tyr	Tryp	Phe	Put	Cad	Spd	Spr
Laboratory A	1	9	82	12	<0.5	5	5	7	14
	2	26	340	21	14	130	26	40	3
	3	22	300	100	66	260	49	30	5
	4	<0.5	86	<0.5	<0.5	76	12	3	8
	5	11	160	7	<0.5	121	16	12	10
Laboratory B	1	15	96	8	<1	9	30	1	23
	2	12	300	1	8	140	9	4	12
	3	28	350	47	44	340	11	4	25
	4	1	77	<1	<1	59	43	1	19
	5	15	140	6	<1	87	30	3	12
Laboratory C	1	14	62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2	23	310	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	3	27	390	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	4	<5	72	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	5	10	120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Laboratory E	1	5	72	n.d.	n.d.	6	<5	n.d.	n.d.
	2	16	320	n.d.	n.d.	130	<5	n.d.	n.d.
	3	17	370	n.d.	n.d.	330	14	n.d.	n.d.
	4	<5	66	n.d.	n.d.	62	<5	n.d.	n.d.
	5	6	140	n.d.	n.d.	89	5	n.d.	n.d.
Laboratory F	1	8	60	12	3	4	6	9	41
	2	23	210	14	6	120	5	9	31
	3	25	230	120	69	320	18	14	42
	4	2	62	11	2	55	5	9	36
	5	8	110	11	9	86	9	7	41

His=histamine, Tyr=tyramine, Tryp=tryptamine, Phe=phenylethylamine, Put=putrescine, Cad=cadaverine, Spd=spermidine, Spr=spermine, n.d.=not determined.

Table 3. Intercomparison study z-scores (histamine and tyramine) in dry sausage-samples.

Laboratory	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	His	Tyr	His	Tyr	His	Tyr	His*	Tyr	His	Tyr
A	-0.9	1.3	2.3	2.2	-0.8	-1.2	-	2.3	0.9	2.4
B	4.4	3.6	-4.2	0.2	1.7	1.1	-	0.8	4.4	-0.5
C	3.5	-1.9	0.9	0.7	1.3	2.9	-	0	0	-1.5
E	-4.4	-0.3	-2.4	1.2	-2.9	2	-	1	-3.5	0.5
F	-1.8	-2.3	0.9	-4.4	0.4	-4.4	-	-1.7	-1.8	-2.4

* not calculated because the levels were <2 mg/kg

Table 2. Biogenic amines in dry sausage-samples - summary of the results.

	Samples	1	2	3	4	5
Histamine, 5 labs:	Mean (mg/kg)	10	21	24	0.5	10
	Repeatability standard deviation (mg/kg)	1.6	1.6	1.4	0.063	1.9
	Reproducibility standard deviation (mg/kg)	4	6.3	4.3	0.7	3.5
	Horwitz acceptable value (%)	11	10	9.9	2.2	11
Tyramine, 5 labs:	Mean (mg/kg)	74	296	327	72	135
	Repeatability standard deviation (mg/kg)	4.1	15	9.7	5.9	6.7
	Reproducibility standard deviation (mg/kg)	14	47	58	9.9	18
	Horwitz acceptable value (%)	6.2	20	22	6.1	10
Tryptamine, 3 labs:	Mean (mg/kg)	10	12	91	3.5	8.1
	Repeatability standard deviation (mg/kg)	1.6	3.4	2.3	0.082	2.5
	Reproducibility standard deviation (mg/kg)	2.3	9.8	36	5.4	3.2
	Horwitz acceptable value (%)	1.1	1.3	7.4	0.46	0.95
Phenylethylamine, 3 labs:	Mean (mg/kg)	1	8	60	0.8	3
	Repeatability standard deviation (mg/kg)	0.082	3.3	4.7	0.082	0.82
	Reproducibility standard deviation (mg/kg)	1.5	5.4	13	1.2	4.6
	Horwitz acceptable value (%)	0.16	1.4	5.2	0.13	0.41
Putrescine, 4 labs:	Mean (mg/kg)	5.7	129	311	63	96
	Repeatability standard deviation (mg/kg)	0.71	5.5	4.9	2.3	8.8
	Reproducibility standard deviation (mg/kg)	1.9	9.4	32	8.6	17
	Horwitz acceptable value (%)	0.72	9.9	21	5.4	7.7
Cadaverine, 4 labs:	Mean (mg/kg)	14	13	23	20	15
	Repeatability standard deviation (mg/kg)	2.8	0.99	1.5	0.83	6
	Reproducibility standard deviation (mg/kg)	13	10	16	18	10
	Horwitz acceptable value (%)	1.5	1.4	2.3	2	1.6
Spermidine, 3 labs:	Mean (mg/kg)	5.7	17	16	4.3	7.4
	Repeatability standard deviation (mg/kg)	2	0.91	2.1	2.9	0.73
	Reproducibility standard deviation (mg/kg)	3.7	18	12	3.9	4.5
	Horwitz acceptable value (%)	0.72	1.8	1.7	0.6	0.9
Spermine, 3 labs:	Mean (mg/kg)	26	15	24	21	17
	Repeatability standard deviation (mg/kg)	0.32	3.8	1.3	0.42	1.4
	Reproducibility standard deviation (mg/kg)	12	13	16	12.6	10.2
	Horwitz acceptable value (%)	2.6	1.6	2.4	2.1	1.8