Influence of Various Pork Fat Types on the Ripening and Characteristics of Dry Fermented Sausage

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Abstract

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The influence of three types of pork adipose tissue (neck, back, and leg) on the quality of dry fermented sausages was evaluated. No statistically significant differences between the most abundantly represented methyl esters of fatty acids (FA), such as C16:0, C18:0 and C18:1n-*9*, were found when the individual FA compositions of the pork adipose tissue samples were compared. The content of polyunsaturated FA was the highest in the neck adipose tissue and the lowest in the leg adipose tissue. These differences were not, however, statistically significant. No differences between the adipose tissue types were found in the sensory, colour, and textural properties, the population of lactic acid bacteria, and the lactic and acetic acids contents in the final products and during the fermentation process. The assertions were not confirmed that pork neck adipose tissue is the best fat type for the production of dry fermented sausages or that pork adipose tissue from the legs is unsuitable because of its insufficient hardness.

Keywords*:* pork adipose tissue; methyl esters of fatty acids; sensory characteristics; lactic acid; lactic acid bacteria

A higher level of polyunsaturated FA can be achieved in pork meat and adipose tissue by altering the fatty acid profile of the diet (Wood *et al*. 2008; Realini *et al*. 2010) and can also be achieved in meat products by the direct application of vegetable oils (Del Nobile *et al*. 2009).

The nutritional aspect is not the only factor or even the decisive factor that influences the sales of the meat products. Sensory properties are at least equally as important, and this is particularly true for dry fermented sausages. Pork adipose tissue plays an important role in the production of such meat products (Stiebing *et al*. 1993). It helps in forming an appropriate sausage mixture structure, which influences the product during the fermentation and ripening phases. It also influences the colour, consistency, and taste of the final product.

The pork adipose tissue used in the production of dry fermented meat products should be firm. Adipose tissue from pigs that are fed primarily with barley is recommended. If soft adipose tissue containing smearing fat is used, this fat is released from the fat cells during mincing and intermingles with the ground meat particles, which become covered with a thin fatty film. The pieces of meat are then unable to adhere and the consistency of sausage remains soft. When the casing is filled with such sausage mixture, the soft fat can

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also infiltrate the casing and restrict its ability to allow the passage of water during drying. When the sausage mixture is being made, particles of adipose tissue contribute to the creation of the appropriate structure by "plumping up" the mass of the sausage mixture (Keim & Franke 2007). This leads to the formation of channels through which water travels from the middle of the product to its surface during drying. If an unsuitable structure and composition of the adipose tissue prevents it from becoming incorporated into the structure of the sausage mixture in the way described above, the lean meat sticks together so strongly that defects may appear while the sausages are drying and the production is impaired. An unsuitable sausage mixture structure can have a negative influence on the fermentation process.

The predominant lactobacilli in the fermented sausage mixture, such as *L. sakei* and *L. curvatus* (Bonomo *et al*. 2008; Cocolin *et al*. 2009), belong to the group of facultative homofermentative species (which also includes *L. plantarum*). Under ordinary conditions, these lactic acid bacteria break down saccharides into lactic acid. In particular situations (such as an unsuitable mixture structure, an inadequate exchange of gases between the product and the environment, or insufficient drying of the product), however, a switch from homofermentation to heterofermentation may occur (Girard *et al*. 1989). Acetic acid or other metabolites that are created have a negative effect on the aroma of the final product.

Herkules sausage is a traditional dry fermented meat product made in the Czech Republic. It was the first sausage to use a starter culture and its production began more than twenty years ago. The original recipe was based on the addition of back fat, lean pork meat (leg), and lean beef. Today, some producers use "cutting fat" in place of adipose tissue from the neck or back. Cutting fat is produced during the deboning of legs or shoulders. It is cheaper than back adipose tissue, although some of its properties are less desirable (soft consistency, lower fat content). These properties can be reflected in the impaired sensory characteristics of the final products that are destined for the market.

The aim of this work was to assess the influence of subcutaneous pork adipose tissue from various parts of the body (neck, back, and leg) on the selected physicochemical, microbiological, and sensory properties of the traditional Czech dry sausage Herkules. The work should answer the following questions:

(a) Were there any substantial differences in the fat content and the proportion of the individual fat?
(b) Were there any substantial differences in how the fat from the meat added to the sausage mixture modified the selected values for the adipose tissue used?
(c) Were there any substantial differences in the physicochemical, microbiological, and sensory properties of individual batches of Herkules sausage?

MATERIAL AND METHODS

Sausage manufacture. Three batches (134 kg per batch) of the dry fermented sausage known as Herkules were made based on the type of pork adipose tissue used. The basic recipe required 77 kg of sow meat (shoulders and neck), 13 kg of lean beef (90% lean), and 38 kg of pork subcutaneous adipose tissue/batch. A mixture of spices with saccharides, the starter culture, and a nitrite salting mixture were added to the sausage mixture during the grinding phase. The sausage mixture was worked into grains of 1–2 mm in size and used to fill collagen casings that measured 75 mm in diameter.

The adipose tissue used in the individual batches was obtained from a group of 25 pigs (gilts Czech Improved White × Landrace with Tempo crosses) that had been fattened up on the same farm with a standard feed batch containing 47% barley, 31% wheat, and 12% triticale and were slaughtered on the same day. The adipose tissue was obtained from pork carcasses on the third day after slaughtering and divided into three groups according to the anatomical location (neck, back, and leg).

Fermentation and ripening occurred in an air-conditioned chamber; the initial temperature was 24°C and was reduced gradually to 15°C. The samples were taken on days 0 (sausage mixture), 3, 7, 14, and 21, when the sausages had achieved shipping ripeness. A portion of the samples was vacuum-packed, with subsequent sampling and analysis after 28 days of storage, i.e., on day 49 from the start of the production. The samples were labelled in such way that the number corresponded to the day of sampling and the type of adipose tissue used (N – neck, B – back, and L – leg).

Chemical analysis. The samples (approximately 250 g) for chemical analyses were homogenised at room temperature using a Grindomix (Retsch, Haan, Germany) (120 s, 3000 rpm) and

were then kept in plastic boxes at –18°C. Nine parallel measurements were made on each sample. The total fat from 50 g of the homogenised sample was extracted with a petroleum ether/acetone solution (2:1) by homogenisation on a Polytron PT-MR 3100 (Kinematica AG, Luyern, Switzerland) (180 s, 10 000 rpm) at room temperature. Triacylglycerols were detected as methyl esters after saponification and ester interchange with methanol. Thirty-seven FA methyl esters (Standard Supelco 37 Component FAME Mix) were analysed using the gas chromatography method on a TRACE GC chromatograph (ThermoQuest, Rodano, Italy). The FA methyl esters were separated on a Supelco SP^{TM}2560 capillary column (100 m × 0.25 mm × 0.2 μm) and detected by flame-ionising detection. HP ChromQuest 3.0 chromatographic software was used for the data collection and processing. The values for each FA were summarised in the groups polyunsaturated FA (PUFA), monounsaturated FA (MUFA), saturated FA (SFA), ω3 (n-3), ω6 (n-6), ω9 (n-9), and *trans* FA, and their proportions in the sample were calculated (%). The total fat content measured in the sample (%) was compared to the amount of total FA as calculated by the FA analysis (g/kg of the sample) and these datas corresponded to each other.

The following parameters were subsequently determined: the amounts of dry matter (ISO 1442:1997) and chlorides (Volhard method, ISO 1841-1:1996), collagen (spectrophotometry at 550 nm, equalised to 4-hydroxyproline content), and pure muscle protein (after the elimination of non-protein N-compounds using hot tannin), the protein content was measured on a KJEHLTEC from TECATOR (Hillerød, Denmark); then pure muscle protein was calculated as the protein content reduced by the amount of collagen. Nitrogen was converted to crude protein using a factor of 6.25. The result stated for each sample is the mean of three measurements.

The degree of lipid oxidation was measured by the reaction with thiobarbituric acid after distillation similarly to the method by Castellini *et al*. (2002) – TBARS value (thiobarbituric acid reactive substance).

Water activity (a_w) and pH value. Water activity was measured using a Novasina LabMaster instrument (Novasina AG, Lachen, Switzerland) and pH measurements were taken using a Double Pore needle probe (Hamilton Bonaduz AG, Bonaduz, Switzerland) and a 340i WTW pH-meter (WTW, Weilheim, Germany).

Lactic and acetic acids determinations. The concentration of D/L lactic acid was determined using an enzymatic test kit (Megazyme, Bray, Ireland). The enzyme D(+)- 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 hydrazine, while the NADH formed was quantified by measuring the absorbance at 340 nm (Megazyme 2011a). Acetic acid was also determined using an enzymatic test kit (Megazyme, Bray, Ireland). Acetyl-coenzyme A synthetase (ACS) in the presence of adenosine-5'-triphosphate (ATP) and coenzyme A (CoA) converts acetic acid (acetate) into acetyl-CoA (1), with the formation of adenosine-5'-monophosphate (AMP) and pyrophosphate. Citrate synthase (CS) converts oxaloacetate into citrate (2) in the presence of acetyl-CoA. The oxaloacetate required in reaction (2) is formed from L-malate and nicotinamide-adenine dinucleotide in the presence of L-malate dehydrogenase (L-MDH) (3). In this reaction, NAD^+ is reduced to NADH. This determination is based on the formation of NADH, which is measured by the increase in absorbance at 340 nm (Megazyme 2011b).

Microbial analysis. The microbial status of the samples was evaluated by determining the lactic acid bacteria (LAB) count. The quantification of LAB was performed using de Man, Rogosa, Sharpe agar (MRS Agar, CM0361; Oxoid, Basingstoke, UK) under anaerobic conditions at 30 ± 1°C for 72 ± 3 h, in accordance with the ISO 13721:1995 guidelines. All analyses were performed in duplicate. The number of colonies was counted and reported as $\log_{10}$ of CFU/g for each sample.

Sensory analysis. The sausages were evaluated by an untrained panel consisting of 12 judges selected from the students and staff members of the Department of Meat Hygiene and Technology, taking into account their habits, acquaintance with the material to be analysed, sensitivity, and ability to reproduce the judgments. The evaluations were performed in individual booths, prepared as described by the ISO 6658:2005. Unsalted crackers and water at room temperature were provided to cleanse the palate between samples. The test was carried out using unstructured 100-mm hedonic scales in which the panellists evaluated various attributes: cut surface appearance, odour, colour, consistency, texture, taste, matrix, fat particles, and ring occurrence (0 = very unpleasant and 100 = very pleasant).

Texture analysis. The samples were tested with a texture profile analysis (TPA) using an Instron Universal Testing Machine (model 5544) (Instron Corporation, High Wycombe, UK). The parameters were obtained using the available computer software (Merlin, High Wycombe, UK). The cylinder samples (1 cm height, 1.25 cm diameter) were compressed twice to 50% of their original height with a compression plate that was 36 mm in diameter. The force times curve was recorded at a crosshead speed of 50 mm per minute. Hardness (N; the peak force required for the first compression) and cohesiveness (the ratio of the positive force area during the second compression to that in the first compression) were evaluated (Szczesniak 2002; Desmond & Kenny 2005).

Data 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 between the different parts of the fat and differences between processing. Significance levels of 0.05, 0.01, and 0.001 were used.

RESULTS AND DISCUSSION

Methyl esters of fatty acids in pork adipose tissue

The results of the FA composition in N, B, and L adipose tissues are given in Table 1. When the proportions of the individual FA in fats from three different anatomical parts of the animal were compared, no statistically significant differences were found for the most abundantly represented FAs, such as palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1n-*9*). Statistically significant differences between the three types of adipose tissue ($P < 0.05$) were detected for the following FA: myristic acid (C14:0), palmitoleic acid (C16:1), and arachidic acid (C20:0). There were also significant differences in the proportions of individual FA groups: the highest proportion of SFA was found in N fat (42.79%), followed by B fat (42.25%), while the lowest concentration was found in L fat (41.16%). In contrast, the largest quantity of methyl esters of monounsaturated fatty acids (MUFA) was found in L adipose tissue (51.43%), followed by B (49.56%) and N adipose tissues (48.71%). The PUFA content was the highest in N adipose tissue (8.50%) and the lowest in L adipose tissue (7.41%). However, the overall quantity of PUFA was lower in all analysed fats than that reported in the literature (Warnants *et al*. 1998). In particular, the quantities of linoleic acid (C18:2n-6 *cis*), linoelaidic acid (C18:2n-6 *trans*), and linolenic acid (C18:3n-3) detected in the present work were lower. The composition of the animal feed has the greatest influence on the composition of FA in meat (Wood *et al*. 2008).

The highest proportion of FA detected in all samples was oleic acid (C18:1n-9 *cis*). As in the work of Maw *et al*. (2003), this FA was present within a range of 443.68–494.12 g/kg of fat (i.e., 45.60–47.92%) in all three types of adipose tissue.

The fat composition of the sausage mixture and the finished product

The individual FA compositions in the mixture and in dry fermented sausages (from neck, back and leg adipose tissue) are shown in Table 2.

Table 2 shows the values for the individual FA composition in the sausage mixture immediately after mixing. It is clear that a certain shift in the proportions of individual groups of FA occurred after the mixing of pork adipose tissue and meat. This was evidently caused by the effect of intramuscular fat, which may differ in terms of the proportions of FA from subcutaneous adipose tissue (Kouba & Sellier 2011). A slight reduction in the amounts of linoleic acid and linolenic acid in the mixture resulted in the reduction of the total PUFA proportions in the sausage mixture: 8.15% (N), 7.05% (B), and 7.19% (L). The proportions of SFA (39.86% (N), 40.41% (B), and 39.35% (L)) were also reduced in comparison with pork adipose tissue (Table 1). A reduction of palmitic and stearic acids (SFA) was observed. In contrast, the addition of meat resulted in an increase of oleic acid content (48.62–49.96%). The proportion of MUFA in the sausage mixture increased up to 51.99% (N), 52.54% (B), and 53.45% (L). When comparing these results with the literature, the sausage mixture in this study showed considerably higher quantities of SFA and MUFA and, in contrast (as for the adipose tissue), a lower quantity of PUFA (Hoz *et al*. 2004). Statistically significant differences ($P < 0.05$) were detected between the three mixtures (N, B, L) in stearic acid (C16:0) and tricosanoic acid (C23:0).

Table 2 shows the proportions of the individual FA in the final products after 21 days of fermenta-

Table 1. The fat and fatty acids profile in the fat adipose tissue – the comparison of samples coming from different anatomic parts (neck/back/leg)

FA	N		B		L		*P*-value
	FA (g/kg fat) ± SD	total FA (%)	FA (g/kg fat) ± SD	total FA (%)	FA (g/kg fat) ± SD	total FA (%)	
C14:0	8.05 ± 0.35^{a}	0.83	10.86 ± 1.75^{a,b}	1.07	12.92 ± 1.42^{b}	1.25	< 0.05
C16:0	248.59 ± 5.65	25.55	251.23 ± 3.56	24.85	255.65 ± 3.60	24.79	ns
C16:1	23.09 ± 1.15^{a}	2.37	24.91 ± 0.56^{a}	2.46	27.98 ± 0.47^{b}	2.71	< 0.05
C17:0	2.69 ± 0.08	0.28	2.76 ± 0.36	0.27	2.39 ± 0.15	0.23	ns
C17:1	1.90 ± 0.23	0.20	1.96 ± 0.05	0.19	2.06 ± 0.07	0.20	ns
C18:0	154.72 ± 9.10	15.90	158.76 ± 3.48	15.70	150.21 ± 5.76	14.57	ns
C18:1n-9	443.68 ± 14.04	45.60	465.18 ± 24.91	46.01	494.12 ± 5.30	47.92	ns
C18:2n-6 *cis*	77.24 ± 2.15	7.94	74.86 ± 3.73	7.40	70.53 ± 3.10	6.84	ns
C18:3n-3	2.81 ± 1.10	0.29	4.78 ± 0.64	0.47	3.78 ± 0.13	0.37	ns
C20:0	0.52 ± 0.06^{a}	0.05	1.22 ± 0.35^{b}	0.12	0.82 ± 0.12^{a,b}	0.08	< 0.05
C20:1	5.27 ± 0.26	0.54	8.84 ± 2.26	0.87	5.96 ± 0.10	0.58	ns
C20:2	1.99 ± 0.08	0.20	2.19 ± 0.19	0.22	1.91 ± 0.04	0.19	ns
C23:0	0.64 ± 0.03	0.07	0.81 ± 0.13	0.08	0.74 ± 0.16	0.07	ns
Total *trans*	1.50	0.15	2.11	0.21	2.16	0.21	ns
Total n-3	3.17	0.33	5.42	0.54	3.92	0.38	ns
Total n-6	77.52	7.97	75.15	7.43	70.61	6.85	ns
Total n-9	443.68	45.60	465.18	46.01	494.12	47.92	ns
Total SFA	416.28	42.79	427.18	42.25	424.36	41.16	ns
Total MUFA	473.95^{a}	48.71	501.08^{a,b}	49.56	530.31^{b}	51.43	< 0.05
Total PUFA	82.69	8.50	82.75	8.19	76.43	7.41	ns
Total fat (%)	79.50		80.40		70.70		
Collagen (%)	4.81		3.12		3.04		

N – neck; B – back; L – leg; FA – fatty acids are expressed as methyl esters of fatty acids; ^{a,b,c}values in a row with different letters are significantly different ($P < 0.05$) (Tukey's test); identical letters indicate no differences for that characteristic; ns – not significant; the groups comprise the sum of FA: SFA (n = 17), MUFA (n = 9), PUFA (n = 11), ω3 FA (n = 4), ω6 FA (n = 5), ω9 FA (n = 3) and *trans* FA (n = 2)

tion and ripening. In comparison with the sausage mixture, a slight increase in SFA (C14:0, C17:0, C18:0 and C23:0) was observed, with a slight decrease in MUFA (C17:1, C18:1n-9 *cis* and *trans*, C20:1) and an increase in PUFA (C18:2n-*6 cis* and *trans*). Statistically significant differences ($P < 0.05$) between the three types of fermented sausage were detected for heptadecanoic acid (C17:0), oleic acid (C18:1n-*9 cis*), linoleic acid (C18:2n-6 *cis*), and linolenic acid (C18:3n-3).

Table 2 shows the individual FA compositions of vacuum-packed products and products stored for 28 days. A slight increase in SFA (C16:0) and a reduction in MUFA and PUFA were observed in comparison with the sausages after ripening (21 days) and sausages after storage. No statistically significant differences were found when comparing the individual FA compositions in the sausages (N, B, L). The highest percentage of fat was found in the sausage produced from N adipose tissue (39.80%), followed by B (39.50%) and L adipose tissues (35.80%).

When comparing the proportions of the individual FA groups during the entire production phase (Table 2), it comes clearly out that the quantity of SFA showed a similar trend of the initial decline and later growth for the B and L fat groups. This trend was similar, although less clear, for the N

Table 2. The fat and fatty acids profile in the sausage emulsion (day 0, 21 and 49) – the comparison of samples coming from different anatomic parts (neck/back/leg)

FA	N		B		L		*P*-value
	FA ± SD (g/kg fat)	total FA (%)	FA ± SD (g/kg fat)	total FA (%)	FA ± SD (g/kg fat)	total FA (%)	
Day 0							
C14:0	9.32 ± 0.34	0.90	7.36 ± 0.15	0.75	8.54 ± 2.80	0.92	ns
C16:0	246.65 ± 6.90^{a}	23.84	239.27 ± 0.75$^{a.b}$	24.40	222.40 ± 7.46^{b}	23.85	< 0.05
C16:1	24.27 ± 0.91	2.35	22.28 ± 0.13	2.27	23.02 ± 1.78	2.47	ns
C17:0	2.19 ± 0.36	0.21	2.29 ± 0.01	0.23	2.41 ± 0.27	0.26	ns
C17:1	2.01 ± 0.01	0.19	1.65 ± 0.03	0.17	1.76 ± 0.21	0.19	ns
C18:0	152.38 ± 3.13^{a}	14.73	146.16 ± 1.23^{b}	14.91	131.76 ± 2.30^{b}	14.13	< 0.01
C18:1n-9	503.09 ± 13.74	48.62	484.40 ± 2.78	49.41	465.8 ± 18.06	49.96	ns
C18:2n-6 *cis*	78.15 ± 2.20^{a}	7.55	65.93 ± 1.07^{b}	6.72	61.97 ± 3.03^{b}	6.65	< 0.01
C18:3n-3	2.83 ± 0.15^{a}	0.27	0.97 ± 0.10^{b}	0.10	2.80 ± 0.20$^{a.c}$	0.30	< 0.001
C20:0	0.40 ± 0.04	0.04	0.36 ± 0.04	0.04	0.53 ± 0.33	0.06	ns
C20:1	8.40 ± 0.23	0.81	6.59 ± 0.23	0.67	7.53 ± 1.66	0.81	ns
C20:2	2.25 ± 0.11	0.22	1.78 ± 0.07	0.18	1.89 ± 0.28	0.20	ns
C23:0	0.43 ± 0.02^{a}	0.04	0.13 ± 0.02^{b}	0.01	0.24 ± 0.11$^{a.b}$	0.03	< 0.05
Total *trans* FA	2.02	0.20	2.41	0.25	2.41	0.26	ns
Total n-3	3.25^{a}	0.31	1.02^{b}	0.10	2.87^{a}	0.31	< 0.001
Total n-6	78.78^{a}	7.61	66.32^{b}	6.76	62.31^{b}	6.68	< 0.01
Total n-9	503.09	48.62	484.50	49.41	465.89	49.97	ns
Total SFA	412.41^{a}	39.86	396.26^{a}	40.41	366.96^{b}	39.35	< 0.05
Total MUFA	537.95	51.99	515.40	52.54	498.40	53.45	ns
Total PUFA	84.28^{a}	8.15	69.11^{b}	7.05	67.07^{b}	7.19	< 0.01
Total fat (%)	28.90		27.70		27.30		
Day 21							
C14:0	12.21 ± 0.10^{a}	1.17	7.47 ± 0.34^{b}	0.75	14.15 ± 0.45^{c}	1.39	< 0.01
C16:0	244.24 ± 2.18	23.42	238.55 ± 6.06	23.89	232.52 ± 5.52	22.84	ns
C16:1	24.70 ± 0.16$^{a.b}$	2.37	22.75 ± 0.92^{a}	2.28	26.59 ± 0.61^{b}	2.61	< 0.01
C17:0	2.61 ± 0.03^{a}	0.25	2.43 ± 0.05^{b}	0.24	2.32 ± 0.06^{b}	0.23	< 0.05
C17:1	0.00 ± 0.00^{a}	0.00	0.00 ± 0.00^{a}	0.00	0.00 ± 0.00^{b}	0.00	< 0.01
C18:0	154.83 ± 2.11^{a}	14.85	153.50 ± 5.30^{a}	15.37	136.52 ± 3.65^{b}	13.41	< 0.05
C18:1n-9	493.04 ± 1.39	47.28	479.96 ± 12.31	48.06	488.15 ± 11.79	47.94	ns
C18:2n-6 *cis*	84.17 ± 0.30^{a}	8.07	74.86 ± 2.33^{b}	7.50	77.30 ± 2.66^{b}	7.59	< 0.05
C18:3n-3	6.11 ± 0.16^{a}	0.59	3.61 ± 0.12^{b}	0.36	6.79 ± 0.17^{c}	0.67	< 0.05
C20:0	1.16 ± 0.05^{a}	0.11	0.72 ± 0.08^{b}	0.07	1.20 ± 0.04^{a}	0.12	< 0.001
C20:1	11.75 ± 0.11	1.13	8.74 ± 0.58	0.88	24.38 ± 16.85	2.39	ns
C20:2	2.74 ± 0.18^{a}	0.26	2.15 ± 0.08^{b}	0.21	2.49 ± 0.03$^{a.b}$	0.24	< 0.01
C23:0	1.21 ± 0.02^{a}	0.12	0.90 ± 0.05^{b}	0.09	1.30 ± 0.03^{a}	0.13	< 0.001
Total *trans*	2.82	0.27	2.77	0.28	2.79	0.27	ns
Total n-3	6.97^{a}	0.67	5.40^{b}	0.54	7.39^{a}	0.73	< 0.001
Total n-6	84.97^{a}	8.15	75.48^{b}	7.56	78.07^{b}	7.67	< 0.05
Total n-9	493.03	47.28	479.96	48.06	488.16	47.94	ns
Total SFA	418.26^{a}	40.11	404.09^{a,b}	40.46	390.60^{b}	38.36	< 0.05
Total MUFA	529.80^{a,b}	50.81	511.26^{a}	51.22	539.62^{b}	53.00	< 0.05
Total PUFA	94.69^{a}	9.08	83.02^{b}	8.31	87.95^{a,b}	8.64	< 0.01
Total fat (%)	39.30		39.10		36.10		

Table 2 to be continued

FA	N		B		L		*P*-value
	FA ± SD (g/kg fat)	total FA (%)	FA ± SD (g/kg fat)	total FA (%)	FA ± SD (g/kg fat)	total FA (%)	
Day 49							
C14:0	13.19 ± 0.42	1.26	12,77 ± 0.26	1.24	13,00 ± 0.26	1.25	ns
C16:0	250.75 ± 3.58	23.95	252.34 ± 3.76	24.56	252.00 ± 1.39	24.25	ns
C16:1	25.80 ± 0.51	2.46	25.56 ± 0.40	2.49	27.39 ± 0.48	2.64	ns
C17:0	2.59 ± 0.08	0.25	2.51 ± 0.05	0.24	2.36 ± 0.06	0.23	ns
C17:1	2.03 ± 0.05	0.19	1.89 ± 0.02	0.18	1.88 ± 0.02	0.18	ns
C18:0	154.56 ± 1.70	14.76	154.34 ± 2.90	15.02	146.68 ± 1.90	14.12	ns
C18:1n-9	496.41 ± 8.40	47.41	483.14 ± 6.08	47.01	498.79 ± 5.66	48.00	ns
C18:2n-6 *cis*	82.48 ± 3.31	7.88	76.32 ± 0.86	7.43	78.07 ± 2.34	7.51	ns
C18:3n-3	4.25 ± 0.05	0.41	4.01 ± 0.09	0.39	4.04 ± 0.04	0.39	ns
C20:0	1.30 ± 0.13	0.12	1.31 ± 0.09	0.13	1.31 ± 0.05	0.13	ns
C20:1	6.46 ± 0.15	0.62	6.46 ± 0.20	0.63	6.57 ± 0.09	0.63	ns
C20:2	2.44 ± 0.04	0.23	2.28 ± 0.07	0.22	2.32 ± 0.02	0.22	ns
C23:0	1.27 ± 0.01	0.12	1.19 ± 0.03	0.12	1.29 ± 0.02	0.12	ns
Total trans	2.57	0.25	2.49	0.24	2.52	0.24	ns
Total n-3	4.70[a]	0.45	4.41[b]	0.43	4.43[b]	0.43	< 0.05
Total n-6	82.88	7.91	76.67	7.46	78.46	7.55	ns
Total n-9	496.41	47.41	483.13	47.02	498.79	48.00	ns
Total SFA	426.03	40.69	426.78	41.53	418.91	40.31	ns
Total MUFA	531.08	50.72	517.46	50.36	535.01	51.49	ns
Total PUFA	90.02	8.60	83.35	8.11	85.21	8.20	ns
Total fat (%)	39.50		39.80		35.80		

N – neck; B – back; L – leg; FA – the fatty acids are expressed as methyl esters of fatty acids; [a,b,c] values in a row with different letters are significantly different ($P < 0.05$) (Tukey's test); identical letters indicate no differences for that characteristic; ns – not significant; the groups comprise the sum of FA: SFA (n = 17), MUFA (n = 9), PUFA (n = 11), ω3 FA (n = 4), ω6 FA (n = 5), ω9 FA (n = 3), *trans* FA (n = 2)

fat. The trend for MUFA differed greatly for the N and L adipose tissues during the phase of sausage mixing. A decrease in MUFA in the sausage mixture was observed during the mixing of meat with L adipose tissue, while an increase in MUFA was observed for the N mixture and to a lesser extent in the B mixture. Further phases of production, however, showed the same trends. The PUFA showed extremely similar behaviour in B and L fats (first an extremely small decrease with the addition of the mixture, followed by a small gradual increase). A small reduction in PUFA was observed during the storage phase of the N fat.

Pig adipose tissue, as part of the recipe, plays an important role in the management of drying. Smearing of fat has to be avoided as this would block the capillaries in the sausage mass and subsequent drying would be severely affected (Feiner 2006). The basic precondition is the processing of hard frozen adipose tissue. The hardness of fat is largely determined by the proportion of FA with double bonds, particularly PUFA (Wood *et al.* 2003). An acceptable product cannot be made from pork adipose tissue if the proportion of PUFA in the fat is higher than 25%. The proportion of PUFA to the total amount of FA in the fat should not exceed 14% for this type of sausage with an intermediate storage period. If such products are intended to be stored for a long time, this proportion should not exceed 12% (Stiebing *et al.* 1993).

The proportion of PUFA was far below the given limit in the test performed. The greatest proportion of PUFA was discovered in the neck adipose tissue (8.50%) and the lowest one in the fat from

the legs (7.41%). This percentage was still lower in the fat obtained from the sausage mixture after all the recipe ingredients had been mixed in (batch N 8.15%, B 7.05%, L 7.19%). In this regard, all the types of adipose tissue used (from this breed and type of feeding) can be recommended for the production of dry fermented sausage.

The influence of the fat on the quality of dry fermented sausages cannot, however, be observed merely from the viewpoint of the content of PUFA, even though this factor is critical. The proportion of total fat and the content of water are also significant. The lowest possible water content in the processed pig adipose tissue is desirable. The content of lipids in pig fat is closely associated with the content of water and is indirectly proportional to it (Wood *et al*. 1989).

Neck and back adipose tissues showed an average fat concentration of 80%, while the average concentration of fat from the legs was 70.7%.

From this viewpoint, the use of neck and back adipose tissues is more suitable for dry fermented sausages. The same recommendation is given by Feiner (2006), who stated that these types of pig fat have the lowest concentration of MUFA and PUFA and therefore do not smear as easily during cutting or mincing as the soft fat from the shoulder or leg. The content of linoleic acid (C18:2), for which, according to Wood *et al*. (1989), a significant dependency exists between the concentration and toughness of the fat (a higher concentration indicates softer fat), was the lowest in the sample of fat from the legs (6.84%) and the highest in the sample of neck adipose tissue (7.94%) in our experiment. Wood *et al*. (1989) analysed samples of the back adipose tissue, which they compared with the fat from the shoulders, and obtained similar results for the content of linoleic acid (C18:2). These results differed only in the dependence on the thickness of the adipose tissue, with thinner tissue having a higher proportion of this FA.

Physicochemical characteristics

The pH value (Table 3) of the sausage mixture corresponded to the figures given in the literature for this type of product (Buckenhüskes 1994) and fell during the course of ripening below 4.8 (value determined on day 3) in all the samples. After a month of storage, a slight increase in these values by approximately 0.15 was recorded. The pH was equal for all batches throughout the course of the experiment, and approached a value of 4.90 after 28 days of storage. The values determined

Table 3. The physicochemical parameters of dry fermented sausages

Sample	pH (–)	a_w (–)	Lactic acid (μmol/g of dry matter)	Acetic acid (μmol/g of dry matter)	Dry matter (%)
N 0	5.85 ± 0.02	0.966 ± 0.002	81.77 ± 16.58	3.65 ± 0.25	50.14 ± 0.36
N 3	4.73 ± 0.02	0.962 ± 0.004	238.97 ± 8.15	16.55 ± 0.63	52.31 ± 0.60
N 7	4.71 ± 0.01	0.957 ± 0.003	233.48 ± 36.19	14.80 ± 0.69	57.39 ± 1.45
N 14	4.77 ± 0.02	0.939 ± 0.002	230.25 ± 29.60	15.12 ± 0.35	61.67 ± 1.89
N 21	4.77 ± 0.03	0.926 ± 0.006	236.31 ± 36.01	18.92 ± 0.97	66.02 ± 2.41
N 49	4.88 ± 0.02	0.897 ± 0.004	219.04 ± 26.66	18.11 ± 0.36	64.37 ± 3.11
B 0	5.84 ± 0.02	0.970 ± 0.002	83.84 ± 11.21	2.19 ± 0.26	50.10 ± 0.41
B 3	4.69 ± 0.01	0.964 ± 0.004	300.43 ± 29.07	8.92 ± 0.38	52.26 ± 0.58
B 7	4.67 ± 0.02	0.956 ± 0.003	266.63 ± 17.67	10.57 ± 0.81	56.72 ± 1.10
B 14	4.72 ± 0.01	0.935 ± 0.004	251.04 ± 36.72	11.57 ± 0.64	63.34 ± 2.46
B 21	4.79 ± 0.01	0.911 ± 0.008	254.59 ± 33.65	18.59 ± 0.72	67.17 ± 1.31
B 49	4.85 ± 0.02	0.878 ± 0.004	222.36 ± 9.97	19.50 ± 0.65	67.46 ± 1.56
L 0	5.80 ± 0.03	0.974 ± 0.002	87.85 ± 14.81	3.83 ± 0.46	47.81 ± 0.48
L 3	4.69 ± 0.01	0.962 ± 0.005	249.25 ± 31.06	22.58 ± 0.64	50.15 ± 0.36
L 7	4.66 ± 0.01	0.955 ± 0.001	256.34 ± 3.77	12.16 ± 0.36	56.17 ± 1.37
L 14	4.74 ± 0.01	0.934 ± 0.003	269.92 ± 5.377	11.24 ± 0.54	60.77 ± 1.78
L 21	4.81 ± 0.01	0.931 ± 0.004	240.44 ± 7.875	20.54 ± 0.56	64.05 ± 2.01
L 49	4.87 ± 0.01	0.894 ± 0.004	248.06 ± 12.60	16.73 ± 0.77	65.71 ± 2.27

N – neck; B – back; L – leg

corresponded to those described in the literature (Komprda *et al*. 2004, 2009).

The values of water activity in the sausage mixture were the highest in the samples containing L adipose tissue. Statistically significant differences were recorded in comparison with the two other types of sample (the sample with N fat, $P \leq 0.01$, and the sample with B adipose tissue, $P \leq 0.05$). After 21 days of ripening, the value of water activity in the samples with L adipose tissue did not decrease below 0.93, which is the critical value for this type of product as set by the Czech legislation (Decree No. 326/2001). Those samples showed statistically significant differences ($P \leq 0.001$) in comparison with the values found in the samples of B adipose tissue.

The content of dry matter in all the samples increased by approximately 17% during the course of ripening. The dry matter content in the sausage mixture containing L adipose tissue was markedly lower, and statistically significant differences ($P \leq 0.01$) were observed in comparison with two other types of fat. This was the case during the entire ripening period, including day 21. This difference, which evidently had an effect on the subsequent value of a_w, may have been caused by the different proportion of fat in the pig adipose tissue used (Table 1). The L adipose tissue contained less fat than N (–8.80%) and B (–9.70%) adipose tissues.

No significant differences between the individual types of sausage were recorded during the assessment of other chemical parameters such as the contents of salt, pure muscle protein, and collagen.

The type of adipose tissue used in the preparation of the sausage had an effect on the fat oxidation process. The individual types of sample differed during ripening in terms of malondialdehyde content, although these differences were not so pronounced in the final product. A sharper increase in these values was observed from the first day of ripening in the samples containing L adipose tissue as compared with other samples. The malondialdehyde content in all the samples increased until day 14 of ripening. At this maximum point, the lowest content was found in the samples containing N adipose tissue, approximately 1 mg/kg compared with the other samples in which the respective values reached 3.5 mg/kg. The progress of oxidation and the values for the malondialdehyde content are recorded in Figure 1. A decrease in these values was observed after 14 days of ripening. A similar course of oxidation changes has also been recorded by García-Iñiguez de Ciriano *et al*. (2010). Statistically significant differences in the malondialdehyde content were recorded on day 14 of ripening between the samples with N and B adipose tissues ($P \leq 0.05$) and N and L adipose tissues ($P \leq 0.05$). Another statistically significant difference was recorded with fresh ripe sausages between the samples containing N and L adipose tissues ($P \leq 0.05$). After a month of storage, statistically significant differences in malondialdehyde content were no longer recorded.

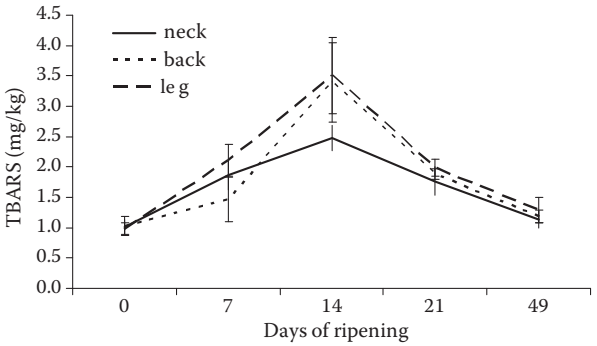

Figure 1. Oxidation process depending on the thiobarbituric acid reactive substance (TBARS) scores

Population of lactic acid bacteria (LAB) and contents of lactic acid and acetic acid

The development of the population of lactic acid bacteria in batches N, B, and L of dry fermented sausages during the course of the experiment is shown in Figure 2. From the initial values of 6.3 log CFU/g (N), 6.4 log CFU/g (B), and 6.7 log CFU/g (L), the population of lactic acid bacteria increased over the course of the following days, peaking on day 7 at 8.7 log CFU/g (N), 8.6 log CFU/g (B), and 8.6 log CFU/g (L), respectively. A decrease in lactic acid bacteria was observed on the following sampling days. During the produc-

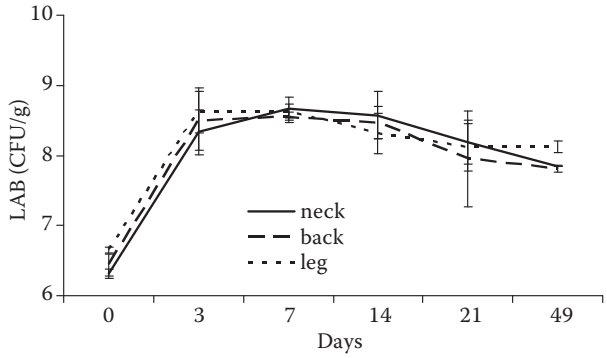

Figure 2. Lactic acid bacteria count (LAB)

tion of dry fermented sausages, lactic acid bacteria are added to the sausage mix as part of starting cultures in batches that guarantee a population of 10^6–10^7 bacterial cells in 1 g of the mix (Lücke & Hechelmann 1985). The reproduction of lactic acid bacteria occurs during the first days of fermentation and the population density peaks at 10^9 cells/g; this figure then remains relatively constant for the duration of the ripening process (Drosinos *et al*. 2005; Silvestri *et al*. 2007). Our results were consistent with these values.

Lactic acid is created by the fermentation of saccharides that are added to the sausage mixture and is produced by the action of lactic acid bacteria in particular (Stahnke & Tjener 2007). Its final concentration in the product depends on the initial amount of saccharides added to the mix, while the speed of fermentation is influenced by the type of starting culture and the temperature of the environment.

The greatest increase in lactic acid content was recorded during the first three days of ripening. During this period, these values increased approximately threefold, achieving concentrations of 239 µmol/g of dry matter (N), 300 µmol/g of dry matter (B), and 249 µmol/g of dry matter (L). These contens did not increase markedly during further ripening. Over a period of one month of storage, a slight decrease was observed to values of 219 µmol/g of dry matter (N), 222 µmol/g of dry matter (B), and 248 µmol/g of dry matter (L). A significant increase in the content of acetic acid during the first three days of ripening was also observed. During further ripening and storage, these values continued to increase slightly until day 21, to a concentration of 18.9 µmol/g of dry matter (N), 18.6 µmol/g of dry matter (B), and 20.5 µmol/g of dry matter (L), and day 49, to a concentration of 18.1 µmol/g of dry matter (N), 19.5 µmol/g of dry matter (B), and 16.7 µmol/g of dry matter (L). The final content of acetic acid after a month of storage was not higher in the samples with L adipose tissue than in those with the other two types of adipose tissue.

List and Klettner (1978) state that as much as 50% of lactic acid is created in the first four days of fermentation, and another 33% on days 4 to 14. In the present study, we discovered that 80% (N), 91% (B), and 76% (L) of the maximum concentration were attained in the given batches on day 3. This fact indicates the high level of activity of the starting cultures that were used. The values of the concentration of lactic acid measured in the types of used product were only insignificantly lower than the levels that were previously discovered by Kameník *et al*. (1992). They were, however, significantly higher than the values published by Girard *et al*. (1989), who tested the influence of pig fat on the process of fermentation and the drying of dry fermented sausages. All the batches of dry fermented sausages showed a pH of approximately 5.1 on day 42; the content of lactic acid ranged from 70 to 110 µmol/g of dry matter. According to Tjener & Stahnke (2007), the level of lactic acid in dry fermented sausages ranges from 0.4 to 3.1 g/100 g of dry matter.

Acetic acid is undesirable in dry fermented sausages as it may have a negative effect on the product taste. A certain quantity of acetic acid is, however, always present in these products, at a level that is smaller by a factor of 10–20 than the concentration of lactic acid. Our results correspond to this. The values we detected were, however, significantly lower than those stated by Girard *et al*. (1989), who measured values of 25–50 µmol/g of dry matter. This higher concentration is evidence for stronger activity of heterofermentative fermentation of saccharides in the product.

Sensory and texture characteristics

The sensory evaluation was performed at the end of ripening (day 21) and during the course of storage of the sausages (day 49). The assessed parameters were cut surface appearance, colour, matrix, fat particles, consistency, texture, taste, odour, and the occurrence of a ring (Figure 3).

At the end of the ripening process, the sensory assessors did not record any significant changes among the three types of adipose tissue used. The aroma of the sausages in which N adipose tissue was used received the worst assessment. After storage, the sausages in which adipose tissue from the legs was used received the worst assessment, particularly for taste and aroma. After storage, the fat particles in the sausages (demarcation of fat tissue) received the worst assessment of all sausages. The sausage in which N adipose tissue was used was assessed as the worst overall.

No differences between the individual fats were detected during the assessment of texture and consistency, not even during the course of storage. Certain differences were, however, recorded by the instrumental analysis of texture. An instrumental assessment, of texture was performed with a

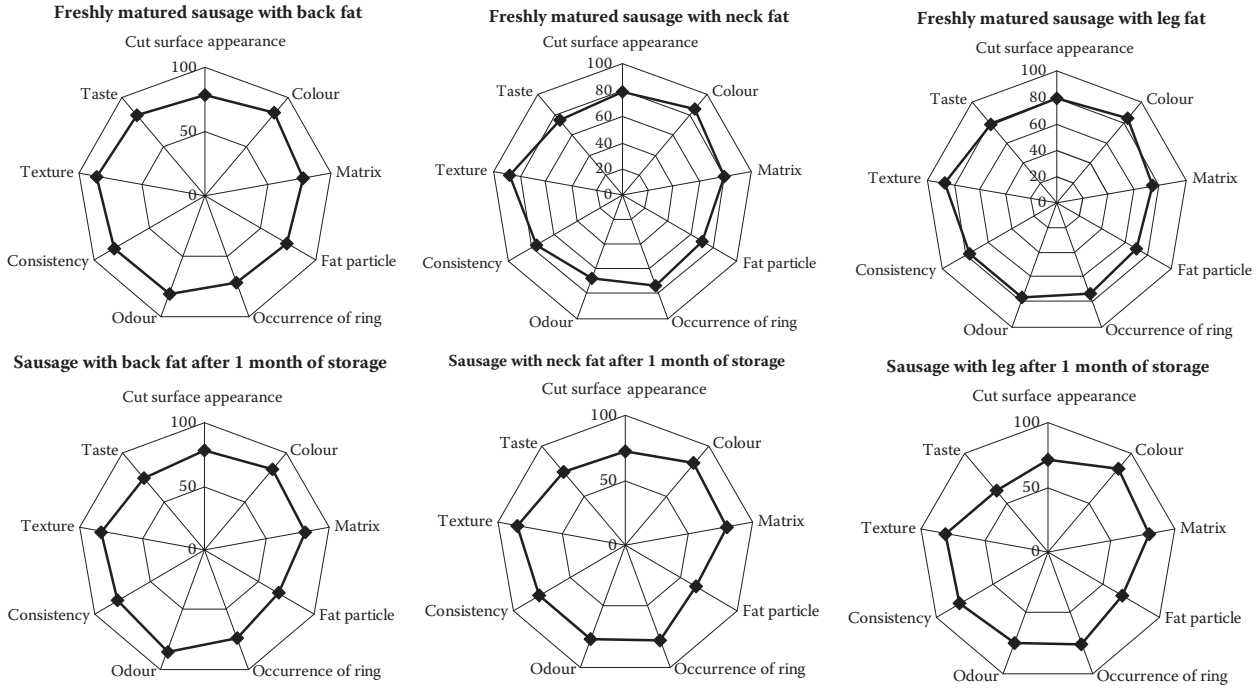

Figure 3. Sensory evaluation of dry fermented sausages

texture profile analysis (TPA). The parameters assessed were maximum force required for the initial compression of the sample (hardness) and cohesiveness (Table 4). The lowest hardness was discovered in the samples with N adipose tissue, and the highest in the samples in which L adipose tissue was used. After storage (day 49), the sausages were tougher in comparison with those at the end of the ripening process. No differences between the various types of adipose tissue or between sausages at the end of ripening and after storage were recorded in terms of cohesiveness.

Table 4. Texture characteristics of dry fermented sausages

Sample	Max. force (N)	Cohesiveness (–)
N 7	31.84 ± 1.77	1.39 ± 0.01
N14	40.73 ± 3.87	1.39 ± 0.02
N 21	40.38 ± 6.19	1.41 ± 0.04
N 49	48.68 ± 4.44	1.39 ± 0.02
B7	39.31 ± 6.95	1.35 ± 0.02
B14	43.03 ± 4.68	1.37 ± 0.02
B 21	42.07 ± 4.25	1.39 ± 0.03
B 49	53.30 ± 4.34	1.38 ± 0.02
L 7	38.90 ± 2.82	1.36 ± 0.01
L 14	37. 86 ±1.65	1.41 ± 0.01
L 21	50.41 ± 4.73	1.39 ± 0.01
L 49	56.39 ± 5.30	1.41 ± 0.02

N – neck, B – back, L – leg

CONCLUSION

(a) The highest percentage of fat (80.40%) was found in the B adipose tissue. The N adipose tissue contained only a slightly lower fat proportion (79.50%), while the L adipose tissue had a markedly lower percentage of fat (70.70%). The individual FA compositions in the fat from three different anatomical parts of the carcass were compared, and no statistically significant differences were found concerning the most abundant FA, such as palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1n-9). The largest proportion of SFA was found in the N adipose tissue (42.79%), followed by the B adipose tissue (42.25%), with the lowest concentration found in the L adipose tissue (41.16%). In contrast, the largest proportion of MUFA was contained in the L, followed by the B, with the N adipose tissue containing the lowest proportion. The content of PUFA was the highest in the N and lowest in the L adipose tissues. These differences were not, however, statistically significant.

(b) A certain shift in the proportions of the individual FA groups occurred after mixting the pig

adipose tissue and the meat. A slight reduction in linoleic acid, linoelaidic acid, and linolenic acid in the mixture resulted in a reduction of total PUFA: 8.15% (N), 7.05% (B), and 7.19% (L). In comparison with pig adipose tissue, the proportion of SFA also decreased: 39.86% (N), 40.41% (B), and 39.35% (L). From the most abundant SFA, a decrease in palmitic and stearic acids was observed. In contrast, the addition of meat resulted in an increase in the dominant oleic acid, so that the MUFA proportion in the mixture increased to 51.99% (N), 52.54% (B), and 53.45% (L).

(c) Fermentation occurred in the standard manner in all three batches of the sausages produced (N, B, L), which can be documented based on the pH values obtained, the contents of lactic and acetic acid, and the population of lactic acid bacteria. At the end of the ripening process, the sensory assessors did not record any significant differences between the batches prepared from the various types of pig adipose tissue. The assertion that N adipose tissue is the best for the production of this type of product was not confirmed and neither was the claim that adipose tissue from the leg is unsuitable because of its insufficient toughness.

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