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THE EFFECT OF SURFACE MOULD APPLICATION TO SELECTED PROPERTIES OF DRY FERMENTED SAUSAGES

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ABSTRACT

Three batches of dry fermented sausages were prepared. A proportion of the products from each batch were surface-treated with a mould starter culture, with the remaining products being smoked without mould. Physico-chemical analysis, including determination of the content of lactic acid and biogenic amines, was performed on day 35 during the ripening and on the final products (day 65). The sausages with surface mould showed a higher pH value, a higher water activity value and a lower content of D/L-lactic acid. The differences were statistically significant ($P \leq 0.001$). A higher content of malondialdehyde (TBARS) was found in products with mould, though the differences determined were not statistically significant. From day 35, statistically significant differences were found in the content of biogenic amines (BA). The highest content was recorded on day 65 in sausages with surface mould, with a content seven times that of the content in the mix immediately after being filled in the casing being recorded. In no case did the sum level of BA exceed 100 mg kg^{-1}.

Keywords: Mould culture, pH value, water activity, lactic acid, biogenic amines, TBARS

INTRODUCTION

Dry fermented sausages with surface mould are traditional meat products in a number of countries of southern Europe, such as Spain, Italy, France and Romania (**Bruna *et al.*, 2000; Sunesen and Stahnke, 2003**). In Italy and France, more than 90 % of fermented sausages are not smoked, and their surface becomes covered with mould during the ripening (**Buckenhüskes, 1994**). These products have been made in the Czech Republic since the middle of the nineteen nineties and are becoming increasingly popular with consumers.
The mould plays an important role during the ripening of dry sausages. Their enzymes influence the product in many ways. They have a generally positive action on the sensory properties of the product (**Bruna *et al.*, 2003**), though they may have a negative effect on the product under certain circumstances (**Sunesen and Stahnke, 2003**). The positive effects of mould mycelia include prevention of excessive drying of the product, the protection of fat against oxidation, colour stability, a marked effect on aroma and taste (the proteolytic and lipolytic effects of mould) and the easier peeling of the casing (**Spotti and Berni, 2007**). Mould extracellular proteases and lipases release amino acids and fatty acids which serve as precursors of volatile compounds having an effect on the aroma of the final product (**Bruna *et al.*, 2001**).
Amino acids are, however, also precursors of biogenic amines (BA) in foodstuffs (**Bover-Cid *et al.*, 1999**). Biogenic amines are formed largely by the action of microbial decarboxylases on free amino acids, and the fermentation of sausages therefore creates a favourable environment for the formation of BA (**Toledo *et al.*, 1997; Ansorena *et al.*, 2002**). In view of the increased proteolytic activity caused by the action of surface moulds, an increased amount of amino acids is released in these products which may potentiate the formation of BA. On the other hand, the aminogenic activity of microorganisms is supported by organic acids, since bacteria release BA as a means of defence against an acidic environment and BA serve to maintain intracellular homeostasis (**Bover-Cid *et al.*, 2008**). The decarboxylation of amino acids is stronger in an acidic environment (**Gençcelep *et al.*, 2007; Pircher *et al.*, 2007**).
In the case of mould sausages, the metabolic activity of surface moulds increases the pH value (**Sunesen and Stahnke, 2003**). The proteolysis here results in a higher concentration of ammonia which reduces the acidity of the environment. Surface moulds also metabolise lactic acid, the content of which in the mix falls, which also leads to an increase in the pH value (**Lücke, 1985**). The specialised literature includes works that describe the influence of moulds on the formation of BA in dry fermented sausages (**Ansorena *et al.*, 2002; Bruna *et al.*, 2003**), though they are restricted to sausages of the Spanish or Italian type, often prepared under laboratory conditions. The influence of mould cultures on the formation of BA in mould sausages in the Czech Republic produced under industrial conditions has not been described to date.
The aim of this work was to determine the influence of surface mould on the physico-chemical properties of dry fermented sausages under Czech conditions and to compare the difference in the content of biogenic amines in final products produced using traditional technologies (smoking) with that in products with surface mould.

MATERIAL AND METHODS

The production of dry fermented sausages

Three batches of sausages of 160 kg each were prepared in a production plant with a capacity of around 200 tons of dry fermented sausages a month. The 3 batches differed in terms of the type and amount of lean meat used:

1. batch 1: 72 kg pork shoulder; 40 kg sow leg; 45 kg back fat
2. batch 2: 0 kg pork shoulder; 107 kg sow leg; 50 kg back fat
3. batch 3: 122 kg pork shoulder; 35 kg back fat

A blend of seasoning containing dextrose, a starter culture (CAX 28, Cargill, France; composition: *Staphylococcus carnosus, Staphylococcus xylosus, Lactobacillus sakei*) and a nitrite salting mixture were added to the sausage mix. The mix was ground to a grain size of 2 – 3 mm and filled in collagen casings of a diameter of 65 mm. A proportion of the products were placed in an air-conditioned smoking chamber in which smoking with cold smoke took place over the course of the first five days. The microclimatic parameters were set to 24°C and 93% relative air humidity, with a gradual fall to a final 17°C and 80% (from day 8 of the beginning of production). The remaining products were placed in another air-conditioned chamber. On the second day after filling, the products were submerged in a suspension of mould spores (starter culture PV 7.1., Cargill,

France; composition: *Penicillium chrysogenum*). Subsequent ripening took place under the same microclimatic conditions as for the smoked sausages.
Samples were taken immediately after the casings were filled (the sausage mixture) and then on days 35 and 65 from all 3 batches. 2 pieces of sausage of 1 kg were considered a sample.

Dry matter, fat, salt, collagen and protein analysis

A drying method (**ISO 1442, 1997**) at 103±2°C for a period of 24 hours was used for the determination of the content of dry matter. The samples were weighed after cooling and the content of dry matter was calculated. The fat content was determined using a SOXTEC instrument (TECATOR, Sweden). Diethyl ether was used as the extraction agent. Samples of a weight of 3 g were left in the drier for 3 hours at 135±2°C and extracted by the agent (diethyl ether) in the instrument for 3 hours. The collagen content was determined spectrophotometrically at a wavelength of 550 nm in a GENESYS^{TM} 6 spectrophotometer (Thermo Electron Corporation, USA) as the quantity of 4-hydroxyproline. The content of hydroxyproline was obtained from the calibration curve and converted into the collagen content. The content of pure muscle protein was calculated as the difference in the content of pure protein and collagen. Pure proteins were determined following the precipitation of non-protein N-substances by hot tannin and subsequent conversion of organic nitrogen to inorganic nitrogen in a KJEHLTEC instrument (TECATOR, Sweden) by the Kjeldahl method. A factor of 6.25 was used for the conversion of the nitrogen content into the protein content. 2 g of sample was weighed and covered with hot water for determination of the salt content. 1 ml of the agent K_2CrO_4 (5 g in 100 ml) was added after thirty minutes and titrated with $AgNO_3$ (29.6 g in 1 l) until a permanent change in colour was attained (yellow – orange).

pH values and water activity

pH values were measured with a Double Pore needle probe (Hamilton, Switzerland) on a 340i WTW pH-meter (WTW, Germany). The water activity was determined in a Novasina LabMaster (Novasina, Switzerland). The sample was fine cut with a knife and placed in a plastic measuring vessel. The water activity was measured at 25°C in the tempered measuring space of the instrument.

Lipid oxidation

The degree of lipid oxidation was measured by reaction with thiobarbituric acid after distillation – TBARS value (thiobarbituric acid reactive substance similarly to **Castellini *et al.* (2002)**. Malonaldehyde was distilled in duplicate from the sample and its absorbance determined at 532 nm in a 1-cm glass cell.

Lactic acid and acetic acid determination

D/L lactic acid was determined using an enzymatic test kit (MEGAZYME, Ireland). The enzyme L(+)- or D(−)-lactate dehydrogenase catalysed the oxidation of L(+)- or D(−)-lactate in the presence of nicotinamide adenine dinucleotide (NAD^+), and the product, pyruvate, was trapped by the conversion of pyruvate to D-alanine and 2-oxoglutarate, with the enzyme D-glutamate-pyruvate transaminase (D-GPT) in the presence of a large excess of D-glutamate, while the NADH formed was quantified by measuring the absorbance at 340 nm (MEGAZYME, 2011).

Determination of biogenic amine (BA) and polyamine (PA) content

BA and PA content were examined in the samples taken on days 35 and 65 of storage. Biogenic amines (tryptamine, 2-phenylethylamine, cadaverine, histamine and tyramine) and polyamines (putrescine, spermidine and spermine) were determined by the method of **Paulsen *et al.* (1997)**. Biogenic amines and polyamines were extracted from the food matrix with 10 % trichloracetic acid and subsequently detected as dansyl derivates. Analysis was performed by the RP-HPLC method with gradient elution and fluorescence detection; histamine was determined on a PDA detector.
The samples were analysed on an Alliance 2695 chromatograph (Waters, USA) with PDA 2996 and fluorescence 2495 detectors using a Zorbax Eclipse XDB C18, 150 x 4.6 mm, 5 µm chromatographic column (Agilent, USA). Gradient elution at a flow rate of 1 ml min^{-1} was used. Mobile phase A consisted of a mixture of 0.1M acetic acid and acetonitrile (90:10), mobile phase B of a mixture of 0.1M acetic acid, acetonitrile and methanol (10:45:45). The injection volume was 10 µl. Fluorescence detection was performed at $\lambda_{ex}/\lambda_{em}$= 330/500 nm, detection within the UV region at 254 nm. Each sample was analysed in at least two parallel tests, with a blank sample in each series. The separated biogenic amines and polyamines were identified and quantified by an external standard method. Measurements were evaluated with the help of Empower 2 software (Waters, USA). The method was validated using EffiValidation software (Effichem, Czech Republic).

Instrumental measurement of colour

Colour was measured by the CIE L*a*b* system using a Minolta CM 2600d (Konica Minolta, Japan). A measuring area of 3 mm, illuminant D65 and 10° standard observer were used. The instrument was standardised using a standard white plate. CIE L* – lightness, a* – redness, b* – yellowness. The structure of sausages was created by fat part and muscle (meat) part. Colour was measured on muscle (meat) part of sausages.

Instrumental measurement of texture

Samples were tested by Texture Profile Analysis (TPA) using an Instron Universal Testing Machine (model 5544) (Instron Corporation, England). Parameters were obtained using available computer software (Merlin). For a TPA cylinder, samples (2 cm high, 2.5 cm in diameter) were compressed twice to 50% of their original height with a compression platen 36 mm in diameter. Force time curves were recorded at a crosshead speed of 50 mm. min^{-1}. Hardness (N) – the peak force required for the first compression was evaluated (**Szczesniak, 2002; Desmond and Kenny, 2005**).

Statistical analysis

Statistical data analyses were conducted using the statistical program *STATISTICA 7 CZ* (StatSoft, Prague, Czech Republic). ANOVA was used for the determination of variability among the groups of samples. Significance levels of 0.05, 0.01 and 0.001 were used.

RESULTS AND DISCUSSION

The results of the physico-chemical analyses

Tables 1 and 2 show the results of the physico-chemical tests conducted on samples of smoked sausages and sausages with surface mould application. It is clear that the smoked sausages lost more water during the course of drying than the sausages with mould. After the first month, the average water loss amounted to 24.2% in sausages without mould as opposed to 15.9% for sausages with mould. After 65 days, the difference between the two groups was not so pronounced (below the level of statistical significance – see table 5), with the overall average water loss amounting to 29.3% in the first group and 25.8% for the products with mould. The fall in the values of water activity during the course of two months of ripening also corresponded to this. From an initial average of 0.97 (freshly filled mix), the average a_w value after 65 days amounted to 0.83 in smoked sausages as opposed to just 0.88 in products with mould.
The average initial pH measured in the mix of the three batches prepared was 5.71. While in sausages without surface mould application a fall to 4.96 was seen during ripening (after 35 days), followed by a slight increase to an average of 5.06 on day 65, a completely different story was seen during the course of fermentation in the sausages with mould. The average pH in sausages with mould increased to 6.34 after 35 days and to 6.26 after two months. The differences in the pH between smoked sausages and products with mould were highly statistically significant on both day 35 and day 65 ($P \leq 0.001$). The content of lactic acid measured corresponded to the pH values. An average content of both isomers of 123.7 µmol.g^{-1} of dry matter was found in the sausage mix. In smoked sausages without surface mould the content of lactic acid showed a pronounced increase during ripening. After a month, the average level in all the test batches amounted to 259.2 µmol.g^{-1} of dry matter, which represented almost twice that of the content in the sausage mix. The content of both isomers, which attained approximately equal proportions, increased. With the exception of batch 2, the level of lactic acid also showed an increase after 65 days, its average value amounting to 266.8 µmol.g^{-1} of dry matter.
In contrast, the average lactic acid content in sausages with mould fell during the ripening process to less than half the initial level in the sausage mix after 35 days (49.0 µmol.g^{-1} of dry matter). It is interesting to note that the content of the L isomer fell, while the level of D-lactic acid rose. After two months, the average values for D/L lactic acid were almost identical (50.6 µmol.g^{-1} of dry matter). As can be seen in table 5, the differences in the content of lactic acid between the two groups of products (smoked sausages and sausages with mould) after 35 and 65 days were highly statistically significant ($P \leq 0.001$).
No statistically significant differences between the two types of products could be demonstrated for the content of malondialdehyde (TBARS), although the sausages with mould showed a higher content than the smoked products.

The results of the instrumental analysis

The results of texture and colour are shown in Table 3. The TPA values were higher in sausages without surface mould; the differences between the two groups were statistically highly significant after 35 and 65 days. The instrumental assessment of colour showed statistically significant differences ($P \leq 0.01$) between the two groups after 65 days for lightness L* (average value 57.5 for sausages with mould and 50.2 for smoked sausages). No differences at the level

of statistical significance were found in the parameters a* and b* between the products with and without mould after 2 months of ripening.

Table 1 Chemical parameters of quality of three batches of sausages with and without mould case application

sample		dry matter [%]	fat [%]	NaCl [%]	collagen [%]	PMP [%]	TBARS [mg.kg^{-1}]
mixture	**1**	50.08 ± 0.57	30.78 ± 0.89	1.17 ± 0.10	1.32 ± 0.10	12.04 ± 0.44	1.05 ± 0.25
	2	48.02 ± 0.36	28.13 ± 1.91	1.49 ± 0.09	1.42 ± 0.11	11.73 ± 0.69	0.95 ± 0.16
	3	44.85 ± 0.03	26.31 ± 0.34	1.69 ± 0.19	1.39 ± 0.13	12.84 ± 0.81	0.96 ± 0.14
with mould 35. day	**1**	65.51 ± 0.33	40.02 ± 1.97	2.93 ± 0.18	1.84 ± 0.14	15.59 ± 0.40	42.93 ± 9.85
	2	62.81 ± 0.43	37.78 ± 2.56	2.85 ± 0.13	1.97 ± 0.03	16.11 ± 1.01	13.86 ± 0.89
	3	62.25 ± 0.47	34.93 ± 1.26	2.98 ± 0.10	1.89 ± 0.05	16.20 ± 0.56	3.55 ± 0.61
with mould 65. day	**1**	73.85 ± 1.63	45.18 ± 0.63	3.56 ± 0.01	1.95 ± 0.26	17.22 ± 0.31	3.34 ± 0.81
	2	73.95 ± 1.64	42.57 ± 1.38	3.50 ± 0.06	2.29 ± 0.04	18.71 ± 0.66	2.59 ± 0.40
	3	72.48 ± 1.63	42.62 ± 1.17	3.98 ± 0.09	2.21 ± 0.07	18.19 ± 0.62	3.03 ± 0.34
without mould 35. day	**1**	71.59 ± 0.25	46.09 ± 1.28	2.95 ± 0.13	1.89 ± 0.12	16.70 ± 0.35	1.61 ± 0.08
	2	72.18 ± 0.25	40.86 ± 1.89	3.22 ± 0.03	2.23 ± 0.04	17.92 ± 0.93	1.31 ± 0.02
	3	71.48 ± 0.40	39.33 ± 1.72	3.59 ± 0.09	2.18 ± 0.12	18.80 ± 0.81	1.52 ± 0.19
without mould 65. day	**1**	77.40 ± 1.16	46.46 ± 2.31	3.44 ± 0.03	1.85 ± 0.05	17.84 ± 0.16	1.35 ± 0.40
	2	77.38 ± 1.47	46.13 ± 1.07	3.70 ± 0.20	1.99 ± 0.05	18.58 ± 0.81	1.01 ± 0.22
	3	76.24 ± 1.23	40.47 ± 0.88	4.03 ± 0.88	2.05 ± 0.08	18.10 ± 0.45	1.16 ± 0.15

Values are given as mean ± standard deviation
TBARS – thiobarbituric acid reactive substance
PMP – pure muscle proteins

Table 2 pH and aw values and lactic acid concentration of three batches of sausages with and without mould

sample		pH	aw	D-lactic acid [µmol.g^{-1} dry matter]	L-lactic acid [µmol.g^{-1} dry matter]
mixture	**1**	5.64 ± 0.00	0.971 ± 0.002	4.27 ± 0.06	127.23 ± 1.77
	2	5.77 ± 0.02	0.974 ± 0.001	11.12 ± 0.10	103.00 ± 0.93
	3	5.72 ± 0.00	0.973 ± 0.002	3.97 ± 0.01	121.40 ± 0.07
with mould 35. day	**1**	6.11 ± 0.01	0.927 ± 0.003	31.48 ± 0.19	44.51 ± 0.26
	2	6.52 ± 0.02	0.941 ± 0.002	26.52 ± 0.07	11.85 ± 0.03
	3	6.40 ± 0.03	0.935 ± 0.003	27.50 ± 0.24	5.16 ± 0.05
with mould 65. day	**1**	6.04 ± 0.07	0.869 ± 0.003	31.34 ± 0.08	43.09 ± 0.11
	2	6.45 ± 0.03	0.876 ± 0.002	22.99 ± 0.01	12.23 ± 0.00
	3	6.30 ± 0.03	0.887 ± 0.002	13.92 ± 0.16	28.33 ± 0.33
without mould 35. day	**1**	4.93 ± 0.02	0.898 ± 0.002	121.19 ± 0.47	120.70 ± 0.46
	2	4.97 ± 0.00	0.898 ± 0.001	121.22 ± 0.48	145.37 ± 0.57
	3	4.98 ± 0.02	0.894 ± 0.000	146.40 ± 0.38	122.58 ± 0.32
without mould 65. day	**1**	5.04 ± 0.01	0.836 ± 0.003	117.61 ± 0.14	132.49 ± 0.16
	2	5.05 ± 0.01	0.834 ± 0.002	117.52 ± 0.12	139.43 ± 0.15
	3	5.08 ± 0.01	0.829 ± 0.002	136.46 ± 0.57	156.77 ± 0.65

Values are given as mean ± standard deviation

Table 3 Parameters of instrumental analysis of three batches of sausages with and without mould

sample		TPA [N]	L*	a*	b*
mixture	**1**	–	52.78 ± 1.66	14.90 ± 0.43	12.37 ± 0.36
	2	–	53.12 ± 3.71	15.85 ± 1.78	14.59 ± 0.92
	3	–	53.88 ± 1.04	14.58 ± 0.16	14.56 ± 0.58
with mould 35. day	**1**	13.95 ± 0.69	54.42 ± 2.90	12.43 ± 0.90	8.43 ± 0.98
	2	12.23 ± 0.94	55.01 ± 3.68	13.22 ± 1.14	9.50 ± 0.32
	3	10.98 ± 1.14	56.42 ± 2.89	11.67 ± 1.41	10.33 ± 1.01
with mould 65. day	**1**	14.72 ± 1.99	59.80 ± 2.81	10.74 ± 0.70	6.28 ± 0.51
	2	15.79 ± 0.93	53.72 ± 2.07	13.20 ± 2.14	7.39 ± 1.58
	3	14.27 ± 2.99	58.89 ± 0.92	10.90 ± 0.92	7.31 ± 1.02
without mould 35. day	**1**	30.37 ± 5.75	55.75 ± 1.57	12.30 ± 0.81	6.84 ± 1.00
	2	41.43 ± 8.49	53.97 ± 1.86	13.57 ± 1.30	7.07 ± 1.07
	3	40.37 ± 5.24	56.29 ± 2.30	11.63 ± 1.56	6.34 ± 0.83
without mould 65. day	**1**	45.34 ± 9.66	50.07 ± 2.66	12.54 ± 1.56	7.10 ± 0.64
	2	50.47 ± 5.44	52.60 ± 3.61	12.60 ± 1.82	7.46 ± 1.98
	3	55.23 ± 8.24	47.96 ± 1.45	13.54 ± 1.42	7.36 ± 1.42

Values are given as mean ± standard deviation
TPA – texture profile analysis
L* – lightness, a* – redness, b* – yellowness

Table 4 Concentration of eight biogenic amines and polyamines of three batches of sausages with and without mould (mg.kg^{-1})

sample		tryptamine	2-phenylethyl-amine	putrescine	cadaverine	histamine	tyramine	spermidine	spermine	total BA 8
mixture	**1**	< *LOD*	< *LOD*	6.21 ± 1.80	0.03 ± 0.01	0.68 ± 0.11	0.71 ± 0.29	0.35 ± 0.08	2.55 ± 0.40	**10.5**
	2	< *LOD*	< *LOD*	6.15 ± 3.13	2.18 ± 6.14	0.42 ± 0.17	0.50 ± 0.65	0.21 ± 0.14	1.60 ± 0.64	**11.1**
	3	< *LOD*	1.40 ± 0.36	7.09 ± 2.01	0.04 ± 0.02	0.54 ± 0.19	1.05 ± 0.26	0.25 ± 0.11	1.86 ± 0.95	**12.2**
with mould 35. day	**1**	18.87 ± 3.69	1.98 ± 0.68	16.46 ± 1.84	0.73 ± 0.13	1.10 ± 0.40	21.27 ± 3.04	0.41 ± 0.29	1.93 ± 0.40	**62.7**
	2	11.12 ± 5.68	0.68 ± 0.75	17.03 ± 7.60	0.84 ± 0.36	0.21 ± 0.41	23.12 ± 10.72	0.29 ± 0.14	1.68 ± 0.81	**55.0**
	3	13.39 ± 3.10	0.50 ± 0.28	16.01 ± 2.95	0.71 ± 0.17	0.10 ± 0.02	20.31 ± 5.17	0.36 ± 0.22	1.83 ± 0.46	**53.2**
with mould 65. day	**1**	10.66 ± 3.07	7.79 ± 1.91	16.93 ± 2.95	1.54 ± 0.38	1.25 ± 0.19	18.96 ± 5.31	0.67 ± 0.54	0.98 ± 0.27	**58.8**
	2	13.92 ± 4.33	6.30 ± 0.99	36.34 ± 0.71	1.76 ± 0.18	0.18 ± 0.05	27.76 ± 0.89	< *LOD*	< *LOD*	**86.3**
	3	19.02 ± 8.94	11.44 ± 5.61	37.53 ± 2.23	1.96 ± 0.65	0.19 ± 0.65	24.22 ± 0.98	< *LOD*	< *LOD*	**94.4**
without mould 35. day	**1**	7.61 ± 0.59	1.03 ± 0.14	5.13 ± 0.89	0.08 ± 0.01	0.06 ± 0.01	5.84 ± 1.12	0.49 ± 0.12	3.11 ± 0.97	**23.4**
	2	1.79 ± 0.32	0.54 ± 0.16	6.53 ± 0.95	0.08 ± 0.02	0.12 ± 0.01	10.60 ± 2.29	0.42 ± 0.13	2.71 ± 0.90	**22.8**
	3	13.71 ± 1.27	6.54 ± 0.92	11.28 ± 2.48	0.24 ± 0.05	0.10 ± 0.04	12.35 ± 2.51	0.50 ± 0.22	3.01 ± 0.94	**47.7**
without mould 65. day	**1**	11.36 ± 3.15	3.54 ± 0.95	13.49 ± 2.16	0.28 ± 0.04	0.19 ± 0.04	17.71 ± 6.01	0.76 ± 0.12	< *LOD*	**47.3**
	2	20.43 ± 5.92	13.29 ±2.19	17.43 ± 4.52	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*	**51.2**
	3	7.17 ± 1.66	1.32 ± 0.20	4.39 ± 0.58	0.14 ± 0.02	0.17 ± 0.04	4.27 ± 0.64	0.36 ± 0.06	1.32 ± 0.14	**19.1**

Values are given as mean ± standard deviation *LOD* = limit of detection (0.032 – 0.092 mg.kg^{-1})

Table 5 Statistically significant differences P-value between sausages with and without mould and days of sampling

	mixture x 35. day W	mixture x 35. day WO	35. day W x 65. day W	35. day WO x 65. day WO	35. day W x 35. day WO	65. day W x 65. day WO
tryptamine	0.001	0.001			0.001	
2-phenylethyl-amine			0.001			0.001
putrescine	0.001			0.01	0.001	0.001
cadaverine						0.05
histamine		0.01	0.001		0.05	0.001
tyramine	0.001	0.001		0.01	0.001	0.001
spermidine			0.05			0.05
spermine		0.05		0.001	0.001	
total BA 8	0.001	0.001		0.05	0.001	0.001
pH	0.01	0.001			0.001	0.001
a_w	0.001	0.001	0.001	0.001	0.001	0.001
dry matter	0.001	0.001	0.001	0.05	0.001	
fat	0.05	0.001				
NaCl	0.001	0.001	0.05			
collagen	0.01	0.001				
PMP	0.001	0.001	0.05		0.05	
total nitrogen	0.001	0.001	0.01	0.05	0.01	0.05
TBARS						
L*						0.01
a*	0.05	0.05				
b*	0.001	0.001	0.05		0.05	
texture	–	–		0.001	0.001	0.001
L-lactic acid		0.001			0.001	0.001
D-lactic acid	0.001				0.001	0.001

W – with mould, WO – without mould

Results for the content of biogenic amines and polyamines

An average content of BA and polyamines (Table 4) of 11.3 $mg.kg^{-1}$ was found in the sausage mix immediately after casing. The largest proportion (more than 50%) was accounted for by putrescine (6.5 $mg.kg^{-1}$), followed by spermine (an average content of 2.0 $mg.kg^{-1}$). The level of BA increased during ripening, this by a factor of almost three after 35 days in sausages without surface mould to an average content of 31.3 $mg.kg^{-1}$ and by a factor of around five in sausages with mould to 57.0 $mg.kg^{-1}$. After 2 months, the value increased still further to 39.2 $mg.kg^{-1}$ (smoked sausages), and far more markedly in sausages with mould (by a factor of seven to an average of 79.8 $mg.kg^{-1}$). The largest proportions of BA found were of tryptamine, putrescine and tyramine; after 65 days ripening the average content of putrescine in products with mould amounted to 30.3 $mg.kg^{-1}$. The differences between the total content of the eight biogenic amines and polyamines monitored in the sausage mix and after 35 days of ripening were statistically highly significant ($P \leq 0.001$), as were the differences between the two groups of products (with mould and without mould) after 35 and 65 days (Table 5).

DISCUSSION

The technological production process for dry fermented sausages makes it possible to select internal and external parameters that can be used to influence the properties of the final product (**Buckenhüskes, 1994**). In this study, which compares the influence of the surface treatment of sausages (smoking or a mould culture) on selected properties of the products, entirely identical internal factors were used for both groups, i.e. the composition of the sausage mix, its structure and type of processing, and the kind of casings used. As far as external parameters are concerned, the microclimatic parameters (temperature, relative air humidity) were identical during the ripening. The only difference was that one group of products was smoked with cold smoke during the first days; the second group of products was not smoked and the surface of the sausages was treated with mould starter culture which in subsequent days formed a white mycelium on the outside part of the casing. It is clear from the results obtained that this surface mould had a significant influence on certain properties of the sausages prepared. **Sunesen and Stahnke (2003)** have described surface mould leading to the oxidation of lactic acid, proteolysis, degradation of amino acids and lipolysis in dry fermented sausages, with a direct influence on the aroma and taste of the final products.

Evidence of the decomposition of lactic acid in the products analysed in this study is provided by the difference in concentrations between the two groups of sausages, with a subsequent direct effect on pH values. A negative correlation ($r = -0.98$) with high statistical significance ($P \leq 0.001$) was found between the content of D/L lactic acid and pH values. The acidity of the products expressed by the pH is a fundamental factor in microbial stability and also contributes to the management of water loss during drying (**Feiner, 2006**). As is clear, mould sausages showed a higher pH, a lower content of dry matter and a higher a_w value in comparison with the smoked products in all cases. The pH and, in particular, the water activity are significant barriers to undesirable bacteria in dry fermented sausages (**Gaier, 1996**). Microorganisms significant from the viewpoint of food safety, such as *Listeria monocytogenes* and *Staphylococcus aureus,* may survive during ripening, particularly in mould sausages (**Gareis *et al.*, 2010**). Fermentation took place in an entirely normal way in the smoked sausages; the pH and lactic acid content found concur with data for products of this type discovered in the past (Kameník et al., 2013). In contrast, higher pH values (> 6.0) are typical of sausages from southern Europe, where surface mould is considered traditional (**Latorre-Moratalla *et al*., 2010**).

Determination of the content of selected biogenic amines can also be used for the assessment of the quality of raw materials and the production process in dry fermented sausages (**Coïsson *et al*., 2004**). Their content in final products may fluctuate from tens to hundreds of mg kg^{-1} (**Suzzi and Gardini, 2003; Kameník *et al*., 2012; Latorre-Moratalla *et al*., 2008**). The samples analysed in this work did not show a content of BA higher than 100 $mg.kg^{-1}$. The sausages with surface mould had a higher BA content than the smoked sausages. Similarly, **Kameník *et al.* (2012)** found lower levels of BA in smoked Nitran sausages in comparison with mould products of the Fuet type or Hungarian products. **Bruna *et al.* (2003)** did not detect statistically significant differences in the BA content in sausages of a diameter of 40 mm covered with *Penicillium camemberti* mould in comparison with a control without a mould covering. The reason for this may have been the fact that they assessed the products no later than on day 22 of ripening when the effect of surface mould on more intensive BA production had not yet been seen.

The greater occurrence of BA in the mould sausages in this work was evidently associated with the proteolytic activity of the mould which releases precursors of BA into the environment. In this case, the pH plays a secondary role (**Latorre-**

Moratalla *et al*., 2010) and it cannot, therefore, be concluded that bacteria produce more BA in an acidic environment to maintain an acid-base balance. The pH values in the dry fermented sausages at the moment of fermentation activity (pH 4.7–4.9) also evidently remain sufficiently high to stimulate the formation of BA. Of the eight selected BA, the highest content was found for putrescine and tyramine, which correlates with the data in the literature (**Komprda *et al*., 2009; Ansorena *et al.*, 2002**). In comparison with previous analyses (**Kameník *et al*., 2012**), an increased content of tryptamine, amounting to a level of around 20 $mg.kg^{-1}$ in two samples after 65 days, was also found. Similar levels of tryptamine were found in European dry fermented sausages by **Latorre-Moratalla *et al.* (2010)**, though only in isolated cases, while **Hernández-Jover *et al.* (1997)** detected even higher levels of tryptamine in Spanish products.

The positive effects of surface mould on the properties of dry fermented sausages also include delaying the onset of rancidity and the stabilisation of colour as a result of catalase activity, oxygen consumption and protection against light (**Sunesen and Stahnke, 2003**). In our case, analysis of the malondialdehyde content did not show a lower content in sausages of the mould type; neither did the instrumental assessment of colour show better parameters in sausages with surface mould. In contrast, a darker colour was found in smoked products according to the L* value. This difference may be associated with the higher water content in mould products. The lower proportion of dry matter was also evidently reflected in lower TPA values in mould sausages. Similarly to our results, a lower value of textural parameters was also found by **Bruna *et al.* (2003)** during the instrumental analysis of mould sausages with a control.

CONCLUSION

The surface mould culture had an effect on selected physico-chemical properties and the characteristics of the instrumental analysis of dry fermented sausages. Certain characteristics (a higher pH, a higher water activity value) of mould sausages may influence the properties of barriers against undesirable bacteria during ripening. Thorough control of the input raw materials must, therefore, be selected for the production of fermented mould sausages from the viewpoint of food safety. Such measures are also in accordance with the monitoring of the content of biogenic amines which was higher in mould sausages than in identical smoked products without surface mould. The sausages with surface mould had a higher BA content than the smoked sausages.

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