potravinárstvo®
Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 14, 2020, p. 149-155
https://doi.org/10.5219/1317
Received: 9. February 2020. Accepted: 2 March 2020.
Available online: 28 March 2020 at www.potravinarstvo.com

ISSN 1337-0960 (online)

COMPARISON OF HEAT-STABLE PEPTIDES USING A MULTIPLE-REACTION MONITORING METHOD TO IDENTIFY BEEF MUSCLE TISSUE

Daniil Khvostov, Natalya Vostrikova, Irina Chernukha

ABSTRACT
Nowadays, proteomics is widely used as an analytical control method. A new method for determining animal tissue species-specificity based on a combination of two effective methods of food analysis, liquid chromatography (LC) and mass spectrometry (MS), was used in this work. Using this approach, it became possible to detect peptides. This work presents a comparison of species-specific, heat-stable peptides for the identification of beef. The objects of the study were native and boiled model mixtures containing beef with concentrations of 8% (w/w) and 16% (w/w). Pork was also added to the recipe to control for false-positive results. A high-performance liquid chromatography technique with mass spectrometric detection (LC-MS/MS) was used. Analysis of finished samples takes 25 minutes and is adapted to detect marker peptides. From the processing of the obtained data, three beef marker peptides were identified that were accepted as the best candidates. Two peptide prototypes, NDMAAQYK and YLEFISDAIIHVLHAK from the myoglobin protein and SNVSDAVAQSAR from the triosephosphate isomerase protein, were selected as potential biomarkers. For all samples, the signal-to-noise ratio (S/N) was set above 10. Temperature was not found to affect the structure and detection of marker peptides in samples with a muscle tissue concentration of 8% (w/w) at p <0.05. This approach is universally applicable for comparing biomarkers of other types of meat and to identify the most suitable candidates.

Keywords: biomarker; LC-MS/MS; prototype peptides; meat authentication; heat-stable peptide

INTRODUCTION
Over the past 15 years, extensive research has been conducted around the world on the study of protein substances in raw meat and meat products, both native and those formed in the process of various technological treatments.

A classic quantification method in proteomics is the use of an isotopic tag, the modification of which has more than 40 species **(Kopylov and Zgoda, 2007)**. There are also techniques that do not use isotopic labels **(Kopylov, Zgoda and Archakov, 2009)**. The sensitivity of protein determination compared with gel electrophoresis increases by several orders of magnitude. More recently, the complexity of the study of phosphorylated proteins has been overcome. Various post-translational modifications of proteins with high sensitivity and specificity are studied by the Selected Reaction Monitoring (SRM) method **(Zav'yalova, et al., 2014)**. Recently, a method of identifying species-specific molecular markers in the field of food analysis has gained strength, based on a combination of two methods, high-performance liquid chromatography (HPLC) and mass spectrometry (MS), used to detect peptides. Using this method, up to 0.5% (w/w) chicken meat was found in meat mixtures **(Sentandreu et al., 2010)**. In more recent studies, in boiled meat products, up to 1.0% (w/w) impurities of beef, pork, chicken, duck and goose were detected **(Montowska and Fornal, 2017)**. Heat treatment products were analysed using marker peptides derived from myosin 1 and 2 light chains. It is very important to determine the limit of detection (LOD) of the method. Using this criterion, one can compare various methods aimed at determining muscle tissue. Indicators of 0.5% and below were set for meat products. As an example, the established quantification limit for buffalo and sheep meat was up to 0.48% (w/w) meat **(Naveena et al., 2017)**. The good thermal stability of the peptides was demonstrated by the authors to identify horse and pork markers a lower limit of 0.24% **(Von Bargen, Brockmeyer and Humpf, 2014)**.

Scientific hypothesis
Using the S/N criterion, it is proposed that peptide markers be compared for the authenticity of raw meat and heat-treated meat. The aim of this work was to establish the best candidates for the species-specificity of beef. The selected biomarkers will be used for a highly specific and reliable method of multivariate identification and quantification of the proportion of muscle tissue.

MATERIAL AND METHODOLOGY

Model mixtures of minced muscle tissue were prepared in accordance with standard industrial procedures. A set of samples with a given recipe was prepared (Table 1). Beef muscle tissue content was 8% (w/w) and 16% (w/w). The calculation of muscle tissue content was carried out according to BEFFE (bindegewebseiweißfreies Fleischeiweiß − meat proteins that do not contain connective tissue) **(Leitsätze für Fleisch und Fleischerzeugnisse, 2016)**. Samples of minced meat mixtures were placed in a collagen shell and cooked to a core temperature of 72 °C.

Reagents and solvents

All reagents used were of U.S.P. purity or higher. All solvents, including water, were used with the LC-MS label.

Protein extraction

A 100.0 ±0.1 mg portion of each sample was weighed on an analytical balance (CP224S, Sartorius, Germany). A 1000 μL volume of denaturing buffer (6 M guanidine chloride) was added to the sample and ground in a mortar until completely dissolved. Samples of homogenized muscle tissue (MagNA Lyser, Roche Applied Science, Germany) were centrifuged at 10,000 rpm for 15 minutes at 4 °C (5430 R, Eppendorf, Germany) and 10 μL of sample was transferred to a 1.5 mL tube (for subsequent hydrolysis).

Protein digestion

Disulphide bridges were restored by adding 2 μL of dithiothreitol (0.5 M in water) and incubating the samples at 37 °C for 60 minutes (Thermomixer comfort, Eppendorf, Germany). Then, sulfhydryl groups were alkylated by adding 5 μL of iodoacetamide (0.5 M in water) and incubating them in the dark for 30 min at room temperature. Ultrafiltration at 13,000 rpm for 15 minutes at 4 °C using bicarbonate buffer was used to eliminate salts and denaturing agents. Protein content was measured by using a Quant-it protein analysis kit (Thermo Fisher Scientific, USA) with a Qubit fluorometer (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Trypsin digestion was carried out by using an enzyme-to-substrate ratio of 1:50 and incubating the reaction for 16 hours at 37 °C. Enzymatic hydrolysis was stopped by adding 1 μL of formic acid. Samples were stored at -20 °C and thawed before analysis.

LC-MS/MS analysis

For chromatographic analysis, a ZORBAX Eclipse Plus C18 column with a fast HD resolution of 2.7 μm (50 × 2.1 mm; Agilent Technologies, Santa Clara, California, USA) was used. Separation was performed by using an Agilent 1260 Infinity HPLC system (USA). The flow rate was set at 0.4 $mL.min^{-1}$, the column temperature was 30 °C, and the sample temperature was 19 °C; eluent A was water with 0.1% (v/v) formic acid, and eluent B was acetonitrile with 0.1% (v/v) formic acid. Gradient elution was performed as follows parameters: 0 min 95% A, 0 – 10 min from 95% A to 40% A, 10 – 15 min from 40% A to 0% A, 15 – 20 min 0% A, 20 – 21 min from 0% A to 95% A, 21 – 25 min 95% A (total analysis time 25 min). The injection volume was 10 μL for all types of samples.

Peptides were detected by using a three-quadrupole mass spectrometer (6410, Agilent Technologies, Santa Clara, California, USA) **(Khvostov et al., 2019)**.

Statistical analysis

STATISTICA 10.0 software was used in this study for statistical analysis. Significant differences were verified by using two-way analysis of variance (ANOVA), $p < 0.05$. Data were extracted from bioprograms in Microsoft Excel (USA).

RESULTS AND DISCUSSION

In this work, we used the **Skyline program (2019)**, capable of theoretically cleaving proteins and listing the SRM for each peptide (Table 2). Protein analysis was performed by using biomodelling. If it is necessary to process complete protein sequences during analysis of LC-MS/MS data, it is possible to use software such as Spectrum Mill (Agilent Technologies, Santa Clara, CA, USA) **(Sarah et al., 2016; Fornal and Montowska, 2019; Montowska and Fornal, 2017; Montowska and Fornal, 2019)**, Protein Lynx Global Server (Waters) **(Naveena et al., 2017)**, Peaks Studio software (Bioinformatics Solutions, Waterloo, ON, Canada) **(Prandi et al., 2017; Prandi et al., 2019)** and MASCOT (Matrix Science, Boston, MA, USA) **(Sentandreu et al., 2010; Naveena et al., 2017; Ruiz Orduna et al., 2015; Ruiz Orduna et al., 2017; Fornal and Montowska, 2019; Montowska and Fornal, 2017; Montowska and Fornal, 2019)**. In our work with the search for parameters for biomarkers on a mass spectrometer, the Skyline program proved to be the best. This is the best choice in the presence of a previously studied peptide sequence for develop of MRM methods. Most often, three transitions were selected. Only y-ions were used. The transition from parent ion (*m/z)* to product ions (*m/z)* occurred from a smaller to a larger one (*m/z)*.

Table 1 Muscle tissue content in the experimental mixtures.

Mixture	Beef (97% (w/w) muscle tissue), % (w/w)	Pork (90% (w/w) muscle tissue), % (w/w)	Pork (50% (w/w) muscle tissue), % (w/w)	Pork (20% (w/w) muscle tissue), % (w/w)	Total muscle tissue, % (w/w)
1	16.0	59.3	0.0	0.0	75.3
2	8.0	0.0	12.4	9.9	30.3
3	0.0	32.1	10.0	0.0	42.1

Table 2 Identification characteristics of beef (*Bos taurus*) heat-stable peptide markers for LC-MS/MS methods.

Protein	Marker peptide sequence	Parent ion (*m/z*), product ions (*m/z*)	Collision energy (V)	Retention time (min ±*SD*)	References*
Myoglobin	HPSDFGADAQAAMSK	766.8 → 1395.6, 949.4, 892.4, 821.4	24.8	6.60 ±0.06	**Claydon et al. (2015); Li et al. (2018) Khvostov et al. (2019)**
		511.6 → 641.3, 635.3, 507.3	13.6		
	NDMAAQYK	470.7 → 580.3, 509.3	15.6	5.73 ±0.07	**Kulikovskii et al. (2019)**
	YLEFISDAIIHVLHAK	623.7 → 797.0, 732.4, 602.4	17.7	9.28 ±0.74	**Kulikovskii et al. (2019)**
Myosin-1	TLALLFSGPASGEAEGGPK	901.5 → 1290.6, 1143.5, 1056.5, 999.5 831.4	28.9	8.64 ±0.03	**Claydon et al. (2015); Fornal and Montowska (2019); Montowska and Fornal (2019)**
Myosin-2	MEIDDLASNVETISK	832.9 → 1061.6, 948.5, 877.5	26.8	8.26 ±0.01	**Montowska and Fornal (2019)**
	TLAFLFSGTPTGDSEASGGTK	1022.5 → 1264.6, 1207.5, 1106.5	32.7	8.19 ±0.25	**Fornal and Montowska (2019)**
Myosin light chain 2f	EASGPINFTVFLNMFGEK	1001.0 → 1446.7, 1185.6, 985.5, 838.4	32.0	10.23 ±0.02	**Fornal and Montowska (2019)**
Stress-induced-phosphoprotein	ALDLDSNC[+57.0]K	518.2 → 851.4, 736.3, 623.2	17.1	7.71 ±0.92	**Wang et al. (2018)**
β-Hemoglobin	LHVDPENFK	549.8 → 848.4, 749.3, 634.3	18.0	7.08 ±0.17	**Li et al. (2018)**
Carbonic anhydrase 3	LVNELTEFAK	582.3 → 837.4, 708.4, 595.3	19.1	7.93 ±0.03	**Li et al. (2018)**
	GEFQLLLDALDK	681.4 → 1028.6, 900.5, 787.5	22.1	8.17 ±0.81	**Li et al. (2018)**
L-Lactate dehydrogenase A chain	DLADEVALVDVMEDK	831.4 → 1019.5, 948.5, 835.4	26.8	9.18 ±1.58	**Li et al. (2018)**
Triosephosphate isomerase	SNVSDAVAQSAR	602.8 → 904.5, 817.4, 702.4, 532.3	19.7	6.08±0.03	**Khvostov et al. (2019)**

Note: * Only the peptide sequence provided from the review article by **Stachniuk et al. (2019)**. The MRM transitions and Collision energy metrics were selected anew.

Peptides presented in a recent review **(Stachniuk et al., 2019)** were selected for comparison of potential biomarkers. Previously submitted peptides by us were analysed **(Khvostov et al., 2019; Kulikovskii et al., 2019)**. One of the criteria for marker specificity is the presence of a sequence of more than six amino acids **(Watson et al., 2015)**. This peptide length provides the species specificity of muscle protein. We decided to use the S/N indicator as the criterion for the comparison of heat-stable peptides.

Chromatograms of SRM peptide markers are shown in Figure 1a and Figure 1b. The four most intense peptides with a signal value of $(50-250)*10^3$ cps are presented in Figure 1a. The remaining peptides in the intensity range of $(10 - 50)*10^3$ cps are indicated in Figure 1b. The chromatogram data were obtained in a sample with a beef concentration of 16% (w/w), subjected to thermal treatment.

The S/N results for a sample of minced meat with 16% beef (w/w) after heat treatment are shown in Table 3.

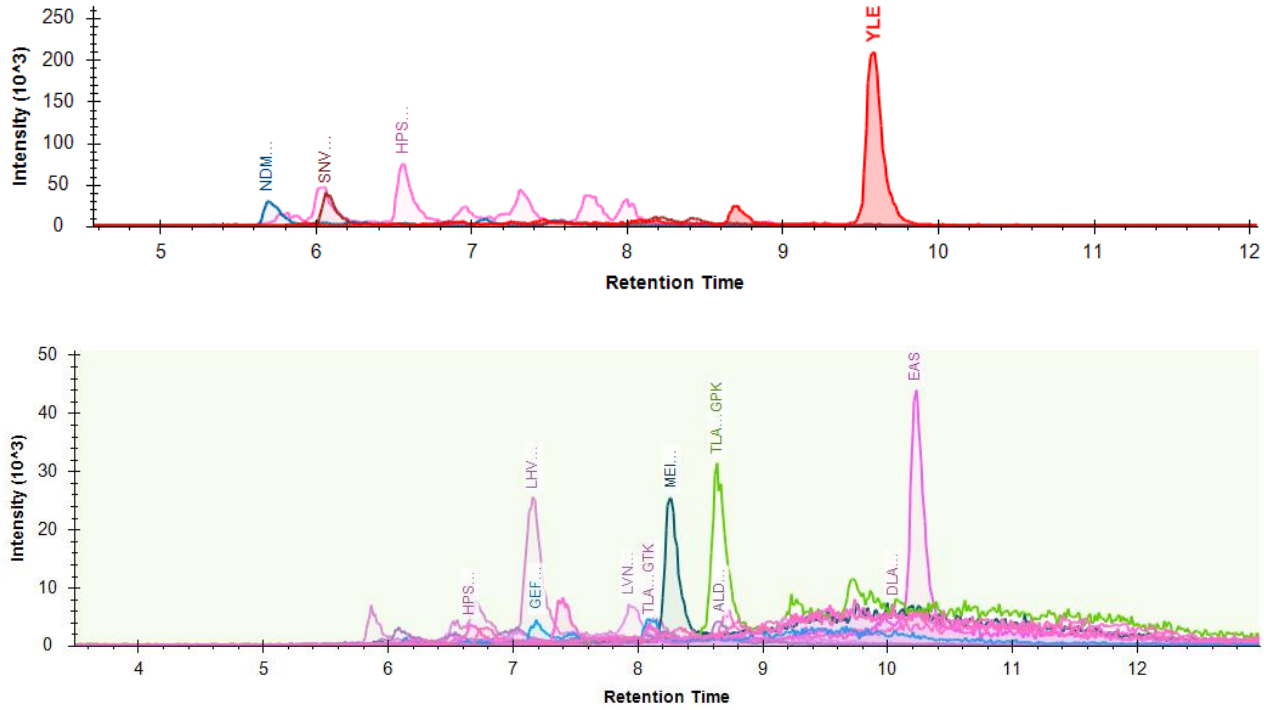

Figure 1 Chromatograms of selected biomarkers responsible for the identification of beef muscle tissue: major peptides (a) and minor peptides (b). Heat-treated mixture with 16% (w/w) beef.

The peptides are arranged in descending order of S/N. The data show that S/N is the highest for the peptide sequences NDMAAQYK **(Kulikovskii et al., 2019)** and YLEFISDAIIHVLHAK **(Khvostov et al., 2019)**, which are myoglobin derivatives. Since beef contains a high level of myoglobin, we obtained the largest number of myoglobin peptide derivatives. The S/N ratio is above 10 for both raw and heat-treated samples. For the peptide HPSDFGADAQAAMSK **(Claydon et al., 2015; Li et al., 2018; Khvostov et al., 2019)**, an additional MRM search was performed. Two parent ions, 766.8 (*m*\z) and 511.6 (*m*\z), were used. The most significant was ion 511.6 (*m*\z). The MRM intensity for this mass increased by 40% ±7.4 compared with ion 766.8 (*m*\z).

Samples were frozen and re-thawed. We evaluated the effect of one freeze/thaw cycle in digested samples on the intensity of the HPSDFGADAQAAMSK peptide in all mixtures. For samples subjected to and without heat treatment, S/N did not change. It was found that one freeze/thaw cycle did not affect the concentration of meat in mixture 1. If the beef content was less than 10% (w/w), the intensity decreased to 52.4 ±15.2. For peptides ALDLDSNC [+57.0] K **(Wang et al., 2018)**, DLADEVALVDVMEDK, and GEFQLLLDALDK **(Li et al., 2018)**, cross-contamination was recorded in a blank sample (no beef) (mixture 3) (Figure 2).

Many peptides did not meet the criterion of S/N >3.

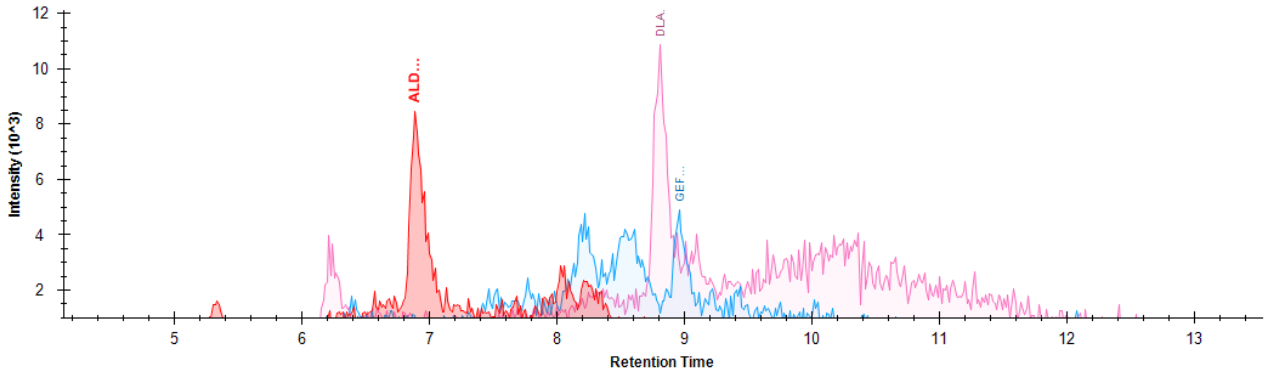

Figure 2 Peptides ALDLDSNC, DLADEVALVDVMEDK and GEFQLLLDALDK identified in samples not containing beef (mixture 3).

Table 3 Comparison of peptide markers with respect to signal-to-noise characteristics for two concentrations of beef muscle tissue and two cooking modes (without and with heat treatment).

Protein	Marker peptide sequence	Mixture 2 with beef 8% (w/w)		Mixture 1 with beef 16% (w/w)	
		not heated (S/N ±*SD*)	heat-treatment, (S/N ±*SD*)	not heated (S/N ±*SD*)	heat-treatment, (S/N ±*SD*)
Myoglobin	NDMAAQYK	12.50 ±2.45	24.61 ±4.82	11.53 ±2.64	127.66 ±12.51
Triosephosphate isomerase	SNVSDAVAQSAR	13.34 ±2.61	10.09 ±1.38	13.02 ±0.23	27.82 ±1.23
Myoglobin	YLEFISDAIIHVLHAK	3.24 ±0.64	7.79 ±0.76	4.64 ±1.97	24.06 ±7.58
	HPSDFGADAQAAMSK_511Freeze	1.36 ±0.27	2.14 ±0.50	1.95 ±0.17	7.78 ±0.42
Myosin-2	MEIDDLASNVETISK	2.47 ±0.48	2.29 ±0.18	3.5 ±0.42	8.33 ±0.79
Myosin-1	TLALLFSGPASGEAEGGPK	1.20 ±0.23	2.15 ±0.21	1.55 ±0.10	8.32 ±1.85
Myoglobin	HPSDFGADAQAAMSK_511	3.70 ±0.73	2.91 ±0.34	1.94 ±1.66	7.43 ±2.05
Stress-induced-phosphoprotein	ALDLDSNC[+57.0]K	2.51 ±0.49	1.74 ±0.51	2.32 ±1.07	4.66 ±0.76
β-Hemoglobin	LHVDPENFK	2.82 ±0.57	2.35 ±0.23	4.05 ±0.61	5.30 ±0.39
Myosin light chain 2f	EASGPINFTVFLNMFGEK	1.31 ±0.26	1.24 ±0.12	1.98 ±0.72	5.09 ±0.87
Myoglobin	HPSDFGADAQAAMSK_766	2.89 ±0.51	1.68 ±0.16	4.96 ±1.76	2.57 ±0.05
Carbonic anhydrase 3	LVNELTEFAK	1.04 ±0.26	0.60 ±0.08	1.78 ±0.48	2.54 ±0.24
	GEFQLLLDALDK	1.36 ±0.22	0.15 ±0.12	1.93 ±0.45	2.42 ±0.20
Myosin-2	TLAFLFSGTPTGDSEASGGTK	5.00 ±0.84	4.35 ±0.43	4.49 ±0.45	1.87 ±0.21
L-Lactate dehydrogenase A chain	DLADEVALVDVMEDK	0.2 ±0.12	1.36 ±0.13	0.58 ±0.13	0.51 ±0.35

Peptides representing from myosin proteins, such as MEIDDLASNVETISK **(Montowska and Fornal, 2019)** TLALLFSGPASGEAEGGPK **(Claydon et al., 2015; Fornal and Montowska, 2019; Montowska and Fornal, 2019)** were sensitive to heat-treated products with 16% muscle tissue (w/w). At lower concentrations, S/N approached 2 – 3. It was not possible to identify the DLADEVALVDVMEDK peptide **(Li et al., 2018)** in all types of samples. The S/N index for all samples was no greater than 1. A one-way analysis of variance (ANOVA) found an insignificant effect of temperature on the intensity of marker peptides at a concentration of 8% (w/w). In previous studies by **Kulikovskii et al. (2019)** and **Khvostov et al. (2019)**, we established a limit of detection (LOD) of 0.29% for the NDMAAQYK peptides and 0.93% for the SNVSDAVAQSAR peptide. From the analysis of species-specific marker peptides, three peptides for determining muscle tissue in beef were selected, taking into account the following factors: high prevalence in muscle tissues (>50 cps), good S/N ratio at low concentrations (S\N >10), high specificity and the presence of trypsin-specific cleavage sites at both ends of the protein chain.

Two-way analysis of variance does not reveal differences in the assessment of the criterion for the influence of heat treatment of mixtures at a concentration of 8% beef, confirmed by statistical calculation of p (<0.71), which is higher than the significance level of alpha (0.05).

CONCLUSION

The developed methodology allowed us to simultaneously identify and compare up to 13 beef peptide biomarker. Using the S/N criterion, it was possible to compare peptide markers for the authenticity of raw meat and heat-treated meat. Considered successful candidates whose signal-to-noise ratio was higher than 3.

From the analysis of species-specific marker peptides, three peptides for determining muscle tissue in beef were finally determined: NDMAAQYK and YLEFISDAIIHVLHAK from myoglobin and

SNVSDAVAQSAR from triosephosphate isomerase protein. For samples with two concentration levels and under cooking conditions at 100 °C, the S/N ratio was set above 10. This approach is universal. It is suitable for comparing meat biomarkers of other animal species. It will be able to identify the most suitable candidates. Selected peptide markers can be used to construct regression curves with good linearity, allowing a quantitative assessment of the types of meat present. The selected peptides can be used effectively to distinguish between accidental contamination (technologically unavoidable impurity) and deliberate falsification.

The developed methodology can aid in the study of the effect of meat protein on meat quality and functional characteristics, as well as the safety of finished meat products.

REFERENCES

Claydon, A., Grundy, H., Charlton, A., Romero, M. 2015. Identification of novel peptides for horse meat speciation in highly processed foodstuffs. *Food Addit Contam—Part A Chem. Anal. Control. Expo. Risk Asses.*, vol. 32, no. 10, p. 1718-1729. https://doi.org/10.1080/19440049.2015.1075256

Fornal, E., Montowska, M. 2019. Species-specific peptide-based liquid chromatography–mass spectrometry monitoring of three poultry species in processed meat products. *Food Chem*, vol. 283, p. 489-498. https://doi.org/10.1016/j.foodchem.2019.01.074

Khvostov, D., Vostrikova, N., Zherdev, A., Zvereva, E., Kurzova, A. 2019. Identifikatsiya i kolichestvennoye opredeleniye myshechnoy tkani na osnove kontrolya prototipicheskikh peptidov s ispol'zovaniyem metoda monitoringa zadannykh reaktsiy (Quantitative identification of muscular tissue by the means of protototic peptides using the multiple reaction monitoring method). *Analitika i kontrol'*, vol. 23, no. 4, p. 580-586. https://doi.org/10.15826/analitika.2019.23.4.012

Kopylov, A., Zgoda, V. 2007. Kolichestvennye metody v proteomike (Quantitative Methods in Proteomics). *Biomeditsinskaia khimiia*, vol. 53, no. 6, p. 613-643. (In Russian)

Kopylov, A., Zgoda, V., Archakov, A. 2009. Kolichestvennyi mass-spektrometricheskii analiz soderzhaniia belkov v biologicheskikh probakh bez ispol'zovaniia izotopnykh metok (Quantitative mass spectrometric analysis of protein content in biological samples without the use of isotopic tags). *Biomeditsinskaia khimiia*, vol. 55, no. 2, p. 125-139. (In Russian)

Kulikovskii, A., Vostrikova, N., Chernukha, I., Khvostov, D. 2019. Quantitative Identification of Muscle Tissue by Means of Biomarker Peptides by Using Method of Multiple Reaction Monitoring. *J. Oriental of Chimistry*, vol. 35, no. 4, p. 1327-1331. https://doi.org/10.13005/ojc/350411

Leitsätze für Fleisch und Fleischerzeugnisse, 2016. Available at: https://www.bmel.de/SharedDocs/Downloads/Ernaehrung/Lebensmittelbuch/LeitsaetzeFleisch.html.

Li, Y., Zhang, Y., Li, H., Zhao, W., Guo, W., Wang, S. 2018. Simultaneous determination of heat stable peptides for eight animal and plant species in meat products using UPLC-MS/MS method. *Food Chem*, vol. 245, p. 125-131. https://doi.org/10.1016/j.foodchem.2017.09.066

Montowska, M., Fornal, E. 2017. Label-free quantification of meat proteins for evaluation of species composition of processed meat products. *Food Chem.*, vol. 237, no. 15, p. 1092-1100. https://doi.org/10.1016/j.foodchem.2017.06.059

Montowska, M., Fornal, E. 2019. Absolute quantification of targeted meat and allergenic protein additive peptide markers in meat products. *Food Chem.*, vol. 274, p. 857-864. https://doi.org/10.1016/j.foodchem.2018.08.131

Naveena, B., Jagadeesh, D., Babu, A., Rao, T., Kamuni, V., Vaithiyanathan, S. Kulkarni, V. K., Rapole, S. 2017. OFFGEL electrophoresis and tandem mass spectrometry approachcompared with DNA-based PCR method for authentication of meat species from rawand cook ground meat mixtures containing cattle meat, water buffalo meat and sheep meat. *Food Chem.*, vol. 233, p. 311-320. https://doi.org/10.1016/j.foodchem.2017.04.116

Prandi, B., Lambertini, F., Faccini, A., Suman, M., Leporati, A., Tedeschi, T., Sforza, S. 2017. Mass spectrometry quantification of beef and pork meat in highly processed food: Application on Bolognese sauce. *Food Contro*, vol. 74, p. 61-69. https://doi.org/10.1016/j.foodcont.2016.11.032

Prandi, B., Varani, M., Faccini, A., Lambertini, F., Suman, M., Leporati, A., Tedeschi, T., Sforza, S. 2019. Species specific marker peptides for meat authenticity assessment: A multispecies quantitative approach applied to Bolognese sauce. *Food Control*, vol. 97, p. 15-24. https://doi.org/10.1016/j.foodcont.2018.10.016

Ruiz Orduna, A., Husby, E., Yang, C., Ghosh, D., Beaudry, F. 2015. Assessment of meat authenticity using bioinformatics, targeted peptide biomarkers and high-resolution mass spectrometry. *Food Addit Contam—Part A Chem. Anal. Control. Expo. Risk Assess.*, vol. 32, no. 10, p. 1709-1717. https://doi.org/10.1080/19440049.2015.1064173

Ruiz Orduna, A., Husby, E., Yang, C., Ghosh, D., Beaudry, F. 2017. Detection of meat species adulteration using high-resolution mass spectrometry and a proteogenomics strategy. *Food Addit Contam—Part A Chem Anal Control Expo Risk Assess*, vol. 34, no. 7, p. 1110-1120. https://doi.org/10.1080/19440049.2017.1329951

Sarah, S., Faradalila, W., Salwani, M., Amin, I., Karsani, S., Sazili, A. 2016. LC-QTOF-MS identification of porcine-specific peptide in heat treated pork identifies candidate markers for meat species determination. *Food Chem.*, vol. 199, p. 157-164. https://doi.org/10.1016/j.foodchem.2015.11.121

Sentandreu, M., Fraser, P., Halket, J., Patel, R., Bramley, P. 2010. A proteomic based approach for detection of chicken in meat mixes. *Journal of Proteome Research*, vol. 9, no. 7, p. 3374-3383. https://doi.org/10.1021/pr9008942

Skyline program. 2019. Available at: https://skyline.ms/project/home/software/Skyline/begin.view

Stachniuk, A., Sumara, A., Montowska, M., Fornal, E. 2019. Liquid chromatography–mass spectrometry bottom-up proteomic methods in animal species analysis of processed meat for food authentication and the detection of adulterations. *Mass Spectrometry Reviews*, vol. 00, p. 1-28. https://doi.org/10.1002/mas.21605

Von Bargen, C., Brockmeyer, J., Humpf, H. 2014. Meat authentication: A new HPLC–MS/MS based method for the fast and sensitive detection of horse and pork in highly processed food. *Journal of Agricultural and Food Chemistry*, vol. 62, no. 39, p. 9428-9435. https://doi.org/10.1021/jf503468t

Wang, G. J., Zhou, G. Y., Ren, H. W., Xu, Y., Yang, Y., Guo, L. H., Liu, N. 2018. Peptide biomarkers identified by LC–MS in processed meats of five animal species. *J. Food*

Comp. Anal., vol. 73, p. 47-54. https://doi.org/10.1016/j.jfca.2018.07.004

Watson, A., Gunning, Y., Rigby, N., Philo, M., Kemsley, E. 2015. Meat authentication via multiple reaction monitoring mass spectrometry of myoglobin peptides. *Anal. Chem.*, vol. 87, no. 20, p. 10315-10322. https://doi.org/10.1021/acs.analchem.5b02318

Zav'yalova, M., Zgoda, V., Kharybin, O., Nikolayev, Y. 2014. Poluchenie peptidnogo standarta dlia srmmetoda s pomoshch'iu fosforilirovaniia *in vitro* (Preparation of a peptide standard for the srm method using *in vitro* phosphorylation). *Biomeditsinskaia khimiia*, vol. 60, no. 6, p. 668-676. https://doi.org/10.18097/PBMC20146006668 (In Russian)

Acknowledgments:

This work was supported by the Russian Foundation For Basic Research, project No. 19-316-90053.

Contact address:

*Daniil Khvostov, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, junior researcher of laboratory, Scientific and methodical work, biological and analytical research, 109316, Moscow, ul. Talalikhina, 26 Tel. +74956767981,
E-mail: d.hvostov@fncps.ru
ORCID: https://orcid.org/0000-0002-3445-4559

Natalya Vostrikova, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, doctor of technical sciences, head of laboratory, Scientific and methodical work, biological and analytical research, 109316, Moscow, ul. Talalikhina, 26 Tel. +74956767981,
E-mail: n.vostrikova@fncps.ru
ORCID: https://orcid.org/0000-0002-9395-705X

Irina Chernukha, V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, doctor of technical sciences, professor, leading research scientist, Experimental clinic — laboratory, Biologically active substances of an animal origin, 109316, Moscow, ul. Talalikhina, 26 Tel. +74956767981,
E-mail: imcher@inbox.ru
ORCID: https://orcid.org/0000-0003-4298-0927

Corresponding author: *