Detection of heart and aorta tissue peptide markers by the multiple-reaction monitoring method

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ABSTRACT Functional, particularly personalized meat-based foods are of more in demand by a consumer today. Functional additives, such as plant components (oils, extracts, fibers) and animal proteins from bovine or porcine tissues have been successfully used wide world. The purpose of the study is meat product composition analysis and special biomarker peptide identification to confirm the presence of heart and aorta tissue in a finished meat product. The best candidate for finding a pork heart in food was VNVDEVGGEALGR peptide. Two marker peptides from serum albumin were selected for the pork aorta: TVLGNFAAFVQK and EVTEFAK.

MATERIALS AND METHODS

Pork aorta and hearts after trimming and chopping in a cutter (50 liters; KG Wetter 258/1336), were transferred to 10 ml falcons and stored in a freezer at -43 °C until analysis.

Meat proteins were extracted and digested with trypsin. After that peptide mixtures were analyzed by triple quadrupole LC/MS system (Figure 1).

For chromatographic separation, the HPLC system Agilent 1260 Infinity II was used with a reverse phase column (50 mm × 2.1 mm, Eclipse Plus C18 Agilent with a fast resolution, 2.7 µm in size). The sample inlet volume is 10 µl, the temperature of the temperature control unit is 30 °C [1].

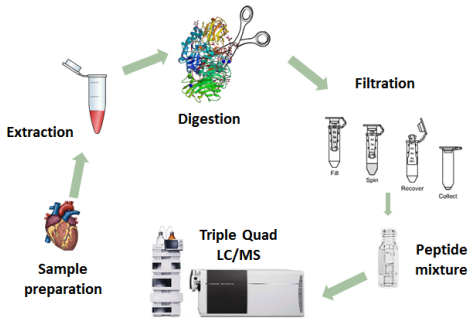

Figure 1. Sample preparation protocol

[1] Khvostov, D., Vostrikova, N., Zherdev, A., Zvereva, E., Kurzova, A. (2019). https://doi.org/10.15826/analitika.2019.23.4.012
[2] Stachniuk, A., Sumara, A., Montowska, M., Fornal, E. (2019). https://doi.org/10.1002/mas.21605
[3] Khvostov, D., Vostrikova, N., & Chernukha, I. (2020). https://doi.org/10.5219/1317

RESULTS

Different methods are used to identify raw materials, including new approaches in peptidomics that are considered the most effective modern methods nowadays. Over 20 amino acid sequences were checked based on earlier obtained data [2]. Those amino acid sequences were analyzed with a high-performance liquid chromatography with mass spectrometric detection as described [1]. The MS settings were selected using the Skyline program [3]. Signal-to-Noise ratio (S/N) over 10 units were used to choose the best peptide candidates (Tab.1). Seven peptides were found in porcine hearts (with S/N more than 20). The best candidate was peptide VNVDEVGGEALGR (S/N - 73.10±5.3) from β-Hemoglobin. Two marker peptides from Serum albumin were selected for pork aorta: TVLGNFAAFVQK (S/N 53.51±2.4) and EVTEFAK (S/N 31.69±4.1). These biomarkers showed the best detection and specificity.

Table 1. Comparison of peptide markers with respect to signal-to-noise characteristics

Peptide (sequence)	Protein (pork)	Pork aorta, S/N	Pork heart, S/N
VNVDEVGGEALGR	**β-Hemoglobin**		**73,10**
YLEFISEAIIQVLQSK	Myoglobin	4,77	65,79
TVLGAPEVLLGILPGAG GTQR	Trifunctional enzyme subunit alpha		42,81
LLSNLFANYAGADTPVE K	Myosin-7		35,79
TVLGNFAAFVQK	**Serum albumin**	**53,51**	
FFESFGDLSNADAVMG NPK	β-Hemoglobin	22,08	31,35
HPGDFGADAQGAMSK	Myoglobin		25,16
EVTEFAK	**Serum albumin**	**31,69**	25,05
FVIER	Serum albumin		14,57
FAGGNLDVLK	Trifunctional enzyme subunit alpha	3,63	10,77

CONCLUSION

The multiple-reaction monitoring method made it possible to identify the most/best specific peptides - biomarkers that could confirm the heart and/or aorta in meat products. The method can be used for comparative research or identification of best peptides that are specific to any type of animal tissue.

ACKNOWLEDGMENTS

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