A direct method for determination of the pure muscle protein content in dry fermented sausages and cooked ham using creatinine adsorption

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Abstract

The aim of this work was to verify the determination of pure muscle proteins (PMP) from the total creatinine content in dry fermented sausages (DFS) during ripening and in cooked ham (CH) using the adsorption of creatinine on a solid adsorbent, and to compare the determined PMP values with the values determined by a reference method. The study also took in the determination of the content of dry matter, fat, total and pure proteins, and collagen. There was an increase in the values of all the indices in DFS. A positive correlation ($P < 0.001$) was found when comparing PMP values in DFS determined by the reference method and by the method of calculation from the total protein content and from the total creatinine content. No differences between the samples were determined in the observed indices for CH of the highest quality ($P > 0.05$). A difference was found in the content of collagen and PMP in CH of standard quality as determined by the reference method between samples CH4 and CH5. A positive correlation ($P < 0.001$) was found between the PMP values in CH determined by the reference method and by the method of calculation from the total creatinine content. The results for the PMP content in DFS and CH determined by the direct method using creatinine adsorption are comparable to the results determined by the reference method. The advantage of this method is the determination of creatinine, which is not part of vegetable protein.

Adulteration, creatine, meat products, non-collagen muscle protein

The most common form of adulteration of meat products is the addition of meat or other materials of lower economic value and lower quality (Meza-Márquez et al. 2010). One of the basic methods of evaluating the quality and authenticity of meat products is the determination of pure muscle proteins (PMP) using the Kjeldahl method. To calculate the PMP, the pure protein (PP) content is determined using the Kjeldahl method and the collagen content is subtracted from it (Hulánková et al. 2018). The minimum PMP content is stipulated for selected meat products by binding legislation in the Czech Republic (Decree No. 69/2016 Sb.).

Although the determination of PMP is currently commonly used in inspection practice, it is an indirect determination method that has its pitfalls and limitations. One of the disadvantages of this procedure is that proteins may not necessarily be precipitated quantitatively. Similarly, in the case of fermented products, the content of muscle protein detected is lower than that corresponding to the amount of meat originally added before the ripening of the meat product. With this method, it is also not possible to determine the origin of the contained protein, but merely its total content (Hajšlová et al. 2018).

One possibility is to determine the PMP content by means of a direct method employing the total creatinine content (2-imino-1-methylimidazolidin-4-one). Creatinine is the end product of the metabolism of creatine and creatine phosphate (Wyss and Kaddurah-Daouk 2000). Creatine and creatinine are characteristic components of muscle tissue and their testing is used to determine the presence of meat in food products. When we

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cook meat, however, the creatine content falls, while the creatinine content rises. The total protein content is usually determined as an indicator of quality, though determination of total creatine (creatine + 1.159 creatinine) should enable estimation of muscle tissue proteins, which are generally the most expensive proteins (Del Campo et al. 1998). If creatinine alone is measured as a quality index, then 20 mg of creatinine/100 g corresponds to 1 g of muscle protein/100 g (Kvasnička and Voldřich 2000). Dvořák (1981) reported that the relationship between total protein content and creatine content was not linear, while the relationship between PMP and creatine was such that 1 g of PMP contained an average of 23 mg of creatine and 1 mg of creatinine.

The possibility of determining total creatinine in meat and meat products spectrophotometrically was described by Kvasnička (2003). Creatinine forms a red-coloured complex with alkaline sodium picrate. The problem during analysis is probably the non-specificity of the Jaffe reaction, and determination may be hampered by a number of interfering substances (Mohabbati-Kalejahi et al. 2012).

The aim of this study was to analyse the possibility of determining total creatinine in dry fermented sausages and cooked ham with the use of creatinine adsorption on a solid adsorbent; subsequently, to verify the possibility of determining the PMP content directly by calculation; and to compare the results obtained using this method with the results found using the indirect reference method. The work also included the monitoring of selected indicators testifying to the composition of the analysed products.

Materials and Methods

Sampling and sample preparation

Samples of dry fermented sausages (DFS) (n = 48) were obtained from two producers in the Czech Republic. Samples were taken as the mix filled in a technological casing and placed in a curing chamber (KRA-Gen3 M, KKT-Lackner GmbH, Klagenfurt, Germany) at the Department of Food Technology at Mendel University in Brno. The chamber regime was set and adjusted during 16 days of drying at a relative air humidity (RH) of 92% and a temperature of 22 °C at the beginning, with a gradual decrease in values to 78% RH and a temperature of 15 °C. Four pieces were taken for the analysis of the sausage mix, after which 4 pieces were taken every 7 days and analysed up to day 35 of ripening. Samples of cooked ham (CH) (n = 18) of two quality grades (standard and highest) were obtained from a producer in the Czech Republic. All chemicals used were of analytical grade. Creatinine 98% (Thermo Fisher Scientific, Geel, Belgium) and halloysite nanoclay (Sigma-Aldrich Co LLC, Merck KGaA, Darmstadt, Germany) were used.

All chemical analyses were performed at the Laboratory of Meat and Meat Product Quality at the Department of Animal Origin Food & Gastronomic Sciences, University of Veterinary Sciences Brno. Samples of DFS and CH (each sample approximately 100 g) were ground and homogenised in a KENWOOD FDM10 two-speed chopper (Kenwood Ltd., Havant, United Kingdom). The content of dry matter, fat, total protein (TP), PP, collagen and total creatinine (TC) were determined in the samples on each day of sampling (0, 7, 14, 21, 28 and 35).

Determination of the dry matter content

A drying method with sea sand (ISO 1442:1997) at a temperature of 103 ± 2 °C for 24 h was used to determine the content of dry matter. After cooling in a desiccator, the samples were weighed and the dry matter content was calculated.

Determination of the fat content

The fat content was determined using a Soxtec™ 2055 instrument (FOSS, Hilleroed, Denmark). Petroleum ether was used as the extraction solvent. Samples weighing 3 g were left in the drier for 3 h at 135 ± 2 °C and extracted with the reagent in the instrument for 86 min.

Determination of the total protein content

The TP content was determined on a Kjeltec™ 2300 instrument (FOSS) using the Kjeldahl method, during which all the nitrogen in the analysed sample is determined and a factor of 6.25 is used to convert the nitrogen content into the protein content. The principle lies in the fact that the sample is first mineralised by boiling with an excess of concentrated sulphuric acid in the presence of a catalyst that accelerates mineralisation. The ammonia formed from nitrogenous substances binds to the sulphuric acid and thereby forms ammonium sulphate. This is then decomposed by sodium hydroxide into ammonia, which is distilled with water vapour in a known standard solution of acid, which must be in a certain excess. The proportion of acid that does not react is determined by back titration with a solution of sodium hydroxide (Kameník et al. 2023).

Determination of the pure protein content

Pure proteins were determined after the precipitation of non-protein N-substances with hot tannin and subsequent conversion of organic nitrogen into inorganic nitrogen on a Kjeltec™ 2300 instrument (FOSS) using the Kjeldahl method. A factor of 6.25 was used to convert the nitrogen content into the protein content.

Determination of the collagen content

The collagen content was determined spectrophotometrically at a wavelength of 550 nm on a GENESYSTM 6 spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA) as the amount of 4-hydroxyproline. The hydroxyproline content was obtained from the calibration curve and converted into the collagen content (f = 8).

Determination of the pure muscle protein content calculated from the total protein – PMP-T

The PMP content was calculated from the TP after subtracting the collagen content.

Determination of the pure muscle protein content by the reference method – PMP-R

Myofibrillar and sarcoplasmic proteins are referred to as PMPs, i.e. the sum of non-collagenous proteins. The PMP content was calculated as the difference between the PP content and the collagen content.

Determination of the pure muscle protein content by direct calculation – PMP-D

The PMP content was calculated from the total creatinine content using the relationship given below.

$$PMP - D\ (\text{g/100 g of sample}) = \frac{creatinine\ content\ in\ sample\ (\text{mg/100 g})}{20}$$

The total creatinine content was determined in an acidic hydrolysate of the sample spectrophotometrically at a wavelength of 495 nm based on the Jaffe reaction using a modified method according to Kvasnička (2003) with the use of a solid adsorbent. Creatinine is adsorbed from the sample onto halloysite nanoclay (SIGMA-ALDRICH, Co., St. Louis, MO, USA) thereby removing interfering components that artificially increase the creatinine content values. The adsorbed creatinine is subjected to a reaction with alkaline sodium picrate with which it forms a red-coloured complex, the intensity of which is proportional to the creatinine concentration.

Statistical analysis

The data were expressed as mean ± SD. The data were compared statistically using the statistical software Statistica version 7.1 (StatSoft, Tulsa, OK, USA). The tests used were one-way ANOVA and *post hoc* Tukey HSD test. The results were compared using the linear regression method and the correlation coefficients between PMP-T and PMP-D and between PMP-R and PMP-D were determined.

Results

Compositional changes in dry fermented sausages during ripening

Over the 35 days of DFS ripening, there was a significant increase ($P < 0.05$) in the content of dry matter from 47.80% to 74.03% (Producer 1; Table 1) and from 43.53% to 70.30% (Producer 2; Table 2). The fat content also increased significantly ($P < 0.05$) along with the content of dry matter from 25.78% to 41.88% and from 22.33% to 36.07%, respectively.

In connection with the increase in dry matter, the TP content also increased significantly ($P < 0.05$) during the course of ripening from 14.37% to 22.41% (Producer 1) and from 14.95% to 24.08% (Producer 2), and the PP content also increased analogously from 13.28% to 19.66% and from 14.12% to 20.72%, respectively. As can be seen from Tables 1 and 2, faster changes in the composition of DFS are seen at the beginning of ripening, when there is more intense drying of the product. During ripening, the collagen content ranged from 1.69% to 2.48% in the case of Producer 1 and from 1.30% to 2.35% in the case of Producer 2.

Changes of total creatinine and pure muscle protein content in dry fermented sausages during ripening

Values for the TC content, PMP-T, PMP-R and PMP-D in DFS are shown in Table 1 (Producer 1) and Table 2 (Producer 2). The TC content in DFS increased significantly ($P < 0.05$) from 0.26% to 0.33% during the course of 35 days of ripening in the case

of Producer 1, while the TC content increased from 0.25% to 0.37% in the case of Producer 2. The values of PMP-T were significantly higher ($P > 0.05$) than PMP-D values throughout the ripening period, except on Day 0 of ripening (Producer 1). The values for the PMP-D content in DFS calculated from the TC content differed ($P > 0.05$) from the values for the PMP-R content on Day 0 of ripening ($P < 0.05$) in the case of Producer 1 and on Day 21 in the case of Producer 2. No differences were found in the content of PMP-D and PMP-R in DFS ($P < 0.05$) on the other days of sampling. When comparing the results of PMP-T and PMP-D over the course of 35 days of ripening of DFS, a positive correlation was found between the two methods of r = 0.630 for

Table 1. Chemical analysis of dry fermented sausages during ripening for 35 days – Producer 1 (mean ± SD).

Indicator (%)	Stage of fermentation (days)					
	0	7	14	21	28	35
Dry matter	47.80±0.11^{a}	57.92±0.24^{b}	64.54±0.21^{c}	70.65±0.31^{d}	72.40±0.20^{e}	74.03±0.12^{f}
Fat	25.78±0.48^{a}	30.67±1.76^{b}	34.79±0.77^{c}	39.62±1.86^{d}	39.48±0.94^{d}	41.88±1.24^{d}
TP	14.37±0.16^{a}	17.19±0.12^{b}	19.88±0.82^{c}	21.36±0.36^{d}	22.00±0.42^{de}	22.41±0.49^{e}
PP	13.28±0.28^{a}	15.47±0.49^{b}	16.82±0.30^{c}	18.52±0.59^{d}	19.21±0.18^{de}	19.66±0.43^{e}
Collagen	1.69±0.16^{a}	1.68±0.09^{a}	2.13±0.10^{bc}	2.12±0.23^{bc}	2.48±0.24^{b}	1.95±0.06^{ac}
TC	0.26±0.01^{a}	0.28±0.01^{a}	0.32±0.01^{b}	0.32±0.01^{bc}	0.35±0.01^{cd}	0.33±0.02^{bd}
PMP-T	12.68±0.11^{Aa}	15.51±0.15^{Ab}	17.75±0.80^{Ac}	19.24±0.46^{Ad}	19.52±0.47^{Ade}	20.46±0.49^{Ae}
PMP-R	11.58±0.44^{Ba}	13.79±0.58^{Bb}	14.69±0.33^{Bb}	16.40±0.55^{Bc}	16.73±0.37^{Bcd}	17.71±0.44^{Bd}
PMP-D	13.19±0.35^{Aa}	14.17±0.72^{Ba}	15.77±0.49^{Bb}	16.21±0.55^{Bc}	17.26±0.29^{Bcd}	16.80±0.62^{Bbd}

TP – total protein; PP – pure protein; TC – total creatinine; PMP-T – pure muscle protein calculated by subtracting collagen content from TP content; PMP-R – pure muscle protein determined by the reference method; PMP-D – pure muscle protein determined by the direct method

$^{a, b, c, d, e, f}$ different superscripts mean that values in the same row are significantly different at $P < 0.05$

$^{A, B}$ different superscripts mean that PMP-R and PMP-D values in the same column are significantly different at $P < 0.05$

Table 2. Chemical analysis of dry fermented sausages during ripening for 35 days – Producer 2 (mean ± SD)

Indicator (%)	Stage of fermentation (days)					
	0	7	14	21	28	35
Dry matter	43.53±0.13^{a}	52.15±0.24^{b}	58.57±0.65^{c}	63.76±0.50^{d}	68.00±0.22^{e}	70.30±0.09^{f}
Fat	22.33±1.50^{a}	25.84±2.43^{b}	32.06±0.85^{c}	32.53±0.39^{c}	36.51±0.69^{d}	36.07±1.45^{d}
TP	14.95±0.12^{a}	17.41±0.22^{b}	19.37±0.37^{c}	21.42±0.45^{d}	23.08±0.46^{e}	24.08±0.44^{f}
PP	14.12±0.12^{a}	15.62±0.13^{b}	17.44±0.51^{c}	19.20±1.05^{d}	19.64±0.23^{de}	20.72±0.73^{e}
Collagen	1.30±0.07^{a}	1.49±0.07^{ab}	1.55±0.07^{b}	2.35±0.11^{c}	2.00±0.11^{d}	2.10±0.18^{d}
TC	0.25±0.00^{a}	0.28±0.00^{b}	0.31±0.01^{b}	0.29±0.02^{b}	0.34±0.02^{c}	0.37±0.01^{d}
PMP-T	13.64±0.07^{Aa}	15.92±0.19^{Ab}	17.82±0.32^{Ac}	19.07±0.51^{Ad}	21.08±0.53^{Ae}	21.98±0.50^{Af}
PMP-R	12.82±0.19^{Ba}	14.13±0.07^{Bb}	15.89±0.56^{Bc}	16.84±1.04^{Bcd}	17.64±0.12^{Bde}	18.62±0.76^{Be}
PMP-D	12.70±0.22^{Ba}	14.19±0.08^{Bb}	15.53±0.72^{Bb}	14.63±0.94^{Cb}	17.11±0.78^{Bc}	18.49±0.40^{Bd}

TP – total protein; PP – pure protein; TC – total creatinine; PMP-T – pure muscle protein calculated by subtracting collagen content from TP content; PMP-R – pure muscle protein determined by the reference method; PMP-D – pure muscle protein determined by the direct method

$^{a, b, c, d, e, f}$ different superscripts mean that values in the same row are significantly different at $P < 0.05$

$^{A, B, C}$ different superscripts mean that PMP-R and PMP-D values in the same column are significantly different at $P < 0.05$

Producer 1 and r = 0.520 for Producer 2 ($P < 0.001$). When comparing the results of PMP-R and PMP-D over the course of 35 days of ripening of DFS, a positive correlation was found between the two methods of r = 0.592 for Producer 1 and r = 0.670 for Producer 2 ($P < 0.001$).

Basic composition of two quality grades of cooked ham

The basic chemical composition of CH is shown in Table 3. No significant difference ($P > 0.05$) was found in any of the analysed parameters between samples of CH of the highest quality. Among CH samples of standard quality, sample CH4 contained less collagen than sample CH5 ($P < 0.05$). The analysed samples of CH of the highest quality contained 25.49% (CH2) to 25.83% (CH1) dry matter, while CH of standard quality contained from 23.06% (CH6) to 24.64 % (CH5) dry matter. The fat content ranged between 2.03% and 2.40% in CH of the highest quality, and between 1.73% and 3.13% in CH of standard quality. Nevertheless, the value found by the post hoc Tukey HSD test was $P = 0.137$, meaning that differences in the fat content of CH were not detected.

The TP content in CH of standard quality ranged from 12.93% to 14.11%, while the TP content in CH of the highest quality was between 17.93% and 19.22%. The collagen content in this study ranged from 0.62% in CH of the highest quality to 1.25% in CH of standard quality.

Table 3. Chemical analysis of two different quality grades of cooked ham (mean ± SD).

Indicator (%)	Highest quality (n = 3)			Standard quality (n = 3)		
	CH1	CH2	CH3	CH4	CH5	CH6
Dry matter	25.83±0.52^{a}	25.49±0.53^{ab}	25.68±0.43^{a}	23.35±1.22^{bc}	24.64±0.84^{abc}	23.06±0.14^{c}
Fat	2.40±0.67	2.05±0.65	2.03±0.30	3.13±0.48	2.99±0.73	1.73±0.10
TP	19.22±0.74^{a}	19.13±0.48^{a}	17.93±1.54^{a}	14.11±0.43^{b}	12.93±0.15^{b}	13.04±0.62^{b}
PP	16.86±0.59^{a}	16.92±0.47^{a}	17.19±0.64^{a}	12.72±0.24^{b}	11.46±0.03^{b}	11.79±0.26^{b}
Collagen	0.62±0.09^{a}	0.67±0.11^{a}	0.68±0.18^{a}	0.82±0.08^{a}	1.25±0.07^{b}	0.99±0.07^{ab}
TC	0.34±0.04^{a}	0.33±0.04^{a}	0.34±0.04^{a}	0.25±0.03^{b}	0.24±0.02^{b}	0.24±0.02^{b}
PMP-T	18.61±0.64^{a}	18.46±0.47^{a}	17.25±1.72^{a}	13.29±0.35^{b}	11.67±0.21^{b}	12.05±0.55^{b}
PMP-R	16.24±0.49^{a}	16.26±0.51^{a}	16.51±0.45^{a}	11.90±0.16^{b}	10.21±0.10^{c}	10.80±0.19^{bc}
PMP-D	16.98±2.03^{a}	16.59±2.38^{a}	17.12±2.18^{a}	12.29±1.25^{b}	11.92±0.87^{b}	11.88±1.21^{b}

TP – total protein; PP – pure protein; TC – total creatinine; PMP-T – pure muscle protein calculated by subtracting collagen content from TP content; PMP-R – pure muscle protein determined by the reference method; PMP-D – pure muscle protein determined by the direct method

$^{a, b, c,}$ Different superscripts mean that values in the same row are significantly different at $P < 0.05$

Content of total ceratinine and pure muscle protein in cooked ham

The results of the measurement of the contents of TC, PMP-T, PMP-R and PMP-D in CH are shown in Table 3. No significant difference ($P > 0.05$) in the contents of TC, PMP-T, PMP-R or PMP-D was found between the samples from the hams of the highest quality. In standard quality hams, a significantly higher ($P < 0.05$) PMP-R content was found in sample CH4 than in sample CH5. The TC content ranged from 0.24% to 0.25% in CH of standard quality, while the TC content ranged from 0.33% to 0.34% in CH of the highest quality. No significant differences ($P > 0.05$) in PMP-T, PMP-R, and PMP-D values were found in any of the analysed samples. Results of the Pearson correlation indicated a highly significant ($P < 0.001$) positive relationship (r = 0.757) between PMP-T and PMP-D values. A positive correlation of r = 0.380 ($P = 0.038$) was found between the PMP-R and PMP-D values in CH.

Discussion

Changes in the chemical composition of dry fermented sausages during ripening

Fat is one of the main components of DFS. The maximum fat content is regulated in selected DFS by legislation in the Czech Republic (Decree No. 69/2016 Sb.). The values of TP and PP in finished DFS are similar to those reported by Kameník et al. (2023). Hughes et al. (2002) report that sarcoplasmic proteins with molecular weights of 16, 29, 50, 97 and 150 kDa disappeared during the first 3 days of ripening as a result of proteolysis or denaturation induced by acid and salt. The fermentation and ripening of dry fermented sausages (DFS) are characterised by three main biochemical processes – glycolysis, proteolysis, and lipolysis. The proteolysis of myofibrillar and sarcoplasmic proteins occurs during the fermentation and ripening of DFS (Hughes et al. 2002; Aro Aro et al. 2010; Ikonić et al. 2013). Native muscle enzymes are primarily responsible for the initial degradation of sarcoplasmic proteins. Intense degradation of the main myofibrillar proteins myosin and actin occurs during ripening. Coagulase-negative staphylococci are particularly active in this regard (di Cagno et al. 2008; Berardo et al. 2017), though lactic acid bacteria also have proteolytic enzymes capable of releasing peptides and amino acids (Ljungh and Wadström 2009). The degradation of actin was fastest in DFS inoculated with *S. carnosus*, with the almost complete degradation of this protein by the end of ripening (Day 35) (Hughes et al. 2002).

Proteolysis generally takes place in DFS in two stages (Berardo et al. 2017). Endopeptidases (proteinases) first break intact proteins down into small peptides. In the second step, the peptides released are then further degraded by bacterial exopeptidases into free amino acids, dipeptides and tripeptides. There are also exopeptidases located in the lysosomes of muscle fibres, e.g. tripeptidyl peptidases and dipeptidyl peptidases. Their action releases tripeptides and dipeptides (Toldrá and Reig 2015). The main lysosomal proteinases are cathepsins B and L (highly active at pH 6.0) and cathepsin D (with an optimal pH in a range of 3.0–5.0) (Toldrá and Reig 2015). Cathepsins are responsible for the initial breakdown of proteins by endopeptidases. These enzymes remain stable in dry meat products for a number of months (Berardo et al. 2017) and cathepsin D was found to be a major contributor to proteolysis in fermented sausages. Cathepsins are muscle fibre enzymes (Toldrá and Reig 2015). Proteolysis in DFS is usually studied by determining the proportions of protein and non-protein nitrogen by electrophoretic techniques or by measuring the amino acids released during ripening. Proteomic techniques that use mass spectrometry now also make it possible to identify peptides released during ripening (Berardo et al. 2017).

The hydrolysis of proteins leads to the formation of polypeptides, peptides, free amino acids (FAA) and other low-molecular-weight substances (Díaz et al. 1997). Sarcoplasmic and myofibrillar proteins are both subject to hydrolysis during the production of DFS. Actin is the second most abundant protein in skeletal muscle (meat) and is subject to intense degradation during the production of DFS (Berardo et al. 2017). Actin is then a source of various peptides in the meat mixture. These are already released by the action of cathepsins B and D during the fermentation process, i.e. in the first days of production. According to the authors Kong et al. (2020), changes were evident on both myosin heavy chains of a molecular weight of 200–220 kDa and myosin light chains of a molecular weight of 21 kDa during the ripening of DFS for a period of 21 days. Ikonić et al. (2013) recorded the hydrolysis of approximately 50% of myosin heavy chains in the Serbian DFS Petrovská Klobása (diameter 55 mm) during 30 days of ripening.

When a tannin solution is used to precipitate the protein fraction in the analysed samples, we are faced with the question from what molecular weight do non-protein nitrogenous organic substances stop forming complexes with tannins (Kameník et al. 2023).

In addition to proteins and peptides, the formation of complexes of tannins with nitrogenous organic compounds has also been demonstrated for the amino acid arginine, as well as for the polyamines putrescine, spermidine and spermine, for the nitrogenous bases adenine, cytosine, guanine, uracil and thymine, and for the aminosaccharides chitin and chitosan. Apart from arginine, Adamczyk et al. (2017) demonstrated no reaction with tannins in any other amino acid.

The content of stromal proteins ranges from 2 to 3% in lean pork meat. Collagen, which is the basic protein in connective tissue, provides strength and support to the muscle structure. Collagen generally represents approximately 5.3% of meat proteins and there are several types (I–V) containing different polypeptide chains (up to 10 α-chains). Type I collagen is found mainly in the epimysium and perimysium, whereas Types III, IV and V are found in the endomysium (Toldrá and Reig 2015). Collagen displays digestive resistance because it has a high percentage of hydroxyproline and glycine which are responsible for a strong tertiary structure (Lee et al. 2021). Hydroxyproline analysis is often used as a measure for determining the total connective tissue in muscle (Kauffman 2012).

The TC values found in our study correspond to the value for the TC content (318.0 mg/100 g) found in DFS using isotachophoretic determination (Kvasnička and Voldřich 2000). The creatine and creatinine content in raw meat was studied by Mora et al. (2008a). The largest creatine content, as was also the case for carnosine, was found in the glycolytic muscles semimembranosus, longissimus dorsi, gluteus maximus and biceps femoris. This could be due to the highest level of dependence on anaerobic metabolism found in glycolytic muscles. In fact, the higher values of creatine may come from its phosphorylated form phosphocreatine which is involved in the transfer of high energy phosphate to adenosine diphosphate. The creatine and creatinine content also increases in this type of metabolism with the glycolytic activities of the muscle. The DFS used in this study are normally ripened under traditional fermentation conditions: fermentation temperature 18–24 °C for more than 40 h to reach pH 5.3; final pH often > 5.0, final a_w < 0.90, total production time > 3 weeks (Holck et al. 2015). According to the Czech legislation, this type of DFS must contain a minimum of 14.0% PMP (Decree No. 69/2016 Sb.). This value was already reliably achieved in the products after 14 days of ripening, both in the case of determination by the reference method and when the new direct determination method with adsorption of creatinine on halloysite nanoclay was used.

Differences in the chemical composition of the two quality grades of cooked ham

Cooked ham is divided into three quality grades in the Czech Republic according to the PMP content (standard, select, and highest quality). The ratio of protein to water is 1:4 for products of very high quality. The yield is around 115%, the high yield being the result of a more than four times higher water content than the protein content (Feiner 2006). Similar values were reported in the production of CH from the quadriceps femoris and semimembranosus muscles by Utrera et al. (2012). The fat content values are higher in CH when biceps femoris is used. Válková et al. (2007) found a fat content from 1.56% to 4.04% in samples of CH from the retail network. Los et al. (2014) determined an average protein content of 14.21% in CH, while only one of their samples did not comply with the standard applicable in Brazil (minimum 14%). Information on the PP content in CH is not usually given in studies and is determined for the calculation of PMP (Válková et al. 2007).

The principal factor determining meat texture is the connective tissue protein collagen. Connective tissue and its structure in muscle are major factors in the variability in ham. The collagen content affects two features of the ham – its slicing characteristics and its texture (Boutten et al. 2000). Válková et al. (2007) analysed the collagen content in CH of three quality classes and the values they found ranged from 0.42% to 0.97%.

The collagen content affects the TP content and depends on the type of muscle used. The collagen content is higher in m. biceps femoris than it is in m. semimembranosus (Boutten et al. 2000).

If the content of creatine and creatinine in skeletal muscle is relatively constant (Dahl 1963), it is evident that CH of the highest quality was made from a larger amount of lean meat than CH of standard quality. As can be seen from the results of this work, no significant differences ($P > 0.05$) were found between the PMP values determined by the reference method and the direct method investigated. The Czech legislation stipulates a minimum content of PMP of 10.0% in CH of standard quality and 16.0% in CH of the highest quality (Decree No. 69/2016 Sb.). Table 3 shows that this requirement is observed in all samples for both PMP-R and PMP-D.

The determination of the creatinine content tends to be associated with the glomerular filtration rate and the diagnosis of renal dysfunction (Kashani et al. 2020). The concentration in the blood is proportional to the muscle mass and is not subject to major changes, with the exception of kidney disease in which it increases (Kotaška et al. 2008). Not many works have considered the content of creatine and creatinine in meat or meat products in connection with their quality, e.g. Dahl (1963), Del Campo et al. (1998) and Kvasnička and Voldřich (2000). Other authors have considered the conversion of creatine and the formation of creatinine during the production of meat products (Mora et al. 2010), during heat treatment (Snider and Baldwin 1981; Mora et al. 2008b), or depending on the type of muscle (Mora et al. 2008a).

When determining the content of TC in meat and meat products, a number of interfering constituents may influence the analysis result. This work clearly demonstrated that with the use of creatinine adsorption on halloysite nanoclay, the action of interfering substances is prevented and the PMP-D content in DFS and CH, which correlates with the PMP-R content determined by the reference method, can be obtained by calculation from the amount of TC. The quality of DFS and CH can therefore be monitored on the basis of determination of PMP-D values. Determination of PMP-D is a direct method of assessing the creatinine content, whereas PMP-T and PMP-R represent an indirect method of determination via the TP or the PP content and the collagen content. Considerable time can be saved by the introduction of this method into laboratory practice. Another advantage of this method is the determination of creatinine, which does not comprise a part of vegetable protein, which means that this method can also be used to detect possible adulteration of meat products and deception of the consumer. The methodology used has been certificated and granted attestation by the State Veterinary Administration of the Czech Republic under No. SVS/2023/175437-G.

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