

1 **Dispersive liquid-liquid microextraction combined with gas chromatography-**
2 **mass spectrometry for *in situ* determination of biogenic amines in meat:**
3 **estimation of meat's freshness**

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8 **Abstract**

9 A dispersive liquid-liquid microextraction (DLLME) gas chromatography-mass spectrometry (GC-MS)
10 technique was developed for the determination of selected biogenic amines (BAs) in samples of
11 poultry, pork and beef. Prior to the extraction process, an appropriate volume of sodium hydroxide
12 solution was added to each of the portioned samples. Next, samples were homogenized, centrifuged
13 and finally sonicated at an increased temperature. After another centrifugation, the supernatant was
14 made up to 50 mL in a calibrated flask. Subsequently, 5 mL of supernatant was separately subjected
15 to a derivatization and extraction procedure. A mixture of methanol (dispersive solvent; 210 μ L),
16 chloroform (extractive solvent; 300 μ L), and isobutyl chloroformate (derivatizing reagent; 100 μ L) was
17 used in the extraction process together with an admixture of pyridine and HCl in order to eliminate the
18 by-products. The application of the method enables fast derivatization and extraction of the BAs and
19 a straightforward and rapid sample enrichment. It displayed good linearity, intra- and inter-day
20 precision and good recoveries. The proposed methodology is characterized by low limits of detection
21 and quantification (0.003-0.009 μ g/g and 0.009-0.029 μ g/g, respectively). The green character of the
22 method was established based on the results of two tools, namely the Analytical Eco-Scale and GAPI.
23 It was successfully used to analyse samples of poultry, porcine and bovine meat. Multivariate statistical
24 data analysis was applied in order to evaluate the potential use of the determined BAs as spoilage
25 markers of particular meat types.

26

27 **Keywords:** biogenic amines; dispersive liquid-liquid microextraction; meat; shelf-life; gas
28 chromatography-mass spectrometry

29 **1. Introduction**

30 The organoleptic qualities of fresh meat and poultry deteriorate during storage. However, sensory
31 analysis is often not sufficient to detect early indications of spoilage, and so methods such as the total
32 viable bacteria counts and the determination of the total volatile basic nitrogen (TVB-N) are used to
33 assess the freshness of meat products [1]. In the latter, the content of ammonia produced during
34 deamination of amino acids is linked to the progress of putrefaction [2]. An alternative approach to
35 the assessment of meat and poultry freshness is the determination of biogenic amines (BAs), as they
36 are formed from precursor amino acids through the enzymatic decarboxylation during storage [3]. The
37 determination of BAs is suitable for detecting early onset of spoilage, as the ones naturally occurring
38 in the animal tissues could be degraded by certain microorganisms [4,5]. Furthermore, apart from

39 being indicators of spoilage, BAs themselves can have a detrimental effect on human health when
40 ingested. Histamine (HIST) has been linked to several outbreaks of food poisoning, while tyramine (TYR)
41 is associated with the hypertensive crisis. The toxicity of HIST is compounded by the presence of
42 cadaverine (CAD), putrescine (PUT) and TYR, and since BAs are the precursors of nitrosamines they
43 should also be considered as potential carcinogens [6].

44 Because of the complexity of the matrix and the nature of amino acids decarboxylation due to
45 microbial enzymes and tissue activity, the concentration of a single BA might not be a sufficient marker
46 of spoilage. For this reason, several meat freshness indices have been proposed. In particular, the
47 Chemical Quality Index (CQI) is the sum of concentrations of CAD, PUT, spermine (SPER), spermidine
48 (SPERM) and HIST [7], and the Biogenic Amines Index (BAI) is the sum of concentrations of HIST, CAD,
49 TYR and PUT [8]. Silva *et al.* have also proposed a chicken meat quality index based on the ratio of
50 SPERM and SPER [9]. However, the reliability of these indices in detecting the early stages of
51 putrefaction relies greatly on the capabilities of the analytical method used for the determination of
52 BAs.

53 The techniques used for the determination of biogenic amines in poultry and meat samples include
54 ion chromatography, capillary electrophoresis, gas chromatography (GC) and high-performance liquid
55 chromatography (HPLC), with the latter being the most popular [10,11]. However, the application of
56 HPLC is often relatively laborious and entails the use of relatively large volumes of organic solvents
57 [12]. On the other hand, the direct determination of BAs in meat samples using GC is difficult due to
58 their relatively low concentration and interferences from e.g. polyphenols [13]. These shortcomings
59 can be alleviated using extraction and derivatization which increases the amines' volatility and
60 facilitates detection using GC. In particular, dispersive liquid-liquid microextraction (DLLME) is
61 relatively inexpensive, easy to perform, rapid and characterised by high enrichment factor and
62 recovery. Moreover, as it requires the use of only small volumes of solvents it conforms to the
63 postulates of green analytical chemistry [14]. DLLME-GC-MS has previously been used for the
64 determination of polycyclic aromatic hydrocarbons in grilled meat [15] and of BAs in food samples [16–
65 19]. However, to the authors' best knowledge a dedicated method involving the use of this technique
66 for the determination of BAs in animal tissues has not yet been described.

67 The aim of this study was to develop and validate a DLLME-GC-MS analytical method for the
68 determination of BAs in meat samples for the purpose of freshness assessment, especially in the
69 context of meat freshness indices. These indices are increasingly being used as a quantitative method
70 to evaluate the shelf-life of fresh meat, and so rapid and reproducible methods for the determination
71 of BAs in this matrix might find immediate application. Particular focus has been placed on the sample
72 preparation procedure. Since the meat samples are solid, additional steps were introduced prior to
73 the extraction stage. Thus, an appropriate volume of sodium hydroxide solution was added to each of
74 the portioned samples. Next, samples were homogenized, centrifuged and finally sonicated at an
75 increased temperature. After another centrifugation, the supernatant was made up to 50 mL in a
76 calibrated flask. Then, 5 mL of supernatant was separately subjected to a derivatization and extraction
77 procedure. In the extraction stage, a mixture of methanol (dispersive solvent; 210 μ L), chloroform
78 (extractive solvent; 300 μ L), and isobutyl chloroformate (derivatizing reagent; 100 μ L) were used in the
79 extraction process together with an admixture of pyridine and HCl in order to eliminate the by-
80 products. Care has been taken to evaluate the impact of the nature and amount of both the
81 derivatizing reagent and the extractive and dispersive solvents, as well as the reaction time. The



82 developed method was used to determine the concentration of selected BAs in the samples of fresh
83 chicken, pork and beef during storage in different containers. Multivariate statistical data analysis was
84 used to determine the applicability of these BAs as meat freshness indicators.

85 **2. Experimental**

86 2.1. Materials and reagents

87 The biogenic amine standards: CAD ($\geq 99.0\%$), dimethylamine (DIMET, 99%), HIST ($\geq 99.0\%$), PUT
88 ($\geq 99.0\%$), SPER ($\geq 99.0\%$), tryptamine (TRP, 99%), TYR ($\geq 98\%$) and 2-phenylethylamine (2-PE, $\geq 98\%$)
89 were obtained, mostly in the form of hydrochloride salts, from Sigma Aldrich (Steinheim, Germany),
90 as was the internal standard (hexylamine, IS). The derivatizing reagents ethyl chloroformate (ECF) and
91 isobutyl chloroformate (IBCF) were also supplied by Sigma Aldrich. High purity grade dispersive
92 solvents acetone and methanol (MeOH) were obtained from Fluka (Buchs, Switzerland). The extractive
93 solvents isooctane, chloroform and dichloromethane of high purity HPLC analysis grade were obtained
94 from Sigma Aldrich. 5 M HCl was obtained from Fluka. Other chemicals were of analytical grade. The
95 solution of alkaline methanol was prepared by dissolving KOH in methanol until saturation. The
96 silanized screw-capped vials with solid PTFE-lined caps were obtained from Supelco (Bellefonte, PA,
97 USA). The manual homogenizer (Bamix ESGE Ltd., Mettlen, Switzerland) at 14.000 rpm was used for
98 homogenization. Centrifuge (Combi-Spin FVL-2400N, Biossan, Latvia) was used for centrifugation
99 performed at 4 °C and 5000 rpm for 15 min. Bandelin SONOREX (Sigma Aldrich, Steinheim, Germany)
100 was used for ultrasonication.

101 2.2. Sampling

102 Samples of fresh chicken breast muscle (*pectoralis major*, 1C-5C), pork loin (*longissimus dorsi*, 1P-5P)
103 and beef loin (*longissimus dorsi*, 1B-5B), five each, were obtained from a local distribution centre in
104 Gdańsk, Poland. Each sample weighed 100 g. All samples were immediately refrigerated and
105 transported in a portable cooler to the laboratory within 30 min, where they were stored at 4°C in
106 three different containers: in a aerobically in a standard PP-R food box (I), polypropylene co-polymer
107 (PP-R) vacuum food box (II), and aerobically in a standard high-density polyethylene (HDPE)
108 refrigerator bag (III). All samples were from adult animals, and pieces were taken for analyses 1, 3,
109 and 5 days post-mortem. Each sample was analysed in triplicate.

110 2.3. Preparation of standards solution

111 Stock solutions (1mg/mL) of BAs were prepared by weighing each analyte standard and dissolving in
112 10 mL of deionized water. A multi-compound working standard solution (1 µg/mL) of each compound
113 was prepared by appropriate dilution. The solutions were stored at 4 °C in silanized screw-capped vials
114 with solid PTFE-lined caps. All calibration and working solutions were prepared by sequentially diluting
115 the stock solutions in an appropriate linear range with a spiked IS on the day of the analysis. The IS
116 solution was prepared at 1 mg/mL and diluted to 0.1 µg/mL with deionized water during sample
117 analysis.

118 2.4. Preparation of samples

119 The sample preparation procedure was the same for each kind of meat. The same amount of meat
120 samples (5 g) was added to 50 mL of 0.1 M NaOH, homogenized using a laboratory mixer and
121 centrifuged for 15 min at 4 °C and 5000 rpm. Samples were then placed in a PTFE vessel and placed for

122 60 min in an ultrasonic bath thermostated at 70 °C. The homogenised mixture was centrifuged at 5000
123 rpm for 3 min, the supernatant was collected and subsequently made up to 50 mL in a calibrated flask.
124 Three aliquots of supernatant (5 mL each) were separately subjected to a derivatization and extraction
125 procedure.

126 2.5. Derivatization and dispersive liquid-liquid extraction methodology

127 For the in-situ derivatization coupled to DLLME, an aliquot of 5 mL of the extract obtained during the
128 previous step was spiked with an internal standard (50 µL of a water solution containing the internal
129 standard) and placed in a glass centrifuge tube with conical bottom containing 0.5 g NaCl. Next, a 5 M
130 HCl solution was added to obtain pH 11. A mixture of methanol (600 µL), pyridine:HCl (100 µL, 1:1 v/v)
131 and isobutyl chloroformate (200 µL) was rapidly injected into the sample tube, and the mixture was
132 again gently shaken for a few seconds. After 10 min, a 1 mL of chloroform was added and after
133 centrifugation for 5 min at 5000 rpm, the extraction solvent was sedimented in the bottom of the
134 conical tube. The bottom layer was transferred to vials with 100 µL inserts. A 5 µL aliquot was injected
135 in the splitless mode into the GC–MS system.

136 The relative response factors (RRFs) was used to express the effectiveness of extraction as well as
137 derivatization procedure and were calculated according to the following equation (1):

$$138 \quad RRF = \frac{A_S \times C_{IS}}{A_{IS} \times C_S} \quad (1)$$

139 AIS: the internal standard peak area,
140 CS: the target analyte concentration (g/mL),
141 AS: the target analyte peak area,
142 CIS: the internal standard concentration (g/mL).
143

144 2.6. Gas chromatography-mass spectrometry method

145 The gas chromatograph 7890A (Agilent Technologies, Santa Clara, CA, USA) equipped with an
146 electronically controlled split/splitless injection port was interfaced with a mass selective detector
147 (5975C, Agilent Technologies, Santa Clara, CA, USA) with EI ionization chamber. GC separation was
148 performed on Zebron ZB-5MS capillary column (30 m x 0.25 mm I.D., 0.25 µm film thickness)
149 (Phenomenex, Torrance, CA, USA). The injection was made in splitless mode (injection pressure 32 ps)
150 at 240 °C. Helium was the carrier gas with a constant pressure of 30 psi. The oven temperature program
151 was as follows: 45 °C held for 2 min, ramped to 160 °C at 15 °C/min and held for 2 min, and ramped to
152 280 °C at 10 °C/min and held 9 min. The total run time was 33 min. The MS transfer line temperature
153 was held at 280 °C. Mass spectrometric parameters were set as follows: electron impact ionization
154 with 70 eV energy; ion source temperature, 250 °C. The MS system was routinely set in SIM mode and
155 each analyte was quantified based on peak area using one target and one or more qualifier ion(s)
156 (Table 1). Agilent ChemStation software was used for data collection and GC-MS control.

157 Table 1. Fragments, relative intensities and retention time (Rt) of BAs obtained by application of GC-
158 MS technique.

Analytes	m/z SIM ions (Relative intensities)	Rt
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DIMET	72 (90)	90 (99)	145 (2)			2.02
Hexylamine (IS)	146 (99)	130 (77)	128 (15)			8.12
SPER	101 (80)	144 (99)	201 (32)	274 (4)		8.51
2-PE	130 (99)	104 (80)	91 (76)	221 (31)	148 (19)	9.99
PUT	170 (99)	130 (64)	288 (11)			12.00
TRYP	130 (99)	143 (59)	260 (19)	187 (4)		13.00
TYR	120 (99)	107 (29)	176 (5)	237 (2)	337 (1)	13.51
CAD	130 (79)	84 (82)	129 (74)	302 (3)		13.71
HIST	194 (99)	238 (17)	138 (26)			14.32

159

160 2.7. Quality assurance

161 Matrix effects (ME) were investigated at two concentration levels, 0.5 and 5 µg/L and were calculated
 162 by comparing the responses (peak area of each analyte against peak area of the IS) for appropriate
 163 solution of analytes prepared in methanol (sets A, n=3) with those measured in blank meat extracts
 164 spiked after the extraction procedure with the same amount of analyte (sets B, n=3). The following
 165 formula was used (2):

$$166 \quad ME[\%] = \frac{B}{A} \times 100\% \quad (2)$$

167 The optimized method was validated for linearity, detection and quantification limits (LOD and LOQ,
 168 respectively), selectivity, accuracy and precision. The method's linearity was investigated by a
 169 regression analysis of the relative area versus the analyte concentration. The relative area was
 170 presented as the ratio between the peak area of a particular BA and the peak area of the IS. The LODs
 171 were calculated as three times the signal-to-noise ratio, while LOQ were calculated as ten times the
 172 signal-to-noise ratio. The intra-day precision was investigated by analysing four replicates of meat
 173 samples spiked at 0.5 µg/L on the same day. Inter-day precision was investigated by means of samples
 174 analysis on two different days over a period of two weeks. The recovery was calculated by comparing
 175 unspiked extract samples to ones spiked at 0.5 µg/L; n=4.

176 2.8. Evaluation of the green profile

177 The developed analytical procedures used for the determination of biogenic amines in meat samples
 178 were subsequently assessed in terms of 'greenness' by two well-established methods: the Analytical
 179 Eco-Scale and the Green Analytical Procedure Index (GAPI).

180 2.9. Multivariate statistical analysis

181 The determined concentration values of BAs in meat samples were used as input data for multivariate
 182 statistical data analysis using a dedicated Python toolkit Orange v.3.13 [20]. Initial data processing
 183 involved standardization (centring by the mean value and scaling by standard deviation). The analysis
 184 of variance within the variables and feature selection was performed using the ReliefF algorithm [21],
 185 as it is more sensitive to feature interactions, especially with discrete features (e.g. days of storage,
 186 packaging material) compared to ANOVA or chi² [22]. The area under the ROC curve, classification



187 accuracy and precision of supervised classification (naïve Bayes) was validated using a 10-fold stratified
188 cross-validation. Missing data (determined concentration below LOQ) was replaced by the value LOD/3.
189 Hierarchical cluster analysis with Ward linkage was performed based on Mahalanobis distances. Height
190 ratio of 66% was assumed for the identification of relevant clusters.

191 **3. Results and discussion**

192 3.1. Optimization of extraction conditions

193 In the DLLME procedure coupled with derivatization process, the fundamental parameters that need
194 to be optimized are the extractive and dispersive solvents, solvents volume, type and volume of the
195 derivatization reagent, and the extraction and derivatization time. These parameters were
196 systematically studied in order to achieve a good sensitivity, selectivity and precision for all BAs
197 determined in the study.

198 *3.1.1. Selection of extractive, dispersive solvents and of the derivatizing agent*

199 For the extractive solvent selection, following requirements were considered: immiscibility with water,
200 density in relation to water, high extraction capability, compatibility with the derivatizing reagent,
201 good solubility of derivatives, and good chromatographic behaviour. Based on these criteria the three
202 following solvents were examined: isooctane (density: 0.83 g/mL), dichloromethane (density: 1.33
203 g/mL) and chloroform (density: 1.48 g/mL). For the selection of dispersive solvent, the miscibility of
204 the dispersive solvent in the extractive solvent as well as in the sample solution were the features
205 taken into account. Two solvents: acetone and methanol (MeOH) were examined.

206 In this study, the group of chloroformates were examined as potential derivatizing agents. It is reported
207 that alkyl chloroformates are a group of derivatizing reagents with very favourable characteristics in
208 regard to the determination of BAs using the GC technique. In addition, these derivatizing agents do
209 not require specific condition during derivatization step which can be performed in a short time.
210 Moreover, they are cheap, commercially available and simple to use. In the present study, two
211 derivatization reagents belonging to this group, namely ethyl chloroformate (ECF) and isobutyl
212 chloroformate (IBCF) were examined.

213
214 For this experiment, extractions were carried out for 15 min from 5 mL of supernatant of meat sample
215 (with pH adjusted to 11) spiked with all the BAs and 100 µL of derivatizing reagent with a combination
216 of 300 µL of MeOH or acetone, 100 µL of mixture of pyridine and HCl (1:1; in order to omit the by-
217 products). After 5 minutes 300 µL of isooctane, dichloromethane or chloroform were added to the
218 solution. The obtained results are listed in Table 2.
219

220
221

Table 2. Information on peak area obtained by GC-MS for analytes of interest by using different method conditions

Analyte	Extractive solvent											
	Dichloromethane				Chloroform				Isooctane			
	Dispersive solvent											
	MeOH		Acetone		MeOH		Acetone		MeOH		Acetone	
	Derivatizing reagent											
	IBCF	ECF	IBCF	ECF	IBCF	ECF	IBCF	ECF	IBCF	ECF	IBCF	ECF
CAD	10053	91124	6745	5683	50006	37234	7142	5987	n.d.	n.d.	n.d.	n.d.
DIMET	105432	109279	4647	n.d.	56675434	51623712	5134	4782	n.d.	n.d.	9102	7893
HIST	n.d.	n.d.	3829	2034	100118	72312	4345	3123	n.d.	n.d.	n.d.	n.d.
PUT	153078	200542	10734	8965	404298	312941	11765	9165	n.d.	n.d.	n.d.	n.d.
SPER	702000	598424	98356	25785	4154005	3334012	100351	41783	n.d.	n.d.	n.d.	n.d.
TRP	385439	219654	10429	10525	1010300	993912	45329	21052	n.d.	n.d.	n.d.	n.d.
TYR	n.d.	n.d.	n.d.	n.d.	267309	200081	5643	4321	n.d.	n.d.	n.d.	n.d.
2-PE	1667432	1300976	678954	457042	8166501	6204192	704952	500040	n.d.	n.d.	100012	18290
IS	997532	975309	27123	11115	3575765	1990998	31098	20843	n.d.	n.d.	8992	7827

CAD, cadaverine; DIMET, dimethylamine; HIST, histamine; PUT, putrescine; SPER, spermine; TRP, tryptamine; TYR, tyramine; 2-PE, 2-phenylethylamine; IS, hexylamine; MeOH, methanol; N.D., not detected

222

223 Based on the results of the analysis it can be observed that the GC-MS responses to the analytes
224 differed significantly from the responses to the solvents. Most of the derivative compounds were not
225 extracted by isooctane, except for DIMET and 2-PE. Both dichloromethane and chloromethane could
226 be successfully used as extractive solvents in the discussed scenario, however, the best extraction
227 results were obtained when chloroform was used as extraction solvent. The extraction efficiency for
228 most of the derivatives was higher when methanol was used compared to acetone. Both IBCF and ECF
229 were used with satisfactory results, with all standards being detected, however, the use of IBCF has
230 led to a higher peak response for all derivatives (Table 2), therefore, only this reagent was tested in
231 the further study. To the authors' best knowledge, no derivatization study for BAs in poultry and meat
232 samples using IBCF (and other alkyl chloroformates) has yet been published. Thus, different volumes
233 of this compound (50 μL , 80 μL , 110 μL , 140 μL) were admixed during 16 experiments carried out at
234 room temperature for 5, 10, 15, and 20 min (Table 3: experiments 1A–4D). The use of the coupling of
235 chloroform and methanol gave the best results and they were chosen as the extraction and disperser
236 solvents, respectively for the following experiments. Based on the above information, the following
237 reagents were used in further studies: MeOH, chloroform and IBCF.

238 3.1.2. Optimization of the volume of dispersive and extractive solvents

239 In order to assess the impact of the extractive solvent volume on the efficiency of the extraction, a
240 constant volume of dispersive solvent (MeOH, 300 μL), as well as the constant volume of pyridine and
241 HCl mixture (100 μL , 1:1 v/v), was subjected to the same procedure. IBCF was used as a derivatizing
242 reagent (100 μL). Different volumes of chloroform (from 100 μL to 500 μL) were examined. Due to the
243 fact that the volume of the upper phase was low in case of an admixture of 100 μL and 200 μL of
244 chloroform, there were issues with reproducibility (replicates were impracticable). However, the
245 volume of the upper phase increased when a higher volume of extractive solvent was used (300, 400
246 and 500 μL). The enrichment factors were calculated using the following equation:

$$247 \quad \text{Enrichment factor} = \frac{\% \text{Recovery} \times \frac{V_{aq}}{V_{sed}}}{100} \quad (3)$$

248 (V_{aq} - the volume of the aqueous phase, V_{sed} - the volume of the sedimented phase)

249 The enrichment factor decreased significantly with the increase of the volume of extracting solvent
250 (Figure 1). Thus, 300 μL of chloroform was selected in order to obtain high enrichment factors and low
251 detection limits.

252 In order to assess the impact of the dispersive solvent volume on the extraction efficiency, different
253 volumes of MeOH (150 μL , 180 μL , 210 μL , 240 μL , 270 μL) containing 100 μL of IBCF and a fixed volume
254 of pyridine and HCl mixture (100 μL ; 1:1 v/v) were examined. The results indicated that with the
255 increase of dispersive solvent volume the extraction efficiency was higher (150 to 210 μL), and then
256 slightly decreased (210, 240, 270 μL) for all derivatives. Thus, based on experimental results 210 μL of
257 MeOH was chosen as the optimum volume for the dispersive solvent. Influence of the volume of
258 methanol on the peak area of BAs by DLLME–GC–MS is shown in Figure 2.

259 3.1.3. Selection of the volume of derivatizing agent and reaction time

260 The concentrations of the target compounds as well as IS (0.5 µg/L) used in each experiment were
 261 constant. The conditions of GC-MS measurement applied during the examination of the impact of
 262 derivatising conditions on the yield of derivatised target compounds were also the same. RRFs were
 263 calculated for the analytes in order to assess the effectiveness of derivatization performed under the
 264 different reaction conditions.

265 Table 3. Different conditions of the derivatization process used for the chemical conversion of the
 266 target compounds by DLLME-GC-MS

Experiment no.	Volume of DR [µL]	Reaction time [min]
1A	50	5
2A		10
3A		15
4A		20
1B	80	5
2B		10
3B		15
4B		20
1C	110	5
2C		10
3C		15
4C		20
1D	140	5
2D		10
3D		15
4D		20

267 Due to the fact that the internal standard is not subjected to derivatization, a higher value of RRFs
 268 indicated an increase in reaction effectiveness. This knowledge was used to compare the effectiveness
 269 of the derivatization processes carried out at different reaction conditions. Information on the
 270 calculated RRFs (as mean value, $n = 3$) calculated based on the GC-MS results of experiments of 1A–4D
 271 for the target compounds are listed in Table 4. The relative standard deviations (RSD) of all RRFs were
 272 <3.3%.

273 Table 4. Information on RRFs (mean value; $n = 3$; RSD < 3.3%) calculated from the obtained GC-MS
 274 results for derivatives of analytes under the chromatographic conditions of experiments 1A–4D, as
 275 shown in Table 3.

DR Experiment	IBCF															
	1A	2A	3A	4A	1B	2B	3B	4B	1C	2C	3C	4C	1D	2D	3D	4D
Analyte	RRF parameters (mean value) ($n=3$) [$\times 10^{-3}$]															
CAD	n.d.	99	134	152	n.d.	102	216	201	n.d.	117	200	187	n.d.	111	199	156
DIMET	115	158	352	509	299	715	1009	911	329	732	917	852	317	672	897	809
HIST	n.d.	172	201	157	101	167	300	243	n.d.	160	272	207	145	142	237	157
PUT	n.d.	143	181	132	n.d.	145	312	293	n.d.	161	291	265	ND	118	263	178
SPER	58	300	414	456	201	506	802	762	199	511	776	748	186	432	743	604
TRP	n.d.	113	331	298	n.d.	276	423	408	n.d.	251	401	382	n.d.	201	378	278
TYR	89	301	519	406	269	645	834	776	201	621	800	678	189	598	748	654
2-PE	n.d.	101	322	218	n.d.	249	421	356	n.d.	219	377	309	n.d.	200	332	265

276

277 Based on the calculated RRFs it can be concluded that the derivatization process with IBCF depends
 278 strongly on the time parameter as well as on temperature. The efficiency of target compound
 279 derivatization with appropriate conditions: 80 μL of IBCF for 15 min (3B) was the highest and thus,
 280 these reaction conditions were selected as the optimum for further study.

281 3.2. Results of quality assurance

282 No statistically significant differences were observed ($P > 0.1$) during the examination of the matrix
 283 effect, and so quantification was performed by internal calibration. The values of correlation
 284 coefficients (R) were good ($R > 0.996$) demonstrating excellent linearity for the studied range. The
 285 LODs ranged from 0.003 to 0.009 $\mu\text{g/L}$ and the LOQs ranged from 0.0099 to 0.029 $\mu\text{g/g}$. Information
 286 regarding these parameters is listed in Table 5. The relative standard deviation for intra-day precision
 287 ranged from 2% to 5%, while the RSD for inter-day precision ranged from 3% to 6%. The EFs were
 288 calculated as shown in Equation 2, and values between 32 and 48 were attained. The values of average
 289 recovery ranged from 79 to 101 % as can be seen in Table 5. The experiment was not carried out
 290 beyond five days of storage, since at this point the changes of the meat's properties can already be
 291 detected using sensory analysis, especially in the case of poultry [23].

292 Table 5. Information on linearity, average recoveries (%), intra-day and inter-day repeatability (%RSD),
 293 limits of detection and limits of quantification obtained with the optimized method in spiked samples,
 294 analyzed by GC-MS ($n = 4$ at each level).

Analyte	Linearity ($\mu\text{g/L}$)	R	Concentration level 0.5 $\mu\text{g/L}$		Inter-day (%RSD)	LOD ($\mu\text{g/g}$)	LOQ ($\mu\text{g/g}$)	EF
			Recovery (%)	Intra-day (%RSD)				
CAD	0.05-10 10-500	0.997	97	4	5	0.003	0.0099	32
DIMET	0.05-10 10-500	0.996	96	5	5	0.004	0.013	48
HIST	0.05-10	0.998	98	2	3	0.006	0.019	42
PUT	0.05-10	0.998	101	3	4	0.005	0.017	38
SPER	0.05-10	0.997	79	4	5	0.009	0.029	33
TRP	0.05-510	0.996	81	3	6	0.007	0.023	35
TYR	0.05-10	0.998	87	4	3	0.007	0.023	42
2-PE	0.05-10	0.996	93	5	5	0.004	0.013	46

LOD, LOQ calculated with respect to the weight of the respective solid matrix

295

296 3.3. Assessment of the noxious impact on the environment using Analytical Eco-Scale and GAPI

297 The concept of Green Analytical Chemistry (GAC) has been introduced to analytical practice due to
 298 concerns connected with a sustainable environment which resulted in a focus being placed on reducing
 299 or completely eliminating the use of solvents and other chemicals which are toxic and hazardous. In
 300 this context, eco-friendly as well as clean practices have been implemented in different fields of
 301 research. As was mentioned previously, the BAs determination in meat samples is mainly carried out
 302 using HPLC after extraction (mainly liquid-liquid extraction) and chemical conversion of analytes, which
 303 are not considered 'green'. The procedure described in this study is based on a micro-scale extraction
 304 technique and GC-MS. To evaluate its 'green' character, the Analytical Eco-Scale and GAPI tools were
 305 applied. In addition, the developed procedure was compared to one based on the ultra-performance
 306 liquid chromatography (UHPC) technique for final determination.

307 The Eco-Scale tool is a semi-quantitative tool, based on assigning penalty points (PPs) to parameters
308 of an analytical process that are not in agreement with an ideal green analysis. It is simple and fast to
309 perform and has well-defined criteria of evaluation. For each analytical protocol, PPs are given if it
310 deviates from desired green parameters which are quantitatively connected to following factors:
311 reagents amount and its hazards, waste production and energy consumption. The fundamental
312 concept of the analytical Eco-Scale is that the ideal green analysis has a value of 100, thus, the closer
313 to the highest score, the greener the procedure [24]. The sum of PPs for the whole evaluated
314 procedure is subtracted from the ideal score of 100 to obtain the Eco-Scale score. The concept of the
315 Analytical Eco-Scale assumes that the score of ≥ 75 represents an excellent green analysis, ≥ 50
316 represents an acceptable green analysis, and < 50 represents inadequate green analysis. Thus,
317 considering PPS given for the described procedure (25 PPs) it can be assumed that it represents a green
318 analysis. The same cannot be said about the reported procedure based on UHPC, where the sum of
319 PPs for the entire methodology is 36 which means that the protocol is merely acceptable in terms of
320 'greenness'. The results of this assessment were confirmed based on the analysis of GAPI pictograms
321 (Figure 3). This index is a 'green' assessment tool of analytical protocols which rates analytical methods
322 against the amount and type of waste, environmental hazard and chemical health, and energy
323 requirements [25]. This tool presents in a pictorial form information on the entire analytical protocol,
324 from sampling, through sample preparation to a final determination.

325 3.4. Analysis of real samples

326 The results of the determination of BAs in samples of pork, beef and poultry are listed in Tables 6-8.
327 These are average values of the results of analysis of five separate samples, each performed in
328 triplicate. They are in agreement with previously reported values [2,5,26,27]. There are noticeable
329 differences between the content of BAs in samples stored in different packaging materials, however,
330 the variance is mostly due to the duration of refrigerated storage. The result of PCA is shown in Figure
331 5. Based on the plot of the two first principal components it can be observed that storing samples in
332 vacuum containers does not produce effects as evident as in the case of modified atmosphere
333 packaging (MAP) [28], although the differences do become more pronounced over time. Based on the
334 cluster analysis (Figure 4), it can be noticed that the BAs can be grouped into three distinct clusters
335 based on the distances between data points in a multi-dimensional space. If only several were to be
336 selected for a meat quality index, they should not be limited to the ones grouped within a single cluster,
337 as this would likely limit the performance of the model.

338

339 The impact of packaging was the greatest in the case of TYR for poultry (AUC 0.720), DIMET and SPER
340 for pork (AUC 0.713) and HIST and SPER for beef (AUC 0.642).

341 In the case of poultry, the four amines which displayed the greatest variance in the terms of storage
342 time were (in decreasing order) CAD, HIST, TYR and PUT which validates the applicability of the BAI
343 index proposed by Veciana-Nogués et al. [29] in chicken meat freshness evaluation. However, perfect
344 classification (AUC 1.000, CA 1.000, precision 1.000) was achieved when using only the concentration
345 values of CAD and HIST as inputs, and the use of CAD alone allowed to obtain a good classification
346 (AUC 0.996, CA 0.903, precision 0.917) which also supports earlier findings [30,31]. It has been
347 suggested that the more rapid increase of the concentration of BAs in poultry meat as compared to

348 pork or beef can be attributed to the presence of shorter protein chains which facilitates the
349 generation of amino acid precursors for their biosynthesis by proteolytic enzymes [30].

350 In pork, the greatest variance during storage was due to the changes in the concentration of CAD, HIST,
351 TYR and 2-PE. All four had to be used as inputs to achieve perfect classification, however, good results
352 were obtained when the amines of the BAI index were considered (AUC 1.000, CA 0.911, precision
353 0.930).

354 Finally, in the case of bovine meat, where the concentration of BAs increased, e.g. poultry, the four
355 best-ranked BAs in terms of variance caused by the duration of storage were CAD, 2-PE, PUT and TRP
356 which allowed for a good classification of samples (AUC 0.967, CA 0.889, precision 0.917).

357 Based on the results of the multivariate statistical analysis it can be assessed that for a general BA-
358 based meat quality index, regardless of the type of sample, the most relevant amines are (in order of
359 decreasing relevance) TRP, CAD, 2-PE and PUT, collectively allowing for a very good classification based
360 on the duration of storage (AUC 0.994, CA 0.941, precision 0.941). A FreeViz projection (linear
361 projection of multivariate data that best separates the instances of a different class [32]) of the entire
362 data set is depicted in Figure 6.

363 Table 6. The concentration of BAs in samples of fresh chicken meat (mg/kg, average±MSE, $n=5$)
364 refrigerated at 4 °C over a period of 5 days in 3 different containers: PP-R food box (I), PP-R vacuum
365 box (II) and an HDPE bag (III)

BA	Container	Day of storage		
		1	3	5
2-PE	I	n.d.	n.d.	0.4506±0.0034
	II	n.d.	n.d.	0.3124±0.0014
	III	n.d.	n.d.	0.5950±0.0015
CAD	I	n.d.	8.706±0.044	10.414±0.048
	II	n.d.	7.818±0.012	9.806±0.047
	III	n.d.	9.150±0.019	11.042±0.032
DIMET	I	0.4828±0.0022	0.4166±0.0030	0.3654±0.0044
	II	0.4840±0.0029	0.4490±0.0052	0.3694±0.0031
	III	0.48140±0.00051	0.3946±0.0021	0.313±0.013
HIST	I	1.4814±0.0046	4.332±0.032	3.806±0.047
	II	1.4800±0.0047	4.114±0.030	3.654±0.037
	III	1.48460±0.00051	5.078±0.048	3.380±0.019
PUT	I	0.9884±0.0019	1.1160±0.0017	1.7958±0.0058
	II	0.9886±0.0018	1.033±0.017	1.5378±0.0052
	III	0.99140±0.00040	1.1498±0.0020	1.977±0.014
SPER	I	14.64±0.14	15.76±0.21	20.56±0.30
	II	14.58±0.14	14.860±0.068	19.620±0.058
	III	14.8±0	16.400±0.084	22.900±0.055
TRP	I	3.044±0.020	2.132±0.014	1.802±0.017
	II	3.038±0.018	2.552±0.030	1.914±0.015
	III	3.0440±0.0075	2.100±0.044	1.184±0.024
TYR	I	n.d.	3.116±0.012	4.100±0.020

II	n.d.	2.418±0.015	3.050±0.038
III	n.d.	4.130±0.011	5.314±0.036

366 Table 7. The concentration of BAs in samples of fresh pork (mg/kg, average±MSE, n=5) refrigerated at
 367 4 °C over a period of 5 days in 3 different containers: PP-R food box (I), PP-R vacuum box (II) and an
 368 HDPE bag (III)

BA	Container	Day of storage		
		1	3	5
2-PE	I	n.d.	n.d.	0.8034±0.0087
	II	n.d.	n.d.	0.495±0.017
	III	n.d.	n.d.	0.8502±0.0017
CAD	I	n.d.	6.330±0.059	8.73±0.11
	II	n.d.	4.870±0.061	7.202±0.033
	III	n.d.	6.768±0.029	9.16±0.11
DIMET	I	0.7790±0.0035	1.328±0.079	3.172±0.090
	II	0.7796±0.0032	1.028±0.024	2.166±0.031
	III	0.7792±0.0036	1.488±0.050	3.806±0.043
HIST	I	1.172±0.027	3.752±0.064	3.786±0.051
	II	1.180±0.028	3.250±0.039	3.390±0.019
	III	1.168±0.031	4.016±0.065	4.056±0.024
PUT	I	n.d.	0.762±0.014	2.254±0.079
	II	n.d.	0.6648±0.0033	1.616±0.035
	III	n.d.	0.9160±0.0021	2.628±0.047
SPER	I	12.34±0.15	10.180±0.086	8.676±0.050
	II	12.40±0.16	9.820±0.073	9.480±0.037
	III	12.38±0.17	9.340±0.040	7.180±0.022
TRP	I	3.346±0.025	4.42±0.11	2.250±0.033
	II	3.330±0.024	3.960±0.053	2.684±0.046
	III	3.312±0.024	4.720±0.062	2.134±0.039
TYR	I	0.2294±0.0027	1.118±0.031	3.280±0.057
	II	0.2328±0.0012	0.9726±0.0026	2.584±0.052
	III	0.2330±0.0021	1.354±0.028	3.406±0.036

369 Table 8. The concentration of BAs in samples of fresh beef (mg/kg, average±MSE, n=5) refrigerated at
 370 4 °C over a period of 5 days in 3 different containers: PP-R food box (I), PP-R vacuum box (II) and an
 371 HDPE bag (III)

BA	Container	Day of storage		
		1	3	5
2-PE	I	n.d.	n.d.	0.2222±0.0078
	II	n.d.	n.d.	0.2134±0.0025
	III	n.d.	n.d.	0.2448±0.0070
CAD	I	n.d.	n.d.	3.468±0.047
	II	n.d.	n.d.	3.228±0.052
	III	n.d.	n.d.	3.684±0.091
DIMET	I	0.61220±0.00020	0.7448±0.0052	0.9808±0.0087

	II	0.61240±0.00024	0.6812±0.0046	0.7880±0.0027
	III	0.61220±0.00020	0.7710±0.0064	1.115±0.040
HIST	I	1.0034±0.0016	1.266±0.046	1.490±0.052
	II	1.0042±0.0015	1.1132±0.0047	1.2156±0.0026
	III	1.0066±0.0016	1.396±0.023	1.630±0.040
PUT	I	n.d.	n.d.	1.522±0.083
	II	n.d.	n.d.	1.096±0.027
	III	n.d.	n.d.	1.716±0.067
SPER	I	27.700±0.063	23.30±0.18	20.68±0.10
	II	27.660±0.060	25.620±0.092	23.500±0.063
	III	27.580±0.058	21.12±0.10	16.82±0.53
TRP	I	6.1220±0.0074	7.582±0.065	8.456±0.047
	II	6.1280±0.0074	7.072±0.029	8.182±0.050
	III	6.1340±0.0060	7.844±0.021	8.814±0.043
TYR	I	0.13080±0.00058	0.2086±0.0065	0.2372±0.0034
	II	0.13100±0.00055	0.1636±0.0025	0.34±0.15
	III	0.13200±0.00063	0.2132±0.0076	0.2572±0.0012

372

373 4. Conclusions

374 The use of the DLLME GC-MS method allows for a relatively simple, rapid and simultaneous
375 determination of BAs in meat products. The efficiency of the procedure for the extraction of BAs from
376 complex meat matrices was confirmed by both, the obtained recovery values and the results of the
377 real samples analysis. The extraction procedure was efficient and highly reproducible. The validation
378 results, namely linearity, recovery, precision and limits of quantification and detection were very
379 satisfactory. The low quantitation limits facilitate the use of BAs concentration values as meat
380 freshness indicators at the early stages of spoilage, before the exponential increase of the
381 concentration of the bacterial metabolites. It can be concluded that the developed procedure is
382 suitable for rapid, reliable and inexpensive determination of BAs in fresh meat samples. Furthermore,
383 it was assessed that tryptamine, cadaverine, 2-phenylethylamine and putrescine should be considered
384 as potential meat freshness indicators when developing freshness indices based on the concentration
385 of BAs.

386 References

- 387 [1] W. Wojnowski, T. Majchrzak, T. Dymerski, J. Gębicki, J. Namieśnik, Electronic noses: Powerful
388 tools in meat quality assessment, *Meat Sci.* 131 (2017) 119–131. doi:10.1016/j.meatsci.2017.04.240.
- 389 [2] J.S. Min, S.O. Lee, A. Jang, C. Jo, C.S. Park, M. Lee, Relationship between the concentration of
390 biogenic amines and volatile basic nitrogen in fresh beef, pork, and chicken meat, *Asian-Australasian*
391 *J. Anim. Sci.* 20 (2007) 1278–1284. doi:10.5713/ajas.2007.1278.
- 392 [3] D. Yang, A. Lu, D. Ren, J. Wang, Rapid determination of biogenic amines in cooked beef using
393 hyperspectral imaging with sparse representation algorithm, *Infrared Phys. Technol.* 86 (2017) 23–34.
394 doi:10.1016/j.infrared.2017.08.013.

- 395 [4] R.G. Leuschner, M. Heidel, W.P. Hammes, Histamine and tyramine degradation by food
396 fermenting microorganisms, *Int. J. Food Microbiol.* 39 (1998) 1–10. doi:10.1016/S0168-
397 1605(97)00109-8.
- 398 [5] C.A. Lázaro, C.A. Conte-Júnior, A.C. Canto, M.L.G. Monteiro, B. Costa-Lima, A.G. da Cruz, E.T.
399 Mársico, R.M. Franco, Biogenic amines as bacterial quality indicators in different poultry meat species,
400 *LWT - Food Sci. Technol.* 60 (2015) 15–21. doi:10.1016/j.lwt.2014.09.025.
- 401 [6] A.R. Shalaby, Significance of biogenic amines to food safety and human health, *Food Res. Int.*
402 29 (1996) 675–690. doi:10.1016/S0963-9969(96)00066-X.
- 403 [7] J.L. Mietz, E. Karmas, Chemical quality index of canned tuna as determined by high-pressure
404 liquid chromatography, *J. Food Sci.* 42 (1977) 155–158. doi:10.1111/j.1365-2621.1977.tb01240.x.
- 405 [8] M.T. Veciana-Nogués, A. Mariné-Font, M.C. Vidal-Carou, Biogenic amines as hygienic quality
406 indicators of tuna. relationships with microbial counts, atp-related compounds, volatile amines, and
407 organoleptic changes, *J. Agric. Food Chem.* 45 (1997) 2036–2041. doi:10.1021/jf960911i.
- 408 [9] C.M. Silva, M.B.A. Glória, Bioactive amines in chicken breast and thigh after slaughter and
409 during storage at 4±1 °C and in chicken-based meat products, *Food Chem.* 78 (2002) 241–248.
410 doi:10.1016/S0308-8146(01)00404-6.
- 411 [10] F.B. Erim, Recent analytical approaches to the analysis of biogenic amines in food samples,
412 *TrAC - Trends Anal. Chem.* 52 (2013) 239–247. doi:10.1016/j.trac.2013.05.018.
- 413 [11] J. Płonka, Food analysis - Samples preparation and chromatographic methods in determination
414 of selected biogenic amines, methylxanthines and water-soluble vitamins, *Anal. Methods.* 4 (2012)
415 3071–3094. doi:10.1039/c2ay25706h.
- 416 [12] E. Dadáková, M. Křížek, T. Pelikánová, Determination of biogenic amines in foods using ultra-
417 performance liquid chromatography (UPLC), *Food Chem.* 116 (2009) 365–370.
418 doi:10.1016/J.FOODCHEM.2009.02.018.
- 419 [13] J. Płotka-Wasyłka, V. Simeonov, J. Namieśnik, An in situ derivatization - dispersive liquid-liquid
420 microextraction combined with gas-chromatography - mass spectrometry for determining biogenic
421 amines in home-made fermented alcoholic drinks, *J. Chromatogr. A.* 1453 (2016) 10–18.
422 doi:10.1016/j.chroma.2016.05.052.
- 423 [14] A. Gałuszka, Z. Migaszewski, J. Namieśnik, The 12 principles of green analytical chemistry and
424 the SIGNIFICANCE mnemonic of green analytical practices, *TrAC Trends Anal. Chem.* 50 (2013) 78–84.
425 doi:10.1016/J.TRAC.2013.04.010.
- 426 [15] M. Kamankesh, A. Mohammadi, H. Hosseini, Z. Modarres Tehrani, Rapid determination of
427 polycyclic aromatic hydrocarbons in grilled meat using microwave-assisted extraction and dispersive
428 liquid-liquid microextraction coupled to gas chromatography-mass spectrometry, *Meat Sci.* 103 (2015)
429 61–67. doi:10.1016/J.MEATSCI.2015.01.001.

- 430 [16] C. Almeida, J.O. Fernandes, S.C. Cunha, A novel dispersive liquid–liquid microextraction
431 (DLLME) gas chromatography-mass spectrometry (GC–MS) method for the determination of eighteen
432 biogenic amines in beer, *Food Control*. 25 (2012) 380–388. doi:10.1016/J.FOODCONT.2011.10.052.
- 433 [17] H. Chen, H. Chen, J. Ying, J. Huang, L. Liao, Dispersive liquid–liquid microextraction followed by
434 high-performance liquid chromatography as an efficient and sensitive technique for simultaneous
435 determination of chloramphenicol and thiamphenicol in honey, *Anal. Chim. Acta*. 632 (2009) 80–85.
436 doi:10.1016/J.ACA.2008.10.068.
- 437 [18] J. Płotka-Wasyłka, V. Simeonov, J. Namieśnik, Characterization of home-made and regional
438 fruit wines by evaluation of correlation between selected chemical parameters, *Microchem. J.* 140
439 (2018) 66–73. doi:10.1016/J.MICROC.2018.04.010.
- 440 [19] J. Płotka-Wasyłka, V. Simeonov, J. Namieśnik, Evaluation of the Impact of Storage Conditions
441 on the Biogenic Amines Profile in Opened Wine Bottles, *Molecules*. 23 (2018) 1130.
442 doi:10.3390/molecules23051130.
- 443 [20] J. Demšar, T. Curk, A. Erjavec, T. Hočevar, M. Milutinovič, M. Možina, M. Polajnar, M. Toplak,
444 A. Starič, M. Stajdohar, L. Umek, L. Zagar, J. Zbontar, M. Zitnik, B. Zupan, Orange: Data Mining Toolbox
445 in Python, *J. Mach. Learn. Res.* 14 (2013) 23492353.
- 446 [21] K. Kira, L. Rendell, The Feature Selection Problem: Traditional Methods and a New Algorithm,
447 in: 10th Natl. Conf. Artif. Intell., AAAI Press, San Jose, 1992: pp. 129–134.
448 <https://dl.acm.org/citation.cfm?id=1867155> (accessed June 19, 2018).
- 449 [22] A. Todorov, An Overview of the RELIEF Algorithm and Advancements, in: M. Windle (Ed.), *Stat.*
450 *Approaches to Gene X Environ. Interact. Complex Phenotypes*, MIT Press, 2016.
- 451 [23] W. Wojnowski, T. Majchrzak, T. Dymerski, J. Gębicki, J. Namieśnik, Poultry meat freshness
452 evaluation using electronic nose technology and ultra-fast gas chromatography, *Monatshefte Für*
453 *Chemie - Chem. Mon.* 148 (2017) 1631–1637. doi:10.1007/s00706-017-1969-x.
- 454 [24] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical Eco-Scale for assessing
455 the greenness of analytical procedures, *TrAC Trends Anal. Chem.* 37 (2012) 61–72.
456 doi:10.1016/J.TRAC.2012.03.013.
- 457 [25] J. Płotka-Wasyłka, A new tool for the evaluation of the analytical procedure: Green Analytical
458 Procedure Index, *Talanta*. 181 (2018) 204–209. doi:10.1016/J.TALANTA.2018.01.013.
- 459 [26] N. Sayem, E. Daher, R.E. Simard, Putrefactive Amine Changes in Relation to Microbial Counts
460 of Ground Beef During Storage, *J. Food Prot.* 48 (1985) 54–58.
- 461 [27] F. Galgano, F. Favati, M. Bonadio, V. Lorusso, P. Romano, Role of biogenic amines as index of
462 freshness in beef meat packed with different biopolymeric materials, *Food Res. Int.* 42 (2009) 1147–
463 1152. doi:10.1016/J.FOODRES.2009.05.012.
- 464 [28] C.C. Balamatsia, E.K. Paleologos, M.G. Kontominas, I.N. Savvaïdis, Correlation between
465 microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored

466 aerobically or under modified atmosphere packaging at 4 °C: Possible role of biogenic amines as
467 spoilage indicators, *Anton. Leeuw. Int. J. G.* 89 (2006) 9–17. doi:10.1007/s10482-005-9003-4.

468 [29] M.T. Veciana-Nogués, A. Mariné-Font, M.C. Vidal-Carou, Biogenic amines as hygienic quality
469 indicators of tuna. relationships with microbial counts, atp-related compounds, volatile amines, and
470 organoleptic changes, *J. Agric. Food Chem.* 45 (1997) 2036–2041. doi:10.1021/jf960911l.

471 [30] G. Vinci, M.L. Antonelli, Biogenic amines: Quality index of freshness in red and white meat,
472 *Food Control.* 13 (2002) 519–524. doi:10.1016/S0956-7135(02)00031-2.

473 [31] W. Wojnowski, J. Płotka-Wasyłka, K. Kalinowska, T. Majchrzak, T. Dymerski, P. Szweda, J.
474 Namieśnik, Direct determination of cadaverine in the volatile fraction of aerobically stored chicken
475 breast samples, *Monatshefte Für Chemie - Chem. Mon.* 149 (2018) 1521-1525. doi:10.1007/s00706-
476 018-2218-7.

477 [32] J. Demšar, G. Leban, B. Zupan, FreeViz—An intelligent multivariate visualization approach to
478 explorative analysis of biomedical data, *J. Biomed. Inform.* 40 (2007) 661–671.
479 doi:10.1016/J.JBI.2007.03.010.

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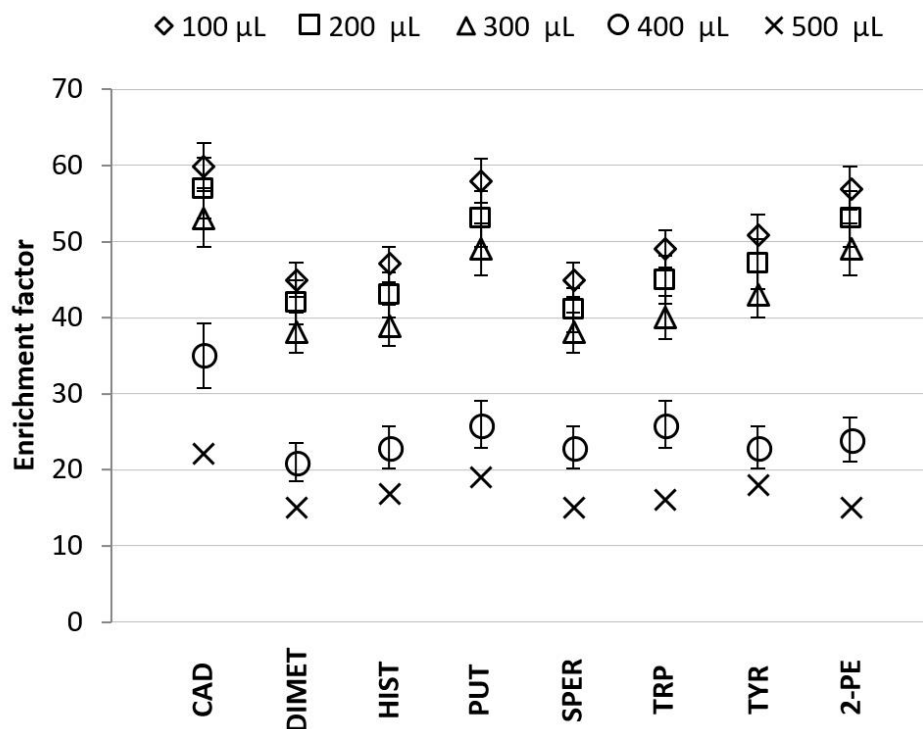
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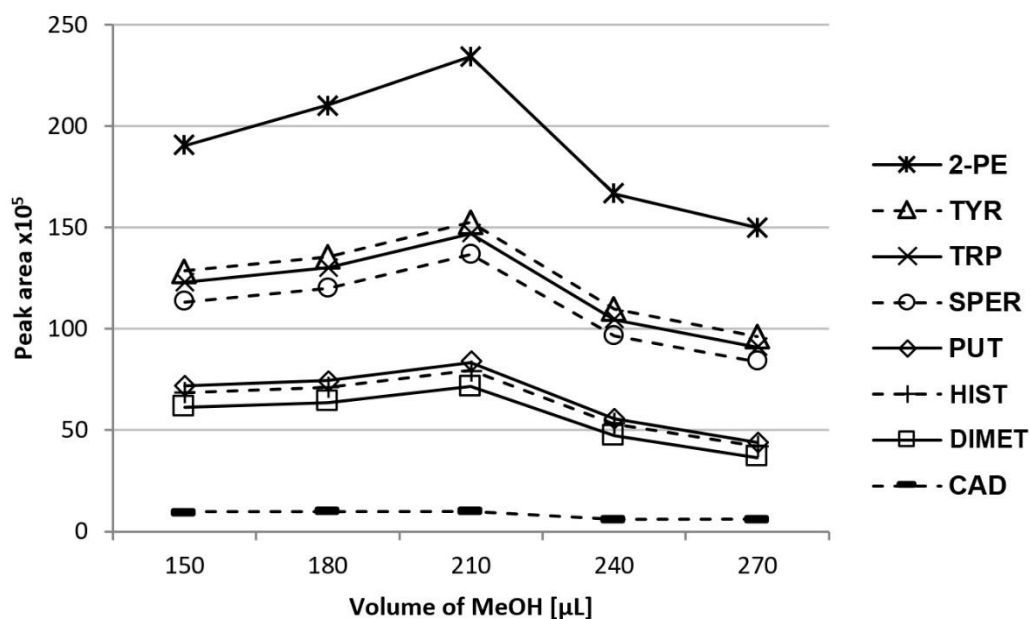
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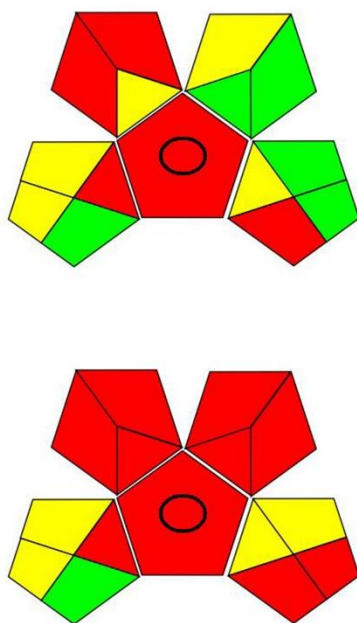
Fig. 1. Enrichment factors obtained using different volumes of extractive solvent, i.e. chloroform (mean value; $n = 3$; RSD < 3.3%).



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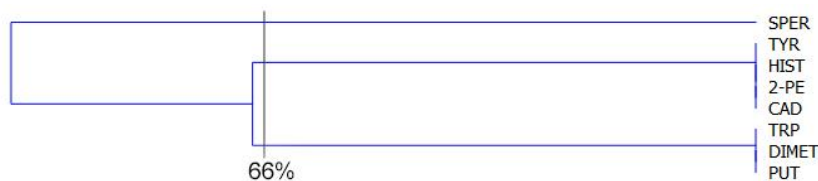
Fig. 2. Impact of the volume of methanol on the peak area of BAs by DLLME-GC-MS.

DLLME-GC-MS	
Reagents	PPs
Pyridine (55 µL)	1
HCl (55 µL)	3
Chloroform (1 mL)	2
Isobutyl chloroformate (200 µL)	6
MeOH (600 µL)	6
NaOH (5 mL)	1
NaCl	0
	Σ 19
Instruments	PPs
Transport	1
GC-MS	2
Occupational hazard	0
Waste	3
	Σ 6
Total PPs: 25	
Score: 75	
UPLC-UV	
Reagents	PPs
Acetonitrile (1.5 mL)	4
Dansyl chloride (2 mL)	8
Proline solution (200 µL)	1
Heptane (3 mL)	8
Acetone (3 mL)	4
Na ₂ CO ₃ (0.75 mL)	0
NaHCO ₃ (0.75 mL)	0
K ₂ CO ₃ (1 mL)	0
HClO ₄ (<100 mL)	2
	Σ 27
Instruments	PPs
Transport	1
UPLC-UV	2
Occupational hazard	1
Waste	5
	Σ 9
Total PPs: 36	
Score: 64	



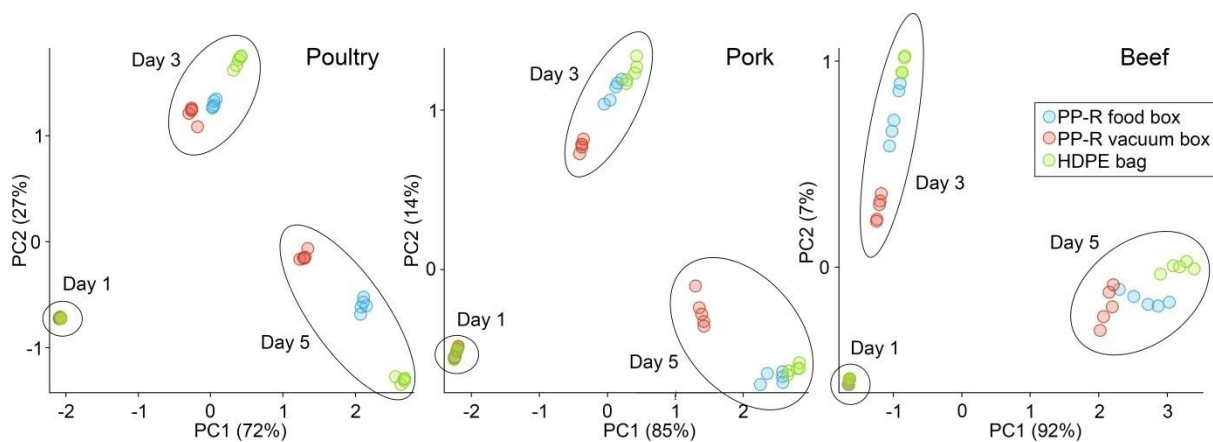
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510 Fig. 3. The penalty points (PPs) for BAs determination in meat samples by in-situ derivatization coupled
 511 to DLLME-GC-MS procedure reported in this study and in a different reported procedure [12].



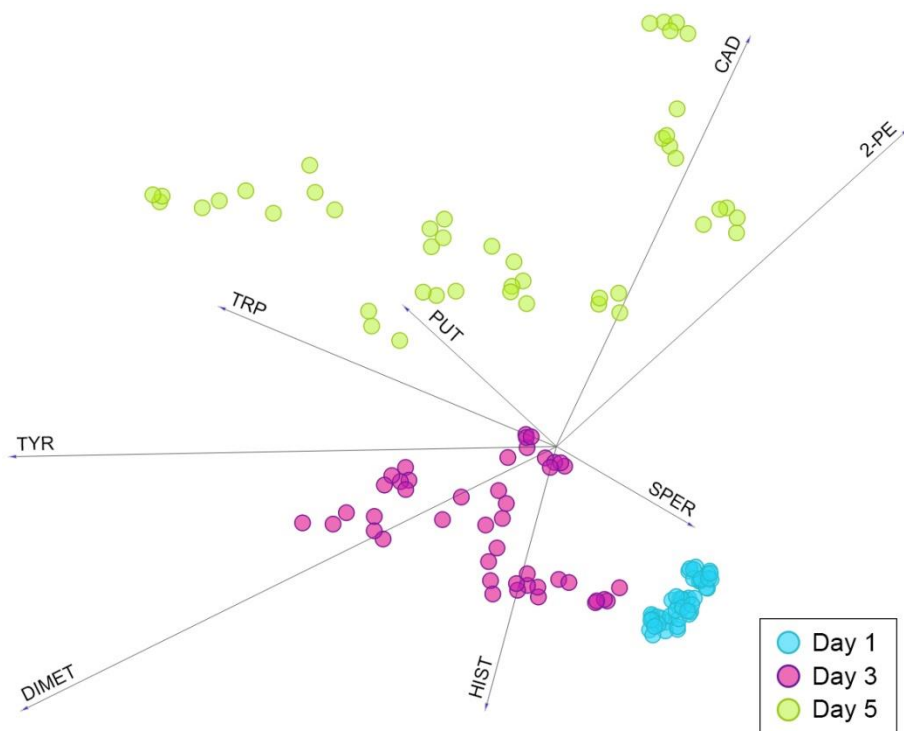
512

513 Fig. 4. Hierarchical cluster analysis of the variables used in the data analysis



514

515 Fig. 5. Principal component analysis of the concentration values of BAs in meat samples according to
 516 the duration of refrigerated storage and packaging material. In all 3 cases, the first two principal
 517 components cover 99 % of the total variance.



518

519 Fig. 6. Linear projection of the variables in the classification of poultry, pork and bovine meat based on
 520 the duration of refrigerated storage.

521

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