potravinárstvo®
Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 13, 2019, no. 1, p. 331-336
https://doi.org/10.5219/1119
Received: 12 March 2019.Accepted: 12 March 2019.
Available online: 28 May 2019 at www.potravinarstvo.com

ISSN 1337-0960 (online)

INFLUENCE OF TECHNOLOGICAL PROCESSING ON LIPID-LOWERING ACTIVITY OF SUBSTANCES CONTAINING IN PORCINE HEARTS AND AORTAS

Elena Kotenkova, Irina Chernukha

ABSTRACT
Edible by-products are a good source of nutrients and bioactive substances and could be used as functional ingredients or for biopeptides production natively contained in raw materials. A wide range of peptides are also formed during the enzymatic hydrolysis or food processing. The comparative results of the effectiveness of isolated certain protein and peptide fractions by ultrafiltration with the same natively presented in raw tissues, as well as the influence of heat treatment on biological activity of origin active substances are presented. The model of rat alimentary hyperlipidemia was developed by adding cholesterol and fat to the standard diet and vitamin D_2 injection *per os*. Serum lipid profile was determined on automatic analyzer BioChem FC-360. Dynamic of changes in serum lipid profile was assessed as corresponding control group medium results in ratio to certain rat data. Two-dimensional electrophoresis (2DE) was performed according to the method of O'Farrell with isoelectric focusing in ampholine pH gradient (IEF-PAGE) with following identification by MALDI-TOF MS and MS/MS mass spectrometry. Consumption of native pig aorta and pig heart during 14^{th} days led to normalization of lipid profile in serum of hyperlipidemic rats, while low molecular weight (LMUF, MW <5 kDa) and medium molecular weight (MMUF, MW = 5 – 30 kDa) ultrafiltrates of pig aorta extract did not strongly influenced on level of triglicerides and, on contrary, elevated high density cholesterol. Consumption of developed product by hyperlipidemic rats during 28^{th} days did not lead to significant changes in serum lipid profile, while on 42^{nd} day all ratios reached ones in group, which were treated with native raw material or isolated active fractions. The stability of developed product was confirmed by proteomic studies. Obtained results open prospects to modernization the technology, presumably use as a matrix dietary meat (e.g. poultry) with incorporated active identified components.

Keywords: by-products; heart; aorta; lipid-lowering activity; functional additives and product

INTRODUCTION
A large number of by-products are left after farm animal's slaughter; edible ones such as internal organs are a good source of nutrients and bioactive substances and could be used as functional ingredients or for biopeptides production **(Toldrá, Mora and Reig, 2016; Alao et al., 2017)**.

Natively contained in raw materials peptides or formed during the enzymatic hydrolysis or food processing demonstrated hypotensive, antioxidant, opioid, immunomodulatory, prebiotic, mineral-binding, cholesterol-lowering and antimicrobial activity **(Bauchart et al., 2006; Mine and Shahidi, 2006; Ahhmed and Muguruma, 2010; Toldrá et al., 2012; Udenigwe and Howard, 2013; Lafarga and Hayes, 2014)** and could be used as a functional ingredient in food processing for specialized purposes. Collagen, hemoglobin and casein are mostly studied, a lot of peptides were isolated, identified and their functions were determined **(Toldrá et al., 2011; Wang et al., 2013; Lafarga et al., 2016; Mohanty et al., 2016; Arrutia et al., 2017; Bamdad et al., 2017; Hongdong and Bo, 2017)**.

Technological processing, especially enzymatic treatment is a good tool for bioactive peptide generation. Nevertheless, it's known that every tissue is characterized uniqe proteome and peptidome, which are involved in maitance of its own normal physiological condition **(Fagerberg et al., 2014)**. Moreover, it was found that besides peptides with defined functions, tissues of living organisms contain powerful peptide background, which mainly consists of fragments of larger molecules or functional proteins **(Chugunov, 2010)**.

In previous studies we confirm lipid-lowering action of raw material (porcine heart and aorta), different protein fractions isolated from aorta, and functional meat product produced from porcine heart and aorta in certain ratio on hyperlipidemic rats **(Chernukha, Fedulova and Kotenkova, 2015; Chernukha, Fedulova and Kotenkova, 2018; Chernukha et al., 2018a, Chernukha et al., 2018b)**.

This paper reports the analytical results of influence of the type of technological treatment on hypolipidemic activity substances naturally presenting in porcine heart and aorta.

Scientific hypothesis

Different technological treatment could influence on biological effectiveness of substances presenting in corresponding tissue. It's known that an enzymatic treatment lead to release different active peptides from some wastes of farm animal's slaughter. On the other hand, specific by-products are already enriched with unique substances with certain biological function and involved in maitance of its own normal physiological condition. In this case we decided to compare the effectiveness of isolated certain protein and peptide fractions by ultrafiltration with the same natively presented in raw tissues. Also we studied the influence of heat treatment on biological activity of origin active substances. All these analytical results could propose final recommendation for further raw material processing to produce functional meat product.

MATERIAL AND METHODOLOGY

Native porcine heart and aorta, low molecular weight (LMUF, MW <5 kDa) and medium molecular weight (MMUF, MW = 5 – 30 kDa) ultrafiltrates of pig aorta extract, meat product containing porcine heart and aorta in ratio 3:1 were objects of study.

Ultrafiltrates production

Aorta tissues were homogenized in a grinder KENWOOD (UK) with a stainless steel plate (3 – 5 mm hole), re-frozed and then homogenized in a cutter KG Wetter 258/1336 (Germany) with the addition of distilled water in the ratio (4:1) and knife shaft speed 2000 rpm. Then homogenate were reconstituted in 0.9% NaCl solution, and extracted during 24 hours with stirrer speed 500 rpm. Extract separation was carried out by centrifugation for 7 – 10 minutes at 3,000 – 3,500 rpm on centrifuge CM-6M (ELMI, Latvia). Supernatant was collected and ultrafiltrated on PES membrane (MWCO 5 and 30 kDa) by tangential filtrationon VivaFlow 200 system (Sartorius, Germany). Low molecular weight (LMUF, MW <5 kDa) and medium molecular weight (MMUF, MW = 5 – 30 kDa) ultrafiltrates were lyophilized in INEY-4 (IPB RAN, Russia) to protein concentration 0.9 $g.L^{-1}$.

Meat product manufacture

Meat functional product was produced on ZAO "Yoshkar-OlinskiyMyasokombinat". Porcine hearts were chopped with a particle size of 2 – 3 mm and salted for 12 h. Porcine aortas were chopped with a particle size of 2 – 3 mm and homogenized in cutter at 3000 rpm for 2 – 3 min. Minced hearts with the juice were quantitatively transferred in the cutter and mixture was then homogenized at 3000 rpm for 6 – 8 min (ratio of aorta to hearts 1:3). Obtained mince was packed in cans and sterilized at 115 °C, a pressure of 0.23 MPa for 40 min. Meat product contained 17.53 ±0.95% protein, 3.82 ±0.13% fat, 0.305 ±0.015% sodium chloride, and 2.35 ±0.25% starch.

Animal experiments

Male Wistar rats (380 ±20 g) aged approximately 12 months were kept under standard conditions (temperature 20 ±3 °C, humidity 48 ±2%, day/night (from 06.00 to 18.00 hours/from 18.00 to 06.00 hours), no more than six rats per plastic cage), and water and feed were available ad libitum. Rats were obtained from Andreevka (Moscow region, Russia), acclimatized for 5 days, and grew up to 12 months of age before use in this study. The model of alimentary hyperlipidemia was developed by adding cholesterol and fat to the standard diet (standard chow (Labkorm, Russia)) and vitamin D_2 injection *per os* **(Chernukha et al., 2018b)**.

Native porcine heart and aorta testing protocol

After modeling the rats were randomly divided into three groups: control (n = 10) animals were administered standard chow, group A (n = 10) - pig heart tissue, group B (n = 10) - pig aorta tissues. All samples were mixed with standard chow in quantity 10 g per kg body weight for 14 days. According to physic-chemical protocol of raw material testing and electrophoretic study results, porcine aorta contained 21.40% protein, including approximately 10% proteins lower 30 kDa and 30% polypeptydes in all nitrogen, therefore experimental animals consumed 10 x 0.214 x 0.4 = 0.86 g target fraction per kg body weight. Porcine heart contained 13.23% protein, including approximately 14% proteins lower 30 kDa and 51% polypeptydes in all nitrogen, therefore experimental animals consumed 10 x 0.1323 x 0.65 = 0.86 g target fraction per kg body weight.

Ultrafiltratesand meat product testing protocol

At the end of modeling animals were randomly divided into four groups: control (n = 10) rats were administered 0.9% solution of sodium chloride, group C (n=10) – LMUF, group D (n=10) – MMUF. All samples were administered *per os* in dose 0.3 mg protein per kg body weight for 14 days. The dose was determined according to the recommended dose of commercial analogue - food bioactive additive containing a mixture of peptides isolated from the vessels of farm animals (Scientific and Production center of Revitalization and Health (SPRH), Russia).

Meat product testing protocol

After modeling, rats in control group were fed with standard chow, in group E – meat product ($8g.kg^{-1}$ b.w.) in mixture with standard chow during 28 and 42 days. The dose was reduced to $8g.kg^{-1}$ b.w. because in raw material experiment animals during all study left about 30 – 40% of standart chow mixrure with testes samples. During meat product testing there was no such observation.

Biochemical analysis

After the experiment, the animals were euthanized (VETtech, UK), blood samples for biochemical studies and were taken. Biochemical investigations were carried out on automatic analyzer BioChem FC-360 (HTI, USA) according to instructions applied to measurement kits

(HTI, USA). Total cholesterol (TCL), triglyceride (TG), cholesterol low-density lipoproteins (CL LDL) and cholesterol high-density lipoproteins (CL HDL) levels were measured in rat serum. Atherogenic index (AI) = (TCL - CL HDL)/ CL HDL. Dynamic of changes in serum lipid profile was assessed as corresponding control group medium results in ratio to certain rat data. Therefore ratio <1 means that experimental group value was higher control mean, ratio >1 – lower.

Proteomic study

Two-dimensional electrophoresis (2DE) was performed according to the method of O'Farrell with isoelectric focusing in ampholine pH gradient (IEF-PAGE). Following reagents were used: urea, acrylamide, methylene bisacrylamide, agarose, Tris, glycine, sodium dodecyl sulfate, ammonium persulfate, Triton X-100, 2-mercaptoetanol, bull serum albumine, ampholines pH 3 – 10, 5 – 8 (Sigma, United States), amberlite IRN-150L (Amersham Biosciences, Sweden). The subsequent detection of the proteins was carried out by staining with silver nitrate (Panreac, Spain) as described previously **(Kovalyov et al., 2006)**. The resulting digital images were edited in a graphic editor and the quantitative protein content was calculated using ImageMaster 2D Platinum version 7 ("GE Healthcare", Switzerland).

Protein fractions were excised from the gel, grinded and undergone trypsinolysis (Sigma, Germany) **(Zvereva et al., 2015)**. Obtained peptides were investigated by MALDI-TOF MS and MS/MS mass spectrometry on Ultraflex MALDI-TOF mass spectrometer (Bruker, Germany) with UV laser (336 nm) in the positive ion mode in molecular weight range of 500 – 8000 Da with calibration according to known peaks of trypsin autolysis.

Bioinformatics analysis

Analysis of obtained tryptic peptides mass spectra was performed using Peptide Fingerprint option in Mascot software (MatrixScience, USA) with MH+ mass determination accuracy of 0.01%; search was performed in databases of the National Center for Biotechnology Information, USA (NCBI).

Statistic analysis

STATISTICA 10.0 software was used in this study for the statistical analyses. The results were calculated as "middle value ± standard error" (M ± SE). Significant differences were tested by one-way ANOVA, followed by the Tukey test. Differences with *p*-values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

The analytical results of dynamic of changes in serum lipid profile are presented in Table 1. Maximum elevation of total CL ratio was noticed in group B (native pig aorta) and was higher value in group A (native pig heart) by 27.5%, while TG ratio was lower by 42.4%. CL LDL ratio, on contrary, in serum of rats group B (native pig aorta) was higher group A (native pig heart) value by 19.3%, as well as CL HDL ratio was higher by 24.3%. Observed changes compensated each other; therefore the AI ratio was the same and showed no statistical difference.

There were no significant changes in total CL, CL LDL, CL HDL and TG ratios between group C (LMWU) and D (MMWU). It was also noticed, that CL HDL and TG elevated control values, because ratios did not exceed 1, while CL LDL was also higer control – corresponding ratios were higher 1. Nevertheless, the AI ratios corresponded with group A (native pig heart) and B (native pig aorta) and was lower control group approximately by 2 fold. This observation was explained previously by phenomenon of CL non-LDL and non-HDL reduction in rat serum, which is also associated with atherogenic lipoprotein fractions, as well as elevation of CL HDL **(Chernukha et al., 2018)**.

Consumption of developed product by hyperlipidemic rats during 28 days did not lead to significant changes in serum lipid profile. Total CL and CL ratio did not differ from control; corresponding ratios did not exceed 1, CL HDL ratio was lower 1. There was a slight increase in TG ratio, as well as in AI ratio. On 42nd day of developed product consumption all ratios reached ones in group A – D, which were treated with native raw material or isolated active fractions. Total CL, CL LDL, CL HDL, TG and AI ratios elevated by 50.5% (p <0.05), 39.0%, 29.8%, 11.4% and 37.0%, respectively.

Summarising, we observed that in group C (LMWU) and D (LMWU) there was not such effect as in group B (native pig aorta), the ratio of TG was lower 1 and therefore higher control value. It could be explained both significantly lower concentration of ultrafiltrates dosage as well as separation of active fraction into two-lower 5 kDa and 5 – 30 kDa. Nevertheless, the ratio of AI in both group

Table 1 Lipid profile in the serum of hyperlipidemic rat model.

Groups	Cholesterols (medium control value in ratioto certain rat data)			Triglycerides	Atherogenic index
	Total	LDL	HDL	(medium control value in ratio to certain rat data)	
Group A	1.31 ±0.10	1.09 ±0.07	1.07 ±0.11	3.09 ±0.92^{a}	1.88 ±0.31
Group B	1.67 ±0.16^{b}	1.30 ±0.05	1.33 ±0.13^{a}	1.78 ±0.33	1.91 ±0.06
Group C	1.30 ±0.09	1.13 ±0.09	0.85 ±0.06^{b}	0.92 ±0.15^{b}	1.90 ±0.09
Group D	1.46 ±0.11^{b}	1.12 ±0.09	0.95 ±0.07^{b}	0.90 ±0.13^{b}	1.98 ±0.19
Group E (28 days)	1.01 ±0.04^{a}	1.05 ±0.04	0.84 ±0.07^{b}	1.32 ±0.18^{b}	1.35 ±0.09
Group E(42 days)	1.52 ±0.15^{b}	1.46 ±0.30	1.09 ±0.03	1.47 ±0.18^{b}	1.85 ±0.25

Note: $^{a-b}$ -significant differences between the experimental groups (p <0.05).

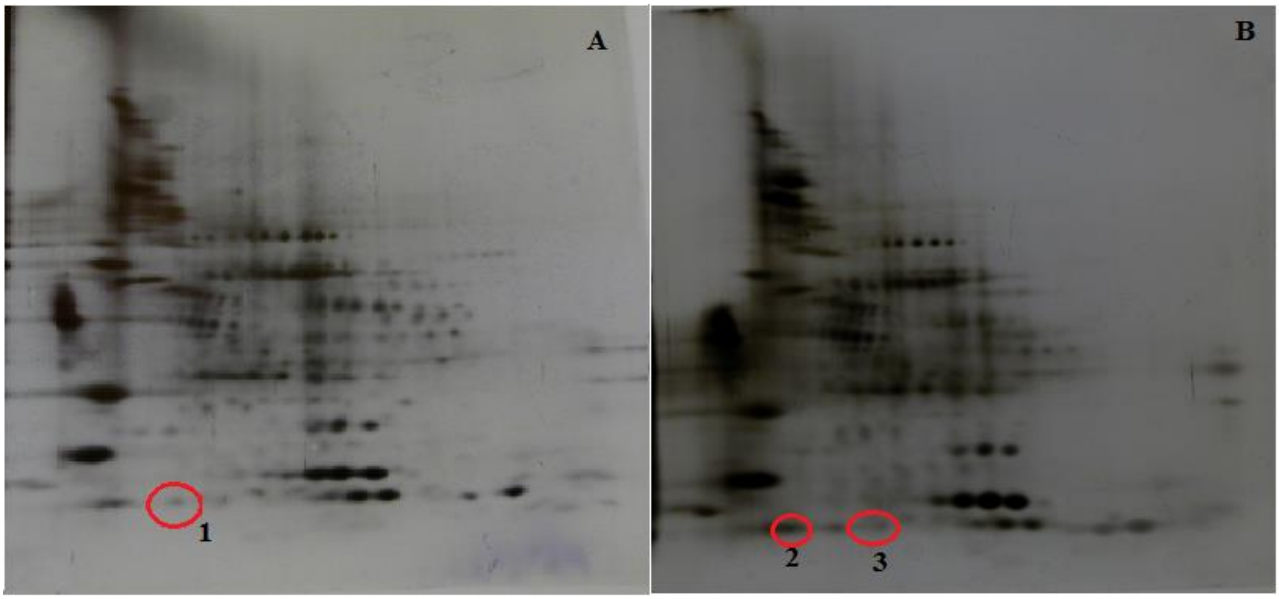

Figure 1 2DE of developed product.
Note: A – 2016 year of processing, B – 2018 year of processing, red round corresponded to fatty acid binding protein.

Table 2 The results of mass spectrometric identification (MALDI-TOF MS и MS/MS) of protein fractions.

№	Protein name; (Gene symbol)	S/M/C *	Мм/pI (exp.)**	Мм/pI (calc.)**
1	heart fatty acid-binding protein (H-FABP) (+ Acetyl (Protein N-term)*****(1)	283/13/68	14.8/5.11	14.8/6.11
2	Mixture of heart fatty acid-binding protein (*H-FABP*)***(1) + Deamidated (96Q,99N), cytochrome c oxidase subunit 5A, mitoch (*LOC100156967*)***(1) + Acetyl (Protein N-term)	122/9/56 38/17/73	15.0/5.25	14,8/6,11 16.7/6.42
3	Mixture of heart fatty acid-binding protein (*H-FABP*)***(1) + Acetyl (Protein N-term) and NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5 isoform X1 (*NDUFA5*)***(1)	120/4/36 113/2/36	15.0/5.60	14.8/6.11 13.3/7.79

Note: * S/M/C: Score – indicator of conformity or «scorecard»; Match peptides – the number of matched peptides; Coverage – % coverage of the entire amino acid sequence of the protein by identified peptides.
**mM/pI (experiment) – scores obtained as a result of electrophoretic mobility on the DE and mM/pI (calculation) – estimates made based on amino acid sequence data with consideration of signal peptide removal, but with no consideration of other post-synthetic modifications using the ExPASy Compute pI/Mw tool software.
***msms – indication of identification by tandem mass spectrometry, the number of sequenced tryptic peptides in parentheses.

C (LMWU) and D (LMWU) was approximately equal to group B (native pig aorta) and A (native pig heart).

In contrast to group C (LMWU) and D (LMWU) in group E there was another dynamic: on 28th day the ratio of TG was higer 1, while the ratio of AI was not so high despite of elevation CL HDL (ratio was lower 1). On 42nd day the ratio of CL HDL was higer 1, the ratio of AI was approximately equal to groups A (native pig heart), B (native pig aorta), C (LMWU) and D (LMWU).

In previous studies, several tissue-specific proteins were identified in porcine heart and aorta, as well as peptides. Mostly observed substances were decomposed during heat treatment of meat product, except fatty acid binding protein and several peptides **(Chernukha et al., 2016)**. However, it was proposed that tissue-specific proteins could be decomposed into active peptides with similar biological action or retained residual activity. In this study analytical study we revealed that meat product characterized by milder hypolipidemic action compare with native raw material or isolated active fractions; a significant effect was observed only after 42 days of consumption. Obtained results confirm our hypothesis.

There are different opinions about the relationship between the content of cholesterol or its atherogenic fractions and the risk of heart attack or stroke. While some scientists argue that there is no established relationship, others link worsening of a person after stroke or heart attack with elevated concentrations of cholesterol in blood, in particular, low-density cholesterol **(Demarin et al., 2010; Nelson, 2013; Ference et al., 2017; Hindy et al., 2018; Matthews, 2018)**. Developed product pronounced as a component of diet. Revealed long-term effect of product meets the requirements for patients receiving traditional medical treatment.

The creation of food products aimed at their constant use and positively affecting on the lipid profile can be considered as an important component of rehabilitation

therapy as accommodating diet therapy in particular for people with risk of stroke or heart attack.

The developed product was processed twice with two year interval. The 2D proteomic maps presented in Figure 1, results of fatty acid binding protein identification presented in Table 2. It was shown that there were no significant changes in major proteins, which indirectly confirm reproducibility of production technology and long shelf life. Moreover, fatty acid binding protein retained during 2-year storage.

Obtained results confirm that developed product contain peptides with residual hypolipidemic activity. On the other hand, native raw material or isolated active fractions demonstrated higher hypolipidemic effect, therefore it would be perspective to modernize the technology, presumably use as a matrix dietary meat (e.g. poultry) with incorporated active identified components.

CONCLUSION

Despite on the decomposition of target proteins and peptides after product processing, a pronounced lipid-lowering effect was noted, but less active than in case of native raw material or isolated active fractions. It was also found, that such technology treatment as ultrafiltration did not affect on activity of target compounds.

The obtained results open wide horizons for modification of the existing technology both in respect of variation of production modes and in respect of matrix changes and dosing of active proteins and peptides.

REFERENCES

Ahhmed, A. M., Muguruma, M. 2010. A review of meat protein hydrolysates and hypertension. *Meat Science*, vol. 86, no. 1, p. 110-118. https://doi.org/10.1016/j.meatsci.2010.04.032

Alao, B. O., Falowo, A. B., Chulayo, A., Muchenje, V. 2017. The Potential of Animal By-Products in Food Systems: Production, Prospects and Challenges. *Sustainability*, vol. 9, no. 7, p.1-18. https://doi.org/10.3390/su9071089

Arrutia, F., Fernández, R., Menéndez, C., González, U. A., Riera, F. A. 2017. Utilization of blood by-products: An in silico and experimental combined study for BSA usage. *Scientific Reports*, vol. 7, p. 1-9. https://doi.org/10.1038/s41598-017-17029-2

Bamdad, F., Shin, S. H., Suh, J. W., Nimalaratne, C., Sunwoo, H. 2017. Anti-Inflammatory and Antioxidant Properties of Casein Hydrolysate Produced Using High Hydrostatic Pressure Combined with Proteolytic Enzymes. *Molecules*, vol. 22, no. 4, p. 1-16. https://doi.org/10.3390/molecules22040609

Bauchart, C., Rémond, D., Chambon, C., Patureau Mirand, P., Savary-Auzeloux, I., Reynès, C., Morzel, M. 2006. Small peptides (<5 kDa) found in ready-to-eat beef meat. *Meat Science*, vol. 74, no. 4, p. 658-666. doi: https://doi.org/10.1016/j.meatsci.2006.05.016

Chernukha, I. M., Fedulova, L. V., Kotenkova, E. A. 2015. Meat by-product is a source of tissue-specific bioactive proteins and peptides against cardio-vascular diseases. *Procedia Food Science*, vol. 5, p. 50-53. https://doi.org/10.1016/j.profoo.2015.09.013

Chernukha, I. M., Fedulova, L. V., Kotenkova, E. A. 2018. The influence of bioactive substances isolated from porcine aorta on the serum fatty acid composition of hyperlipidemic rats. *Theory and practice of meat processing*, vol. 3, no. 4, p. 16-22. https://doi.org/10.21323/2414-438X-2018-3-4-16-22

Chernukha, I. M., Fedulova, L. V., Kotenkova, E. A., Shishkin, S. S., Kovalyov, L. I., Mashentseva, N. G., Klabukova, D. L. 2016 Influence of heat treatment on tissue specific proteins in the Sus Scrofa cardiac muscle and aorta. *Russian Journal of Biopharmaceuticals*, vol. 8, no. 6, p. 38-44.

Chernukha, I., Fedulova, L., Kotenkova, E., Akhremko, A. 2018a. Hypolipidemic action of the meat product: in vivo study. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 566-569. https://doi.org/10.5219/959

Chernukha, I. M., Fedulova, L. V., Kotenkova, E. A., Takeda, S., Sakata, R. 2018b. Hypolipidemic and anti-inflammatory effects of aorta and heart tissues of cattle and pigs in the atherosclerosis rat model. *Animal Science Journal*, vol. 89, no 5, p. 784-793. https://doi.org/10.1111/asj.12986

Chugunov, A. O. 2010. Unknown peptides. *Nauka i zhizn*, no. 10, p. 26-32.

Demarin, V., Lisak, M., Morović, S., Cengić, T. 2010. Low high-density lipoprotein cholesterol as the possible risk factor for stroke. *Acta Clinica Croatica*, vol. 49, no. 4, p. 429-439.

Fagerberg, L., Hallström, B. M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., et al. 2014. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Molecular and Cellular Proteomics*, vol. 13, no. 2, p. 397-406. https://doi.org/10.1074/mcp.M113.035600

Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., et al. 2017. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *European Heart Journal*, vol. 38, no. 32, p. 2459-2472. https://doi.org/10.1093/eurheartj/ehx144

Hindy, G., Engström, G., Larsson, S. C., Traylor, M., Markus, H. S., Melander, O., Orho-Melander, M. 2018. Role of Blood Lipids in the Development of Ischemic Stroke and its Subtypes: A Mendelian Randomization Study. *Stroke*, vol. 49, no. 4, p. 820-827. https://doi.org/10.1161/STROKEAHA.117.019653

Hongdong, S., Bo, L. 2017. Beneficial Effects of Collagen Hydrolysate: A Review on Recent Developments. *Biomedical Journal of Scientific & Technical Research*, vol. 1, no. 2, p. 1-4. https://doi.org/10.26717/BJSTR.2017.01.000217

Kovalyov, L. I., Kovalyova, M. A., Kovalyov, P. L., Serebryakova, M. V., Moshkovskii, S. A., Shishkin, S. S. 2006. Polymorphism of delta3,5-delta2,4-dienoyl-coenzyme A isomerase (the ECH1 gene product protein) in human striated muscle tissue. *Biochemistry (Moscow)*, vol. 71, no. 4, p. 448-453. https://doi.org/10.1134/S0006297906040146

Lafarga, T., Hayes, M. 2014. Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. *Meat Science*, vol. 98, no. 2, p. 227-239. https://doi.org/10.1016/j.meatsci.2014.05.036

Lafarga, T., Rai, D. K., O'connor, P., Hayes, M. 2016. Generation of Bioactive Hydrolysates and Peptides from Bovine Hemoglobin with In Vitro Renin, Angiotensin-I-Converting Enzyme and Dipeptidyl Peptidase-IV Inhibitory Activities. *Journal of food biochemistry*, vol. 40, no. 5, p. 673-685. https://doi.org/10.1111/jfbc.12259

Matthews, S. 2018. 'No evidence' having high levels of bad cholesterol causes heart disease, claim 17 physicians as they

call on doctors to 'abandon' statins. Available at: https://www.dailymail.co.uk/health/article-6176151/No-evidence-having-high-levels-bad-cholesterol-causes-heart-disease.html

Mine, Y., Shahidi, F. 2006. *Nutraceutical Proteins and Peptides in Health and Disease*. USA : Taylor & Francis Group, CRC Press, 688 p. ISBN 9781420028836. https://doi.org/10.1201/9781420028836

Mohanty, D. P., Mohapatra, S, Misra, S, Sahu, P.S. 2016. Milk derived bioactive peptides and their impact on human health - A review. *Saudi Journal of Biological Sciences*, vol. 23, no. 5, p. 577-83. https://doi.org/10.1016/j.sjbs.2015.06.005

Nelson, R. H. 2013. Hyperlipidemia as a risk factor for cardiovascular disease. *Primary Care*, vol. 40, no. 1, p. 195-211. https://doi.org/10.1016/j.pop.2012.11.003

Toldrá, F., Aristoy, M. C., Mora, L., Reig, M. 2012. Innovations in value-addition of edible meat by-products. *Meat Science*, vol. 92, no. 3, p. 290-296. https://doi.org/10.1016/j.meatsci.2012.04.004

Toldrá, F., Mora, L., Reig, M. 2016.New insights into meat by-product utilization. *Meat science*, vol. 120. p. 54-59. https://doi.org/10.1016/j.meatsci.2016.04.021

Toldrà, M., Parés, D., Saguer, E., Carretero, C. 2011. Hemoglobin hydrolysates from porcine blood obtained through enzymatic hydrolysis assisted by high hydrostatic pressure processing. *Innovative Food Science and Emerging Technologies*, vol. 12, no. 4, p. 435-442. https://doi.org/10.1016/j.ifset.2011.05.002

Udenigwe, C. C., Howard, A. 2013. Meat proteome as source of functional biopeptides. *Food Research International*, vol. 54, no. 1, p. 1021-1032. https://doi.org/10.1016/j.foodres.2012.10.002

Wang, J., Su, Y., Jia, F., Jin, H. 2013. Characterization of casein hydrolysates derived from enzymatic hydrolysis. *Chemistry Central Journal*, vol. 7, no. 1. 62 p. https://doi.org/10.1186/1752-153X-7-62

Zvereva, E. A., Kovalev, L. I., Ivanov, A. V., Kovaleva, M. A., Zherdev, A. V., Shishkin, S. S., Lisitsyn, A. B., Chernukha, I. M., Dzantiev, B. B. 2015. Enzyme immunoassay and proteomic character-ization of troponin I as a marker of mammalian muscle compounds in raw meat and some meat products. *Meat Science*, vol. 105, p. 46-52. https://doi.org/10.1016/j.meatsci.2015.03.001

Acknowledgments:
This work was supported by the Russian Science Foundation (project No. 16-16-10073).

Contact address:
*Elena Kotenkova, V. M. Gorbatov Federal Research Center for Food Systems of RAS, Experimental-clinical research laboratory of bioactive substances of animal origin, Talalikhina st., 26, 109316 Moscow, Russia, Tel.: +79031684478,
E-mail: lazovlena92@yandex.ru
ORCID: http://orcid.org/0000-0003-1864-8115
Irina Chernukha, V. M. Gorbatov Federal Research Center for Food Systems of RAS, Experimental-clinical research laboratory of bioactive substances of animal origin, Talalikhina st., 26, 109316 Moscow, Russia, Tel.: +79859248446,
E-mail: imcher@inbox.ru
ORCID: http://orcid.org/0000-0003-4298-0927

Corresponding author: *