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THEORY AND PRACTICE OF MEAT PROCESSING

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Original scientific paper

A METHOD DEVELOPMENT FOR IMPROVING THE STABILITY OF VEGETABLE POLYPHENOL COMPLEXES FOR SEMI-FINISHED MINCED MEAT PRODUCTS WITH ANTIOXIDANT EFFECT

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Key words: *semi-finished minced meat products, offals, dried cowberry squeezing extract, total antioxidant content, polyphenols, sorption, losses during heat treatment, peroxide value, storage*

Abstract

Polyphenolic vegetable complexes are active antioxidants and play an important role in the processes of free radicals quenching in the human body. The aim of the work was to develop an immobilization method as a way for improving the stability of polyphenols in dried cowberry squeezing extract (DCSE) and to develop semi-finished minced meat products with antioxidant effect without changing their nutritional value. Experiments were carried out to study the nutritional value of DCSE. The presence of a large number of polyphenolic compounds in DCSE and a high radical-binding ability of the extract were revealed. Based on the mathematical planning method, an offal paste composition was obtained, which was used to obtain pellets, and the formulation of semi-finished minced meat products with pellets was optimized. It was established that preliminary immobilization of DCSE on offal pellets components allows to increase the level of total antioxidant content in the minced meat. Subsequent heat treatment of semi-finished minced meat products produced from such minced meat showed an increase in stability and, thus, bioavailability of antioxidants and the possibility of obtaining a finished product with antioxidant effect. The antioxidant effect of cowberry squeezing extract in the meat system is proved: accumulation of lipid oxidative decomposition products in the meat system during storage of frozen semi-finished minced meat products was inhibited to a greater extent when using offal pellets with immobilized compounds of cowberry squeezing extract.

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Introduction

In meat production, quick-frozen semi-finished minced meat products play a significant role. The advantage of this type of food is the possibility of long-term storage at low temperatures and quick preparation of a hot dish with high nutritional value.

At the same time, meat products do not have enough micronutrients in their composition and contain a certain amount of fat undergoing oxidative changes during storage, which may negatively affect the consumer properties of the product.

Constantly growing impact of chemical and non-chemical factors on the human body may decrease overall resistance of the body as a result of adaptive and protective mechanisms decompensation. Currently, one of the main cellular mechanisms of adaptation, i. e. the antioxidant system of the body, is of increasing interest of researchers.

The main source of antioxidants are products of vegetable origin: vegetables, fruits, juices, tea, etc. The antioxidant potential of products may be considered not only as functionality aspect, but also as one of the aspects of maintaining the quality of food products [1,2,3,4,5,6,7,8,9,10]. It should be noted that various profiles of the biologically active substances in vegetables, fruits and other vegetable products are explained not only by genetics, but also by other factors, including climatic, agricultural and other ones.

So, in [11,12], the authors present the results of experimental studies evaluating the antioxidant activity of spicy herbs and onion. It is shown that the content of phenolic compounds in spicy herbs varies depending on the type of spice and on the extent of ripening. The authors conclude that the studied spicy herbs and onion have the ability to quench free radicals, prevent lipid oxidation, and exhibit reducing properties. The addition of spicy herbs (dill, parsley, basil, rosemary, oregano) and onion in food, including meat, allows enriching them with natural phenolic compounds.

Analysis and comparative evaluation of antioxidant activity and the content of prooxidant factors in various types of food raw materials and food products, primarily of vegetable origin, i. e. vegetables, fruits, drinks, juices, various crops, bakery and confectionery products, are given in a number of works [13,14].

The work [15] studies total antioxidant content in water/alcohol infusion of rose hips and barberry fruits and their

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use as food additives with antioxidant effect in the production of meat products.

Natural antioxidants have significant advantages over artificial ones: they contain a natural complex of biologically active substances in an available and digestible form. Such sources of antioxidants include many berries, for example, cowberry and cranberry, which are especially abundant in the Siberian forests [16].

One of the methods of berries processing is the production of juices, in the production of which 20–35% of waste is formed consisting of peel and seeds, i. e. so-called squeezing. In the best case, they may be used as livestock feed. In the worst case, they may be disposed. Although squeezing has the greatest antioxidant potential, since it contains a complex of vitamins (C and B), organic acids (benzoic, citric, malic, sorbic), minerals (Na, K, Ca, Mn) and an increased content of dietary fiber [17,18,19].

Recycled berry processing products have already widely used in wine industry in the form of pastes obtained from frozen squeezing [20]. It is proposed to use dried and crushed squeezing to enrich confectionery, bakery and meat products [21,22,23]. In the work, it was found that in terms of antioxidant properties (reducing strength by the FRAP method, antioxidant activity in the linoleic acid system, antioxidant activity by the DPPH method), squeezing is the best, while juices are significantly inferior to berries. Thus, squeezing is a valuable product containing more antioxidants than the initial raw material and having a high rate of antioxidant activity.

We have developed a technology for producing dried cowberry squeezing extract (DCSE) with antimicrobial and pronounced antioxidant effects [24]. In [25], the effect of dried extract from cranberry squeezing on the antioxidant and consumer properties of sugar cookies was investigated. The finished product, enriched with cranberry extract, was characterized by a high content of water-soluble antioxidants and had good consumer properties.

The level of bioflavonoids in food products depends both on the production technology and on storage duration and conditions of both raw materials and the products obtained from them. Cooking has a significant impact on the level of polyphenolic components. Simple peel cutting-off from fruits, vegetables, and berries may lead to a significant decrease in the content of bioflavonoids in them, which are usually present in significant quantities in the outer, and not in the inner part of fruits, berries, and vegetables [26].

Thus, the study of the actual content of compounds with antioxidant effect in finished products (after technological processing, cooking), increasing the efficiency and real assessment of the enriched dietary products contribution to the antioxidant status of the human body is extremely relevant.

The aim of this work was to develop a method for improving the stability of polyphenol complex isolated from cowberry squeezing and to formulate semi-finished minced meat products with high nutritional value and antioxidant effect.

Materials and methods

To conduct experimental studies, frozen beef offals from Buryat cattle were selected. The objects of research at the first stage were the beef offals, i. e. rumen, lungs, and diaphragm. The objects of research at the second stage were the dried cowberry squeezing extract (DCSE), minced meat and semi-finished minced meat products.

At the first stage of the experiment, the nutritional value of beef offals was investigated; offal paste formulation was optimized, which included alginate-containing food additive as a texture-forming component, KF Stabipro FAT in the amount of 4%. KF Stabipro FAT food additive was preliminarily subjected to hydration before its usage.

At the second stage, DCSE properties were studied, which was obtained by the following technology: squeezing formed after the juice was squeezed from cowberry growing in Transbaikalia was dried under infrared radiation at a temperature of 40 °C for 50 min to 10% moisture. Then, dried squeezing was subjected to grinding and further extraction with a water-alcohol solution using a microwave field. The obtained extracts were filtered, concentrated on a rotary evaporator at a temperature of 45 °C and then dried to obtain a powder [24].

At the next stage, in the course of the experiment, minced meat samples were prepared according to the formulation, which consisted of 2-grade trimmed beef and semi-fat trimmed pork (Control 1). Test 1 — samples of steaks from minced meat, into which 0.2% DCSE was added. Control 2 — steaks from minced meat with pellets (based on ground beef rumen, lungs, diaphragm, alginate-containing additive). Test 2 — steak sample with pellets, on the components of which 0.2% DCSE was preliminarily immobilized. Pellets have been used to replace basic raw materials and to study the possibility of DCSE polyphenols sorption on protein molecules. Alginate-containing additive was used as an enhancer of functional and technological characteristics.

To evaluate the antioxidant activity of the studied samples, the amperometric measurement of total antioxidant content (TAC) in quercetin equivalent was performed. To determine TAC, sample preparation was carried out by aqueous extraction of the test samples with bidistilled water to isolate water-soluble compounds with antioxidant effect. Total antioxidant content was determined by amperometric method using Tsvet Yauza-01-AA instrument. To construct calibration graphs, quercetin solutions were used [27].

The extraction efficiency was estimated by the number of phenolic compounds isolated with spectrophotometric method using the Folin-Ciocalteu reagent. The content of benzoic acid was investigated using the HPLC method. The pH value was determined by potentiometric method.

A spectrophotometric method with 2,2-diphenyl-1-picryl hydrazyl chromogen radical (DPPH) was used to determine the radical-binding ability (RBA) of the extracts. The method is based on the reaction of DPPH dissolved in ethanol with an antioxidant sample. Radical-binding ability was calculated

as EC_{50}, i. e. the concentration of the initial extract required for quenching of 50% DPPH radicals [28].

During the experiments, the physical and chemical parameters characterizing the quality of semi-finished meat product were studied. Protein content was evaluated by the Kjeldahl method. Collagen content was evaluated by the amount of oxyproline (treating the analyzed sample with a solution of perchloric acid for 4 hours under heating conditions in a water bath at 100 °C, neutralization of the reaction mixture with sodium hydroxide, addition of propanol-2 and a solution of chloramine-T in acetate-citrate buffer, followed by treatment with a solution of paradimethylaminobenzaldehyde in a mixture of 57% solution of perchloric acid and propanol-2, heating for 25 minutes at 60 °C and photometric analysis of the resulting colored solution). Elastin content was evaluated by gravimetric method, and fat by Soxhlet method. Moisture was evaluated by drying to constant weight, ash by dry ashing, and amino acid composition by chromatographic method.

Sensory analysis of semi-finished minced meat products were carried out according to a 9-point scale. Peroxide value in the samples was determined by a method based on the interaction of the oxidation products of fatty raw materials (peroxides and hydroperoxides) with potassium iodide in a solution of acetic acid and chloroform, followed by quantitative determination of the iodine released by the sodium thiosulfate solution with the titrimetric method.

The experiments were performed in triplicate; statistical processing of the obtained experimental data was carried out using the Microsoft Excel software.

Results and discussion

In order to improve stability of the antioxidant polyphenolic complex from cowberry squeezing, offal paste based pellets were developed, into which an extract was added to sorb biologically active substances of DCSE on the protein components of the paste. When developing the formulation of pellets, the aim was to adequately replace part of raw meat with offals. Currently, low-value offals, such as lungs, rumen, etc., are either sold frozen or transferred to the production of feed. Although it is known that offals contain valuable proteins. Table 1 presents the content of the main components in the studied offals obtained when slaughtering Buryat cattle.

Table 1. The chemical composition of beef offals

Parameters	Offals		
	Lung	Rumen	Diaphragm
	Mass fraction,%		
moisture	73.85 ± 1.56	75.51 ± 1.18	72.53 ± 1.41
total protein, including	18.43 ± 0.91	17.09 ± 0.78	18.84 ± 1.12
— collagen	6.64 ± 0.22	12.03 ± 0.52	5.15 ± 0.21
— elastin	1.02 ± 0.03	0.61 ± 0.04	0.3 ± 0.01
fat	5.85 ± 0.10	5.65 ± 0.11	7.08 ± 0.11
ash	1.87 ± 0.12	1.77 ± 0.11	1.74 ± 0.12

The data in Table 1 indicate that the moisture content in rumen is 1.66% higher than in lungs and 2.98% higher than in diaphragm. Protein content is quite high in all types of offals and amounted to 68 to 77%, the difference was within statistical error. Offal proteins include both complete ones and low-value connective tissue proteins, which play a role in the gastrointestinal processes.

To analyze the protein composition of beef offals, comparative diagrams were constructed (Fig. 1), which clearly show that complete proteins, such as myoglobin, albumin, globulin and others, prevail in the lungs and diaphragm, which amounted to 63.4 to 71.1%. Rumen generally contains low-value proteins, mainly collagen (70.4%), however, complete proteins are present, and their mass fraction is almost 30%.

In accordance with the aim, the possibility of creating pellets based on offal paste for the adequate replacement of minced meat and the immobilization of biologically active substances from dried cowberry squeezing extract on pellet protein components to increase their stability during processing and heat exposure was studied.

To achieve this, studies were carried out to replace a portion of the meat in the formulation of minced steak with pellets developed on the basis of offal paste and alginate-containing additive. The replacement is justified not only by the rational use of animal by-products, but also by reducing the cost of the finished product and the need to introduce polyphenolic micronutrients into the meat product in an effective dose. To preserve the nutritional value of the product and adequate meat replacement, the method of mathematical

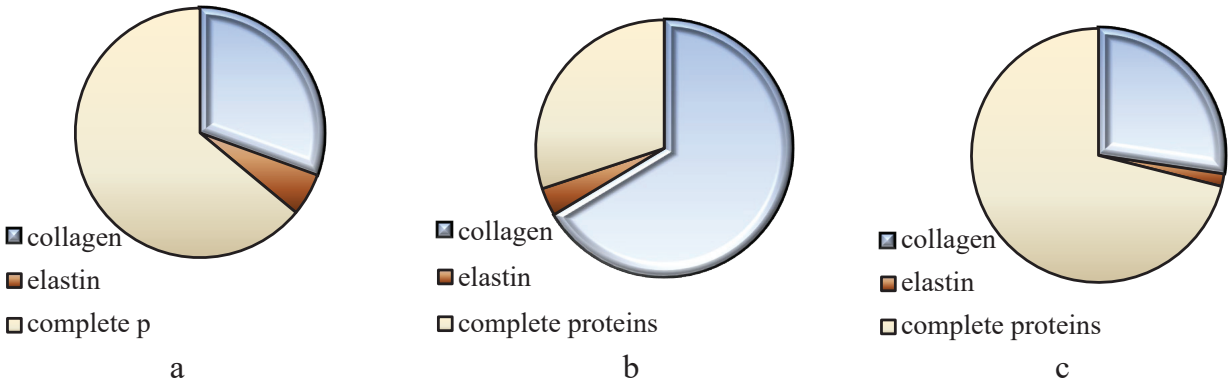

Figure 1. The ratio of complete and low-value proteins in offals obtained from slaughter of Buryat cattle: a) lungs, b) rumen, c) diaphragm

simulation of minced steak formulation and optimization of the chemical composition was used.

Offals were prepared by thawing, cleaning and washing, then finely ground to a paste-like consistency. To determine the optimal ratio of offals in the composition of the paste, its formulation was optimized using the «Solution Search» tool in Microsoft Excel software, which resulted in the following ratio: diaphragm 40%, rumen 30%, lung 30%. The paste was red, without any off-odor. To improve the functional and technological characteristics of the paste used, KF Stabipro FAT food additive containing sodium alginate, calcium pyrophosphate and calcium sulfate was introduced. To stabilize the texture of the resulting mixture, it was kept in a refrigerator at a temperature of 5 °C for 12 hours. The obtained hardened mass had an elastic consistency, red color and no off-odor. Then it was passed through a meat grinder (3 mm diameter) and so-called pellets were obtained, which were added into the composition of minced meat for steaks.

Formulation of minced steaks with pellets was developed by computer optimization using an integrated model that takes into account the chemical and amino acid composition of the ingredients.

To carry out the calculation of the formulation, a mathematical model was developed that included both the initial (protein, fat, amino acid content — Table 2) and output data (optimal ratio of the formulation ingredients and the value of the optimality test).

Table 2. The content of the main nutrient components

Parameters	2-Grade trimmed beef *	Semi-fat trimmed pork*	Pellets
Protein content,%	18.8	12.6	8.4
Fat content,%	7.5	33.5	4.6
Essential amino acids, g/100 g protein:			
Valine	10.9	9.2	2.2
Isoleucine	8.2	6.9	1.9
Leucine	16.6	9.9	3.8
Lysine	16.7	12.1	3.4
Methionine + cystine	6.1	4.2	1.6
Threonine	8.2	6.1	1.5
Tryptophan	2.2	1.8	0.4
Phenylalanine + tyrosine	14.8	10.2	2.9
Total essential amino acids	83.7	60.4	17.7

* data from literature

The conditions in the mathematical model of the optimal steaks composition are described as a system of inequalities, where:

x_1 is 2-grade trimmed beef;

x_2 is semi-fat trimmed pork;

x_3 is offal pellets.

The limitations for the system of inequalities are presented by the indicators of biological value and chemical composition (Table 3).

Table 3. Limitations for the mathematical model of formulation

Parameters	Content	
	min	max
Protein content,%	14.0	58.0
Fat content,%	0	20.0
Essential amino acids, g/100 g protein:		
Valine	5.0	no limitation
Isoleucine	4.0	no limitation
Leucine	7.0	no limitation
Lysine	5.5	no limitation
Methionine + cystine	3.5	no limitation
Threonine	4.0	no limitation
Tryptophan	1.0	no limitation
Phenylalanine + tyrosine	6.0	no limitation
Total essential amino acids	36.0	no limitation

The system of inequalities in the mathematical model (complex model) of formulation for the designed finished product is presented as follows:

1. $14.0 \le 18.8\ x_1 + 12.6\ x_2 + 8.4\ x_3 \le 58.0$ (by protein content)
2. $0 \le 7.5\ x_1 + 33.5\ x_2 + 4.6\ x_3 \le 20.0$ (by at content)
3. $5.0 \le 10.9\ x_1 + 9.2\ x_2 + 2.2\ x_3$ (by valine content). etc.

To simplify the inequalities, the following is assumed: $x = x_i/100$, where $i = 1 \div 3$. Thus, the following natural requirement for obtaining a production unit arises:

$$x_1 + x_2 + x_3 = 1.0$$

The objective function is as follows:

$$F_{ц}(\text{Total essential amino acids}) = 83.7\ x_1 + 60.4\ x_2 + 17.7\ x_3 \rightarrow M$$

As a result of problem solving, the formulation options of the finished product were obtained presented in Table 4.

Table 4. Formulation options

Formulation ingredients	Options						
	1	2	3	4	5	6	7
2-Grade trimmed beef	70.0	65.0	60.0	55.0	50.0	45.0	40.0
Semi-fat trimmed pork	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Pellets	10.0	15.0	20.0	25.0	30.0	35.0	40.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

In accordance with Table 4, samples of minced meat and minced steaks were produced and their sensory characteristics were analyzed. The analysis showed that the addition of pellets at the level of more than 15% reduces the overall sensory quality of the finished products; therefore, for the further experiments, option 2 formulation was chosen.

The predicted quality indicators of the developed minced steaks with pellets, which were obtained when solving the inequalities of the mathematical model, are shown in Table 5.

Table 5. Quality indicators obtained when solving the mathematical model

Parameters	Content	
	Steaks (Control 1)	Steaks with pellets (Control 2)
Protein content, %	17.56	16.00
Fat content, %	12.70	12.27
Total essential amino acids, g/100 g protein:	79.00	69.14

The data showed that as a result of formulation development for minced steaks with pellets, their composition is close to the content of the main substances in meat steaks.

At the next stage, the possibility of effective enrichment of semi-finished minced meat products with DCSE compounds was studied. The dry extract is obtained from by-products of juice production: the remaining squeezing after separation of the liquid pulp is not further processed and is considered waste. Squeezing, which consists of cowberry peel and seeds contains the largest number of biologically active compounds with high pharmacological characteristics [25].

DCSE preparation technology is presented in [24]. DCSE is a free-flowing mass of sweet and sour, harsh flavor with cowberry aroma and maroon color. The dry texture of the concentrated extract provides a high concentration of biologically active substances, so even a small dose of DCSE in a meat product may provide it with functional properties; however, some technological difficulties are associated with this.

Table 6 presents physical and chemical characteristics of squeezing extract from cowberry growing in Transbaikalia.

Table 6. Physical and chemical characteristics of dried cowberry squeezing extract

Parameters	Value
pH value	3.23 ± 0.07
Total antioxidant content, mg/g	382.5 ± 8.4
Content,%:	
moisture	4.51 ± 0.07
polyphenols	6.62 ± 0.03
benzoic acid	1.33 ± 0.01

The data in Table 6 indicate that cowberry squeezing extract has a low pH of 3.23 due to the high concentration of organic acids in DCSE. Significant total antioxidant content was found, which amounted to 382.5 mg/g of dry extract. The main components with antioxidant properties are polyphenols, the concentration of which is up to 6.62%. More than half of the polyphenols in cowberry are anthocyanins (3.59%), which are able to quench almost all types of reactive oxygen and nitrogen species four times more effective than ascorbate and α-tocopherol.

A high content of benzoic acid (1.33%) was found in DCSE, which is typical for cowberry fruits. That is why the berries may be stored for a long time without spoilage. Benzoic acid is known to have antimicrobial and even bactericidal effects.

Free radicals that occur in the body as a result of a homolytic rupture of chemical bonds due to a number of conditions (temperature, radiation, stress, improper nutrition, etc.) have a maximum destructive effect on human cells. Level of free radicals binding characterizes the ability of the antioxidant to resist the development of the so-called oxidative stress. Among several methods for studying antiradical activity, the method using 2,2-diphenyl-1-picryl hydrazyl is widely used and is the most acclaimed [29]. Figure 2 shows radical-binding ability of dried cowberry squeezing extract.

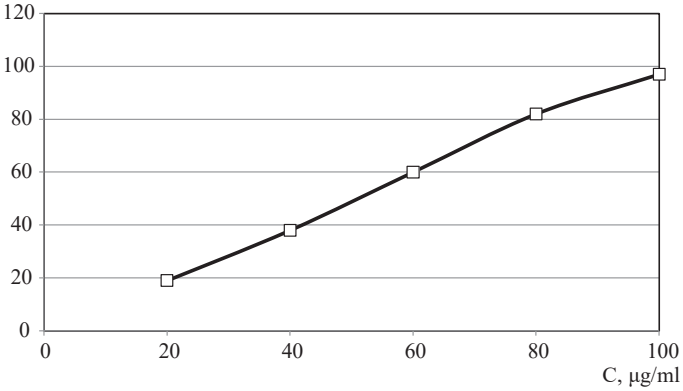

Figure 2. Radical-binding ability of DCSE

According to the data obtained, 50% of DPPH radical binding is achieved at a concentration of cowberry extract of 50.5 μg/ml, and 80% at 80.3 μg/ml. The high antiradical activity of apple and grape squeezing, which exceed the corresponding indices of berries and juices, is reported by the authors in [30].

To enrich semi-finished minced meat products with antioxidants, experimental studies were conducted on the addition of DCSE in the amount of 0.2% in dry form, hydrated in process water, and preliminarily sorbed on offal paste ingredients. Samples of minced meat were prepared and total antioxidant content was studied in minced meat and finished minced steaks after steam treatment at 80–85 °C for 20 minutes (Figure 3).

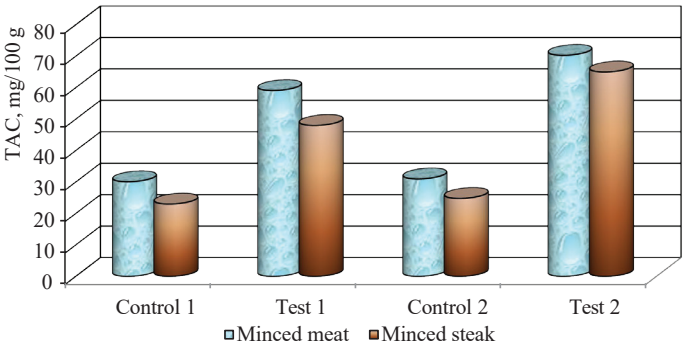

Figure 3. Total antioxidant content in minced meat and semi-finished minced meat products: Control 1 — steaks; Test 1 — steaks with 0.2% DCSE; Control 2 — steaks with pellets; Test 2 — steaks with pellets containing 0.2% sorbed DCSE

The data presented in Figure 3 indicate that antioxidants are present in minced meat of steak (Control 1) in an amount of 30.1 mg/100 g of minced meat. With the introduction

of 0.2% DCSE (Test 1), TAC increased almost twice and amounted to 59.1 mg/100 g of minced meat.

When adding pellets without DCSE to minced meat (Control 2), the antioxidant content remained almost unchanged compared to Control 1 and amounted to 31.0 mg/100 g of minced meat. With the addition of 0.2% DCSE sorbed on pellets (Test 2), TAC increased 2.27 times and amounted to 70.3 mg/100 g of minced meat. The increase in total antioxidant content with preliminarily immobilization of DCSE compounds compared to simple addition of the dry extract was 19%, which indicates the efficiency of polyphenolic compounds sorption when pre-incubated with pellets. Proteins of connective tissue in offals, mainly collagen, have high sorption abilities. Fine grinding of offals to a paste also provides optimal conditions for sorption of polyphenols. The collagen molecule contains a significant amount of diamino and amino dicarboxylic acids with a large number of side polar groups that are able to bind other molecules, e. g. polyphenols. Furthermore, the introduction of alginate-containing additive suggests the possibility of retaining polyphenolic compounds by sodium alginate polymolecules.

Further, antioxidants losses during heat treatment of minced steaks were studied. Since these are products without a cover, there is a high probability of TAC losses in finished products intended for consumption. The data presented in Figure 3 indicated that all samples showed a decrease in TAC after heat treatment. Thus, in the finished product (control 1), there was a decrease in antioxidant content by 23.6% compared with minced meat. In Test 1, with the addition of 0.2% DCSE, the losses were less (18.8%), apparently due to the initial high TAC level (almost 2 times higher compared to control).

Based on the data in Figure 3, TAC reduction after heat treatment in the finished product with pellets (Control 2) was 20%. A significant reduction in TAC losses in the finished product (Test 2) should be noted, in which DCSE was preliminarily sorbed on pellets. Losses amounted to only 7.5%.

The data presented prove the effectiveness of DCSE polyphenols immobilization, as losses in Test 2 decreased by 2.5 times compared with Test 1.

In accordance with the current «Recommended levels of consumption of food and biologically active substances. Guidelines. MR 2.3.1.1915–04» (approved by Rospotrebnadzor 07/02/2004), the average consumption rate of antioxidant polyphenols for a healthy population is 250–300 mg/day. In our proposed formulation of minced steaks containing pellets with immobilized DCSE, the TAC level is 70.3 mg/100 g of the finished product, which provides 23.4 to 28.1% of the daily requirement for antioxidants and represents a functional food with antioxidant effect.

The sensory characteristics of finished minced steaks were investigated (Figure 4).

As shown in Figure 4, the studied samples almost did not differ in their sensory characteristics. Test 1 sample was as close as possible to Control 1 sample. Control 2 and Test 2 had more dense texture, elastic, pleasant to the taste. As a result, overall estimates of indicators characterizing the consumer properties of the finished product turned out to be at the same level in the control and test samples.

Polyphenols also have antioxidant activity; in this connection, the effect of DCSE on the dynamics of peroxide value during storage of minced steaks was investigated. For a comparative study, a steak sample with pellets (Control) and a steak sample with pellets containing immobilized DCSE (Test) were selected. Figure 5 shows the influence of DCSE on the dynamics of fat oxidation process in the control and test minced steaks.

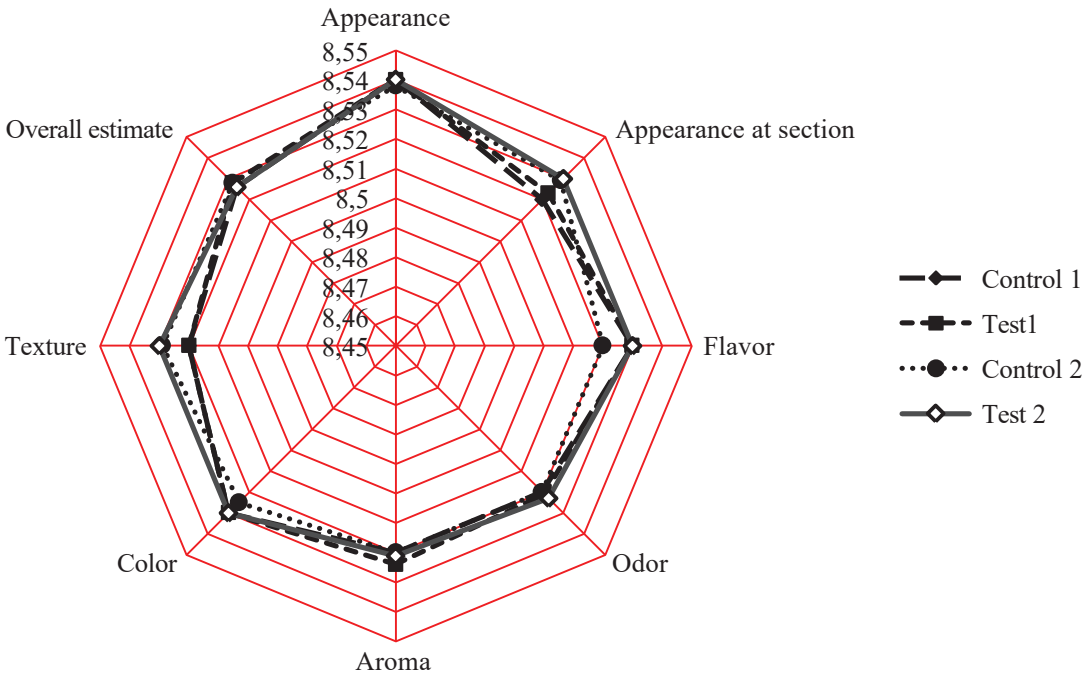

Figure 4. Sensory parameters of semi-finished minced meat products: Control 1 — steaks; Test 1 — steaks with 0.2% DCSE; Control 2 — steaks with pellets; Test 2 — steaks with pellets containing 0.2% sorbed DCSE

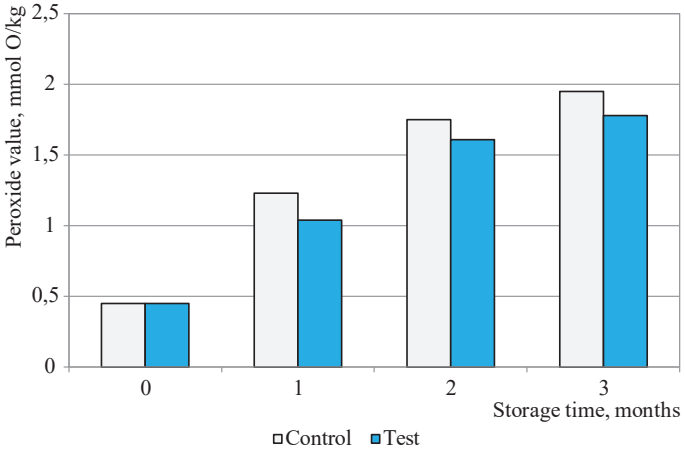

Figure 5. Effect of DCSE on the change in peroxide value of semi-finished minced meat products during storage

Quick-frozen semi-finished minced meat products in sealed packaging may be stored for three months at a temperature of minus 18 °C. According to the data presented in Figure 5, there is an irreversible process of fat self-oxidation in semi-finished products with the accumulation of primary fat degradation products. Immediately after production, peroxide value in test samples was 0.45 mmol O/kg. After storage of the frozen finished products at a temperature of minus 18 °C for one month, the peroxide value increased in the control up to the level of 1.23 mmol O/kg, and in the test sample up to 1.04 mmol O/kg. After two months of oxidative process, peroxide value in the control was already 1.75 mmol O/kg (3 times increase), and in the test 1.61 mmol O/kg (2.5 times increase). After months, when the shelf life of frozen semi-finished minced meat products was reached, the peroxide value in the control sample increased to 1.95 mmol O/kg, while remaining within the prescribed range. In the test sample, after three months, the peroxide value was 1.78 mmol O/kg. Based on the data obtained, the effect of lipid self-oxidation inhibition in the meat system with the addition of DCSE was found. The beneficial effect of natural antioxidants on oxidative reactions inhibition during storage of both minced semi-finished meat products and finished products was noted in [31].

Conclusion

Thus, as a result of experimental studies, it was found that the addition of DCSE, which has a high antioxidant effect, into the formulation of semi-finished minced meat products allows obtaining a finished product with an antioxidant effect. It was established that preliminary mixing of DCSE with offal-based pellets and subsequent ageing of the mixture make it possible to increase the content of antioxidants both in semi-finished and in finished products due to sorption of DCSE polyphenols on ingredients of offal paste. Sorption of polyphenols allows improving antioxidant stability during the technological and thermal processing of minced steaks, thereby providing 23.4 to 28.1% of the daily requirement for antioxidants when consuming 100 grams of the finished product. The use of pellets allows not only to reduce the consumption of basic raw materials, but also to ensure adequate preservation of the nutritional value of the finished meat product. Antioxidants of DCSE have antioxidative properties and reduce the accumulation of primary lipid degradation products, thus ensuring high consumer properties of the finished product and the possibility of shelf life extending for quick-frozen semi-finished minced meat products.

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REFERENCES

1. Tohma, H., Gülçin, İ., Bursal, E., Gören, A.C., Alwasel, S.H., Köksal, E. (2017). Antioxidant activity and phenolic compounds of ginger (Zingiber officinale Rosc.) determined by HPLC–MS/MS. *Journal of food measurement and characterization*. 11(2), 556–566. DOI: 10.1007/s11694–016–9423-z
2. Zhirkova, E.V., Skorokhodova, M.V., Martirosyan, V.V., Sotchenko, E.F., Malkina, V.D., Shatalova, T.A. (2016). Chemical composition and antioxidant activity of corn hybrids grain of different pigmentation. *Foods and raw materials,* 1(4), 85–91. DOI: 10.21179/2308–4057–2016–2–85–91
3. Lisitsyn, A.B., Chernukha, I.M., Lunina, O.I. (2018). Modern trends in the development of the functional food industry in Russia and abroad. *Theory and practice of meat processing,* 3(1), 29–45. DOI: 10.21323/2414–438X-2018–3–1–29–45 (In Russian)
4. Kakouri, E., Daferera, D., Paramithiotis, S., Astraka, K., Drosinos, E.H., Polissiou, M.G. (2017). Crocus sativus L. tepals: The natural source of antioxidant and antimicrobial factors. *Journal of applied research on medicinal and aromatic plants*, 4, 66–74. DOI: 10.1016/j.jarmap.2016.09.002
5. Ayoub, Z., Mehta, A., Mishra, S.K., Ahirwal, L. (2017). Medicinal plants as natural antioxidants: a review. *Journal of botanical society,* 48.
6. Baiano, A., del Nobile, M.A. (2015). Antioxidant compounds from vegetable matrices: Biosynthesis, occurrence, and extraction systems. *Critical reviews in food science and nutrition,* 56(12), 2053–2068. DOI: 10.1080/10408398.2013.812059
7. Li, Y., Zhang, J.J., Xu, D.P., Zhou, T., Zhou, Y., Li, S., Li, H.B. (2017). Bioactivities and health benefits of wild fruits. *International journal of molecular sciences,* 17(8), 1258. DOI: 10.3390/ijms17081258
8. Chan, E.W.C., Tan, Y.P., Chin, S. J., Gan, L.Y., Kang, K.X., Fong, C.H., How, Y.C. (2014). Antioxidant properties of selected fresh and processed herbs and vegetables. *Free radicals and antioxidants,* 4(1), 39–46. DOI: 10.5530/fra.2014.1.7
9. Esan, Y.O., Sade, O.O., Victor, E.N., Oluranti, O.O. (2018). Functional and antioxidant properties of raw and popped Amaranth (Amaranthus cruentus) seeds flour. *Annals. Food science and technology*, 19(2), 399–408.
10. Akbarirad, H., Ardabili, G.A., Kazemein, i S.M., Khaneghah, M.A. (2016). An overview on some of important sources of natural antioxidants. *International food research journal*, 23(3), 928–933.
11. Borisova, A.V., Makarova, N.V. (2013). Rating antioxidant activity of herbs and onions. *Storage and processing of farm products*, 8, 32–35. (In Russian)
12. Oswel, N.J., Thippareddi, H., Pegg, R.B. (2018). Practical use of natural antioxidants in meat products in the U.S.: A review. *Meat Science,* 11, 145:469–479. DOI: 10.1016/j.meatsci.2018.07.020
13. Yashin, Ya.I., Ryzhnev, V. Yu., Yashin, A. Ya., Chernousova, N.I. (2009). Natural antioxidants. Content in food products and impact on human health and aging. Moscow: Translit. — 212 p. ISBN 978–5–94976–727–6 (In Russian)

14. Kebede, M., Admassu, Sh. (2019). Application of antioxidants in food processing industry: options to improve the extraction yields and market value of natural products. *Advances in food technology and nutritional sciences,* 5(2), 38–49. DOI: 10.17140/AFTNSOJ-5–155
15. Zabalueva, Yu. Yu., Meleshkina, N.V., Bazhenova, B.A., Danilov, M.B. (2017). One of the ways of enrichment of meat products by natural antioxidants. *Vsyo o myase,* 2, 12–15. (In Russian)
16. Bazarnova, Yu.G., Polyakov, K. Yu. (2009). Study of antioxidant activity of natural substances. *Storage and processing of farm products,* 3, 31–36. (In Russian)
17. Izosimova, I.V. (2004). Scientific and practical basis for the rational use of cowberry (*Vaccinium vitis-idaea*) and cranberry (*Oxycoccus palustris*). Author's abstract of the dissertation for the scientific degree of Candidate of Biological Sciences. Krasnoyarsk: Krasnoyarsk State Trade and Economic Institute. — 24 p. (In Russian)
18. Lutikova, M.N. (2013). Composition studying of biologically active ingredients in wild berries of *Vaccinium vitis-idaea* and *Oxycoccus palustris* depending on their degree of maturity and storage conditions. Author's abstract of the dissertation for the scientific degree of Candidate of Chemical Sciences. Surgut: Surgut State University. — 24 p. (In Russian)
19. Bazarnova, Yu.G. (2012). Biologically active substances of wild-growing raw materials: stabilization and application in food technologies. St. Petersburg: St. Petersburg State University of Low-Temperature and Food Technologies. — 215 p. (In Russian)
20. Kolman, O. Ya., Ivanova, G.V. (2013). Methods for preserving the secondary raw materials of wild cowberries and cranberries. *Bulletin of KrasGAU,* 5(80), 218–223. (In Russian)
21. Kolman, O. Ya., Ivanova, G.V., Tsuglenok, N.V. Reduced calorie muffins. Patent RF, no.2012117932. 2013. (In Russian)
22. Kolman, O. Ya., Ivanova, G.V., Tsuglenok, N.V. Marmalade and berry masses. Patent RF, no.2012118185. 2013. (In Russian)
23. Izosimova, I.V., Ivanova, G.V., Sergacheva, O.M. (2001). The use of environmentally friendly secondary vegetable raw materials in the production of pastes. *IV International Symposium «New and unconventional plants and prospects for their use»*, 3,469–471. (In Russian)
24. Zambulaeva N.D., Zhamsaranova, S.D. A method of obtaining a dry cowberry or cranberry squeezing extract. Patent RF, no. 2626565. 2017. (In Russian)
25. Zambulaeva N.D., Zhamsaranova, S.D. (2016). Dry extract from squeezing of wild berries as a promising ingredient in functional nutrition products. *XV International Conference of Young Scientists «Food Technology and Biotechnology». Conference proceedings,* 107–109 pp. ISBN 978–5–98946–172–1 (In Russian)
26. Tutelyan, V.A., Lashneva, N.V. (2013). Biologically active substances of plant origin. Flavonols and flavones: prevalence, dietary sources and consumption. *Voprosy Pitaniia*, 82(1), 4–23. (In Russian)
27. Yashin, A. Ya. (2008). Injection-flow system with amperometric detector for the selective determination of antioxidants in foods and beverages. *Russian chemical journal*, 52(2), 130–135. (In Russian)
28. Majewska, M., Skrzycki, M., Podsiad, M., Czeczot, H. (2011). Evaluation of antioxidant potential of flavonoids: an in vitro study. *Acta Poloniae Pharmaceutica*, 68(4), 611–615.
29. Makarova, N.V., Valiulina, D.F., Azarov, O.I., Kuznetsov, A.A. (2018). Comparative studies: content of phenolic compounds, flavonoids and antioxidant activity in apples of different varieties. *Khimija Rastitel'nogo Syr'ja,* 2, 115–122. (In Russian)
30. Bykova, T.O., Makarova, N.V., Shevchenko, A.F. (2015). Chemical composition and antioxidant properties of apple and grape processing products, i.e. juice and squeezing. *Storage and processing of farm products,* 11, 20–22. (In Russian)
31. Yurikova, E. V., Savvateev, E. V., Savvateeva, L. Yu. (2013). Properties of additives for meat products. *Food Industry,* 11, 60–62. (In Russian)

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Review paper

BIOLOGICALLY ACTIVE PEPTIDES OF MEAT AND MEAT PRODUCT PROTEINS: A REVIEW

PART 1. GENERAL INFORMATION ABOUT BIOLOGICALLY ACTIVE PEPTIDES OF MEAT AND MEAT PRODUCTS

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Key words: *biologically active peptides, autolysis of meat raw materials, enzymatic treatment of muscle tissue, ACE-I*

Abstract

Over many years, proteins and polypeptides have aroused scientific-practical interest due to multiple functions in the metabolic processes in the body upon vital activities. Biologically active substances of protein origin have wide application in different industries, including the food industry and medicine. At present, many studies are directed towards investigation of mechanisms of formation of such physiologically valuable food components as biologically active peptides and methods of their recovery from meat raw materials and meat products. A large part of literature data confirms that mechanisms of formation of such peptides are similar irrespective of methods of their generation. Their basis is enzymatic hydrolysis of muscle tissue proteins under the action of intracellular enzymes during autolysis, digestive enzymes of the human gastrointestinal tract or commercial enzyme preparations used in laboratories or in the industry. The method of culinary and/or technological processing also affects the process of biopeptide formation in meat products, namely, their recovery and availability.

Introduction

Animal proteins in a human diet are physiologically active compounds; they have a direct action or are a substrate for enzymatic hydrolysis in processing and consumption of food. Biologically active peptides (BAPs) can be obtained through hydrolysis by intrinsic enzymes of meat raw materials, endogenous enzymes of the human gastrointestinal tract or microbial enzymes used in a technological process.

The integrity and structure of meat proteins change during autolysis or long-term storage of meat raw materials in the frozen state. A great number of peptides with the physiological activity are released during meat processing. The biological activity of food components is widely studied *in vitro*, and nowadays the attempts are made to study their *in vivo* effect on healthy people or patients with different pathologies.

This paper presents a complex review of the methods of biologically active peptide formation in meat raw materials and products.

Main part

The mechanisms of BAP formation in meat and meat products are similar (Table 1). During autolysis of muscle tissue, the proteolytic activity conditioned by endogenous enzymes (calpains and cathepsins) is a key process, which influences protein destruction and, consequently, generation and release of a large number of peptides and free amino acids [1,2].

Bauchart et al [4] in the study of aged beef found an increase in the content of BAPs <5 kDa in meat after 14 days of storage at a temperature of 4 °C compared to their quantity in fresh meat. Fu et al. [23] also demonstrated that bioactive peptides with a size of about 3 kDa can be generated in longissimus dorsi and semitendinosus muscles during meat aging after 20 days of proteolysis.

Generation of peptides can also be caused by the oxidation processes during meat storage [24]. The oxidative status can regulate the endogenous enzymatic activity and, consequently, a degree of degradation of myofibrillar and sarcoplasmic proteins [25]. Changes in temperature and pH can affect the content of bioactive peptides due to changes in the activity of endogenous enzymes [3,26,27].

It is known that peptides with the biological activity are naturally formed in the mammalian gastrointestinal tract during metabolism of meat diet proteins under the action of the digestive enzymes such as pepsin, trypsin, chymotrypsin, elastase, and carboxypeptidase [3,28,29,30]. Therefore, to generate such potentially biologically active peptides, researchers model a process that simulates gastrointestinal digestion. The process is based on enzymatic hydrolysis with the use of different commercial exogenous proteinases obtained from animal tissues (pepsin and trypsin), plants (papain, ficin, and bromelain) and microbial sources (alcalase®, flavourzyme®, neutrase®, collagenase or proteinase K) [30,31,32]. In addition to the meat sources, several BAPs are generated by enzymatic hydrolysis of collagen from meat or slaughter by-products (trimmings, organs, blood hemoglobin) as was highlighted in several studies [24,33].

For example, the release of potential BAPs — angiotensin-I-converting enzyme (ACE-I, EC3.4.15.1), renin (EC3.4.23.15)

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Table 1. Brief characteristics of the processes of generation of meat biologically active peptides [3]

Product	Process	Carrier/Regulation	Functionality	Peptide sequence	Reference
Muscle tissue	Proteolysis, oxidation	Endogenous enzymes	ACE*-I activity	APPPPAEVPEVHEEVH, PPPAEVPEVHEEVH, IPITAAKASRNIA, LPLGG, FAGGRGG, APPPPAEVP	[4,5,6]
	Enzymatic hydrolysis	Exogenous enzymes	ACE-I, antioxidant, antithrombotic, antimicrobial and antitumor activities	KRQKYD, EKERERQ, KAPVA, PTPVT, RPR, GLSDGEWQ, GFHI, DFHING, FHG	[7–15]
	Preparation	High temperature	ACE-I	SPLPPPE, EGPQGPPGPVG, PGLIGARGPPGP	[4]
Collagen	Enzymatic hydrolysis	Bacterial collagenase, exogenous enzymes, proteases from Aspergillus oryzae	ACE-I and antioxidant activities	AKGANGAPGIAGAPGFPGARGPSGPQGPSGPP, PAGNPGADGQPGAKGANGAP, GAXGLXGP, GPRGF, VGPV, QGAR, LQGM, LQGMH, LC	[16–19]
Dry-cured products	Proteolysis	Endogenous enzymes	antioxidant activity	DSGVT, IEAEGE, EELDNALN, VPSIDDQEELM, DAQEKLE, ALTA, SLTA, VT, SAGNPN, GLAGA, DLEE	[20,21]
Fermented products	Proteolysis	Presence of starter cultures	antioxidant activity	FGG, DM	[22]

*** ACE — Angiotensin converting enzyme**

and dipeptidyl peptidase-IV (DPP-IV, EC3.4.14.5) — from bovine and porcine proteins including hemoglobin, collagen and serum albumin, was assessed in the study of 2014 [34] using the *in silico* methods, peptide databases and software as well as chemical synthesis and *in vitro* analysis to confirm bioactivity of peptides. These proteins are usually found in meat by-products such as bones, blood and meat trimmings, and play a key role in the control of hypertension, development of type-2 diabetes and other diseases associated with the metabolic syndrome. New peptides included ACE-I inhibitory tripeptide Ile-Ile-Tyr and DPP-IV inhibitory tripeptide Pro-Pro-Leu corresponding to the sequences f (182–184) and f (326–328) of porcine and bovine serum albumin, which can be released after hydrolysis by the enzymes papain and pepsin, respectively.

In another work [35], the inhibitory and antioxidant activities of ACE-I sarcoplasmic proteins extracted from the pectoral muscle (*Pectoralis profundus*) of cattle (*Bos taurus*) and hydrolyzed by papain at 37 °C for 24 hours were studied. Sarcoplasmic protein hydrolysates were subjected to membrane ultrafiltration and filtrates 10 kDa and 3 kDa were obtained. As a result, 11 peptides were characterized from the total hydrolysate fraction: 15 from the fractions of 10 kDa filtrate, 9 peptides from the fractions of 3 kDa filtrate. The similarity between amino acid sequences of peptides identified in this investigation and known antioxidant and ACE-I inhibitory peptides described in the database of biologically active peptide sequences BIOPEP was found [36].

A promising source of BAPs are porcine myofibrillar proteins [37]. The enzymes pepsin, trypsin and chymotrypsin were used for *in silico* proteolysis. In intact proteins and after simulation of gastrointestinal digestion, the inhibitory peptide sequences of dipeptidyl peptidase-IV were observed most frequently. In total, the authors found 399 peptides with the antioxidant, hypotensive, antiamnesic and stimulating or regulating different body functions activities, as well as enzyme inhibitors [38].

Other mechanisms, such as the processes of freezing and cooking can affect release and availability of meat BAPs. Freezing can denature proteins due to different chemical and physical stress mechanisms including ice formation, pH changes and low temperature [39], which leads to an increase in BAPs. Cooking can influence peptide generation and their biological activity [23,27] due to changes in the native conformation (denaturation) and disruption of intramolecular bonds caused by heating [40].

It was shown that several BAPs are also released from meat products during drying or aging [41]. Proteolytic degradation, which occurs during aging of dry cured ham or during sausage fermentation and forms aroma and texture, results in generation of peptides with low molecular weight (3–5 kDa) and free amino acids [7,42]. In fermented meat products, protein degradation is influenced by different variables such as product composition, processing conditions and starter cultures. Proteolytic degradation by endogenous enzymes and lactic acid bacteria affects the peptide content. In particular, the presence of lactic acid bacteria causes a decrease in pH, which leads to higher activity of muscle endogenous proteases [43].

Generation of bioactive peptides in meat autolysis

Biologically active peptides can be generated under the action of endogenous proteases in the process of meat autolysis. Proteolysis by endogenous enzymes is the most important phenomenon that takes place during meat aging. Endopeptidases, such as calpains and cathepsins, first of all, are responsible for hydrolysis of proteins into large fragments and oligopeptides, which influences meat texture during aging and the initial stages of the rigor mortis processes. Later due to exopeptidases, such as aminopeptidases and carboxypeptidases, small peptides and free amino acids will be formed [44].

Meat aging influences its taste, tenderness, moisture binding capacity (MBC), color and juiciness. A detailed

study of the biochemical processes occurring during meat aging improves the understanding of their development. Monitoring of these processes allows revealing biomarkers of meat product quality [45]. Di Luca et al. [46] studied changes in proteome of muscle exudate during the normal period of meat aging (seven days) in genetically similar pigs from one population with the same meat quality characteristics. It was found that several quality meat indicators significantly changed during autolysis especially at the latest stage of aging. For example, from the 3rd to the 7th day, meat tenderness significantly increased, color parameter CIE b* of muscles also changed compared to the period of rigor mortis, and cooking losses changed as well. These data illustrate structural changes that take place in pork muscles during their aging, which was significantly reflected on their proteomic profiles. Three key protein groups (stress proteins, metabolic enzymes and structural proteins), which significantly changed during meat aging, were identified [47].

Another significant peculiarity resided in the fact that quantity of proteins associated with stress reduced. Monitoring of these changes is usually performed using myofibrillar or sarcoplasmic proteomic fractions. These observations in the more available substrate, that is, in the muscle exudate, allows complimenting previous studies, showing, for example, that vinculin correlates with the moisture binding capacity and peroxiredoxin-6 with meat tenderness. These protein biomarkers have potential for monitoring fresh meat quality and predicting a course of autolysis [47,48].

Hydrolysis by enzymes of different origin

The most common methodology of BAP generation is hydrolysis of proteins by commercial enzymes of microbial, plant or animal origin. In meat and meat products, Flavourzyme from *Aspergillus oryzae*, as well as Neutrase and Alcalase from *Bacillus subtilis* and *Bacillus lincheniformis*, respectively, are most widely used for generation of bioactive peptides. In addition, proteases of plant origin, such as bromelain and papain, were described as interesting enzymes for meat protein hydrolysis due to their role in meat tenderization. These enzymes show wider specificity compared to other enzymes, such as trypsin and pepsin, cleaving peptide bonds from a wide spectrum of areas and often acting either as endopeptidases or as exopeptidases hydrolyzing amino acids from N- and C-terminal ends [45].

Conclusion

Meat and meat products are one of the main sources of biologically active peptides.

With proteins as the main meat components, BAP generation occurs either under the action of endogenous muscle enzymes in the processes of autolysis and aging, or exogenous enzymes during digestion in the gastrointestinal tract, or with the use of commercial enzymes in laboratories or industrial processes under controlled conditions. During meat storage, peptide formation can also be caused by the oxidation processes. In addition, freezing processes and cooking can affect recovery and availability of meat BAPs. BAPs can be released from meat products during drying or aging. Mechanisms of BAP formation in meat and meat products are similar.

However, despite clear identification of BAPs, there is a growing need for studying interactions of a food matrix, especially when the aim is to use bioactive peptides as a functional ingredient. Qualitative assessment of these peptides for the better understanding of their impact on health and bioavailability is necessary for advance in this field. We will discuss this question in the second part of the review.

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REFERENCES

1. Sentandreu, M.A., Coulis, G., Ouali, A. (2002). Role of muscle endopeptidases and their inhibitors in meat tenderness. *Trends in Food Science and Technology,* 13(12), 400–421. DOI: 10.1016/S0924–2244(02)00188–7
2. Toldrá, F., Aristoy, M.-C., Mora, L., Reig, M. (2012). Innovations in value-addition of edible meat by-products. *Meat Science,* 92(3), 290–296. DOI: 10.1016/j.meatsci.2012.04.004
3. Albenzio, M., Santillo, A., Caroprese, M., Malva, A.D., Marino, R. (2017). Bioactive Peptides in Animal Food Products. Review. *Foods.* 6(5), 35. DOI: 10.3390/foods6050035
4. 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,* 74(4), 658–666. DOI: 10.1016/j.meatsci.2006.05.016
5. Fu, Y., Young, J.F., Therkildsen, M. (2017). Bioactive peptides in beef: Endogenous generation through postmortem aging. *Meat Science,* 123, 134–142. DOI: 10.1016/j.meatsci.2016.09.015
6. Zhang, W., Xiao, S., Ahn, D.U. (2013). Protein oxidation: Basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition*, 53(11), 1191–1201. DOI: 10.1080/10408398.2011.577540
7. Arihara, K. (2006). Strategies for designing novel functional meat products. *Meat Science,* 74(1), 219–229. DOI: 10.1016/j.meatsci.2006.04.028
8. Katayama, K., Anggraeni, H.E., Mori, T., Ahhmed, A.M., Kawahara, S., Sugiyama, M., Nakayama, T, Maruyama, M., Muguruma, M. (2008). Porcine skeletal muscle troponin is a good source of peptides with angiotensin-I converting enzyme inhibitory activity and antihypertensive effects in spontaneously hypertensive rats. *Journal of Agricultural and Food Chemistry,* 56(2), 355–360. DOI: 10.1021/jf071408j
9. Escudero, E., Sentandreu, M.A., Arihara, K., Toldrá, F. (2010). Angiotensin I-converting enzyme inhibitory peptides generated from in vitro gastrointestinal digestion of pork meat. *Journal of Agricultural and Food Chemistry*, 58(5), 2895–2901. DOI: 10.1021/jf904204n
10. Escudero, E., Toldrá, F., Sentandreu, M.A., Nishimura, H., Arihara, K. (2012). Antihypertensive activity of peptides identified in the in vitro gastrointestinal digest of pork meat. *Meat Science,* 91(3), 382–384. DOI: 10.1016/j.meatsci.2012.02.007
11. Saiga, A., Tanabe, S., Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry*, 51(12), 3661–3667. DOI: 10.1021/jf021156g
12. Arihara, K., Ohata, M. (2006). Functional Properties of Bioactive Peptides Derived from meat Proteins. In book: Advanced Technologies for Meat Processing, Publisher: CRC Press (Boca Raton). pp. 245–273.

13. Morimatsu, F., Ito, M., Budijanto, S., Watanabe, I., Furukawa, Y., Kimura, S. (1996). Plasma cholesterol-suppressing effect of papain-hydrolyzed pork meat in rats fed hypercholesterolemic diet. *Journal of Nutritional Science and Vitaminology*, 42(2), 145–153. DOI: 10.3177/jnsv.42.145
14. Shimizu, M., Sawashita, N., Morimatsu, F., Ichikawa, J., Taguchi, Y., Ijiri, Y., Yamamoto, J. Antithrombotic papain-hydrolyzed peptides isolated from pork meat. *Thrombosis Research,* 123(3), 753–757. DOI: 10.1016/j.thromres.2008.07.005
15. Jang, A., Jo, C., Kang, K.-S., Lee, M. (2008). Antimicrobial and human cancer cell cytotoxic effect of synthetic angiotensin-converting enzyme (ACE) inhibitory peptides. *Food Chemistry,* 107(1), 327–336. DOI: 10.1016/j.foodchem.2007.08.036
16. Banerjee, P., Shanthi, C. (2012). Isolation of novel bioactive regions from bovine Achilles tendon collagen having angiotensin I-converting enzyme-inhibitory properties. *Process Biochemistry*, 47(12), 2335–2346. DOI: 10.1016/j.procbio.2012.09.012
17. Fu, Y., Young, J.F., Lokke, M.M., Lametsch, R., Aluko, R. E. Therkildsen, M. (2016). Revalorisation of bovine collagen as a potential precursor of angiotensin I-converting enzyme (ACE) inhibitory peptides based on in silico and in vitro protein digestions. *Journal of Functional Foods,* 24, 196–206. DOI: 10.1016/j.jff.2016.03.026
18. Saiga, A., Iwai, K., Hayakawa, T., Takahata, Y., Kitamura, S., Nishimura, T., Morimatsu, F. (2008). Angiotensin I-converting enzyme-inhibitory peptides obtained from chicken collagen hydrolysate. *Journal of Agricultural and Food Chemistry,* 56(20), 9586–9591. DOI: 10.1021/jf072669w
19. Li, B., Chen, F., Wang, X., Ji, B., Wu, Y. (2007). Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography and electrospray ionization–mass spectrometry. *Food Chemistry*, 102(4), 1135–1143. DOI: 10.1016/j.foodchem.2006.07.002
20. Escudero, E., Mora, L., Fraser, P.D., Aristoy, M.-C., Toldrá, F. (2013). Identification of novel antioxidant peptides generated in Spanish dry-cured ham. *Food Chemistry,* 138(2–3), 1282–1288. DOI: 10.1016/j.foodchem.2012.10.133
21. Xing, L.-J., Hu, Y.-Y., Hu, H.-Y., Ge, Q.-F., Zhou, G.-H., Zhang, W.-G. (2016). Purification and identification of antioxidative peptides from dry-cured Xuanwei ham. *Food Chemistry,* 194, 951–958. DOI: 10.1016/j.foodchem.2015.08.101
22. Broncano, J.M., Otte, J., Petrón, M.J., Parra, V., Timón, M.L. (2012). Isolation and identification of low molecular weight antioxidant compounds from fermented «chorizo» sausages. *Meat Science,* 90(2), 494–501. DOI: 10.1016/j.meatsci.2011.09.015
23. Fu, Y., Young, J.F., Therkildsen, M. (2017). Bioactive peptides in beef: Endogenous generation through postmortem aging. *Meat Science*, 123, 134–142. DOI: 10.1016/j.meatsci.2016.09.015
24. Lafarga, T., Hayes, M. (2014). Bioactive peptides from meat muscle and by-products: Generation, functionality and application as functional ingredients. *Meat Science,* 98(2), 227–239. DOI: 10.1016/j.meatsci.2014.05.036
25. Zhang, W., Xiao, S., Ahn, D.U. (2013). Protein oxidation: Basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition,* 53(11), 1191–1201. DOI: 10.1080/10408398.2011.577540
26. Korhonen, H., Pihlanto-Leppälä, A., Rantamäki, P., Tupasela, T. (1998). Impact of processing on bioactive proteins and peptides. *Trends in Food Science and Technology*, 9(8–9), 307–319. DOI: 10.1016/S0924–2244(98)00054–5
27. Leygonie, C., Britz, T.J., Hoffman, L.C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science,* 91(2), 93–98. DOI: 10.1016/j.meatsci.2012.01.013
28. Adje E.; BaAdje, E.Y., Balti, R., Kouach, M., Guillochon, D., Nedjar-Arroume, N. (2011). α 67–106 of bovine hemoglobin: A new family of antimicrobial and angiotensin I-converting enzyme inhibitory peptides. *European Food Research and Technology,* 232(4), 637–646. DOI: 10.1007/s00217–011–1430-z
29. Bauchart, C., Morzel, M., Chambon, C., Mirand, P.P., Reynès, C., Buffière, C., Rémond, D. (2007). Peptides reproducibly released by in vivo digestion of beef meat and trout flesh in pigs. *British Journal of Nutrition,* 98(6), 1187–1195. DOI: 10.1017/S0007114507761810
30. Pihlanto, A., Korhonen, H. (2003). Bioactive peptides and proteins. *Advances in Food and Nutrition Research*, 47, 175–276. DOI: 10.1016/S1043–4526(03)47004–6
31. Cheung, I.W.Y., Nakayama, S., Hsu, M.N.K., Samaranayaka, A.G.P., Li-Chan, E.C.Y. (2009). Angiotensim-I converting enzyme inhibitory activity of hydrolysates from oat (Avena sativa) proteins by in silico and in vitro analyses. *Journal of Agricultural and Food Chemistry,* 57(19), 9234–9242. DOI: 10.1021/jf9018245
32. Lafarga, T., O'Connor, P., Hayes, M. (2015). In silico methods to identify meat-derived prolylendopeptidase inhibitors. *Food Chemistry,* 175, 337–343. DOI: 10.1016/j.foodchem.2014.11.150
33. Vercruysse, L., Van Camp, J., Smagghe, G. (2005). ACE inhibitory peptides derived from enzymatic hydrolysate of animal protein: A review. *Journal of Agricultural and Food Chemistry*, 53(21), 8106–8115. DOI: 10.1021/jf0508908
34. Lafarga, T., O'Connor, P., Hayes, M. (2014). Identification of novel dipeptidyl peptidase-IV and angiotensin-I- converting enzyme inhibitory peptides from meat proteins using in silico analysis. *Peptides,* 59, 53–62. DOI: 10.1016/j.peptides.2014.07.005
35. Di Bernardini, R., Mullen, A.M., Bolton, D., Kerry, J., O'Neill, E., Hayes, M. (2012). Assessment of the angiotensin-I-converting enzyme (ACE-I) inhibitory and antioxidant activities of hydrolysates of bovine brisket sarcoplasmic proteins produced by papain and characterisation of associated bioactive peptidic fractions. *Meat Science,* 90(1), 226–235. DOI: 10.1016/j.meatsci.2011.07.008
36. Database BIOPEP [Electronic resource: http://www.uwm.edu.pl/biochemia/index.php/en/biopep. For access to information resources require authorization. Access date 20.09.2019]
37. Kęska, P., Stadnik, J. (2016).Porcine myofibrillar proteins as potential precursors of bioactive peptides—an in silico study. *Food and Function*, 7(6), c. 2878–2885. DOI: 10.1039/c5fo01631b
38. Chernukha, I.M., Mashentseva, N.G., Vostrikova, N.L., Kovalev, L.I., Kovaleva, M.A., Afanasev, D.A., Bazhaev, A.A. (2018). Generation of bioactive peptides in meat raw materials exposed to proteases of different origin. *Sel'skokhozyaistvennaya biologiya*, 53(6), 1247–1261. DOI: 10.15389/agrobiology.2018.6.1247rus (in Russian)
39. Christensen, L., Ertbjerg, P., Løje, H., Risbo, J., van den Berg, F.W.J., Christensen, M. (2013). Relationship between meat toughness and properties of connective tissue from cows and young bulls heat treated at low temperatures for prolonged times. *Meat Science,* 93(4), 787–795. DOI: 10.1016/j.meatsci.2012.12.001
40. Yu, T.-Y., Morton, J.D., Clerens, S., Dyer, J.M. (2017). Cooking-induced protein modifications in meat. *Comprehensive Reviews in Food Science and Food Safety*, 16(1), 141–159. DOI: 10.1111/1541–4337.12243
41. Stadnik, J., Keska, P. (2015). Meat and fermented meat products as a source of bioactive peptides. *Acta Scientiarum Polonorum, Technologia Alimentaria*, 14(3), 181–190. DOI: 10.17306/J.AFS.2015.3.19
42. Toldra, F. (2004). Dry. In book: Encyclopedia of Meat Sciences. Three-Volume Set Volume 1–3. Edited by Jensen, W.K., Carrick Devine, C., Dikeman, M. New York: Elsevier. pp. 360–365. ISBN: 978–0–12–464970–5
43. Kato, T., Matsuda, T., Tahara, T., Sugimoto, M., Sato, Y., Nakamura, R. (1994). Effects of Meat-conditioning and Lactic Fermentation on Pork Muscle Protein Degradation. *Bioscience, Biotechnology, and Biochemistry*, 58(2), 408–410. DOI: 10.1271/bbb.58.408
44. Mora, L., Gallego, M., Toldra, F. (2018). ACEI–Inhibitory peptides naturally generated in meat and meat products and their health relevance. Review. *Nutrients*, 10(9), 1259. DOI: 10.3390/nu10091259
45. Zamaratskaia, G., Li, S. (2017). Proteomics in meat science—current status and future. *Theory and Practice of Meat Processing*, 2(1), 18–26. DOI: 10.21323/2414–438X-2017–2–1–18–26 (in Russian)
46. Di Luca, A., Elia, G., Mullen, A.M., Hamill, R.M. (2013). Monitoring post mortem changes in porcine muscle through 2-D DIGE proteome analysis of Longissimus muscle exudate. *Proteome Science,* 11(1), 9. DOI: 10.1186/1477–5956–11–9
47. Chernukha, I.M., Kovalev, L.I., Mashentseva, N.G., Kovaleva, M.A., Vostrikova, N.L. (2019). Detection of protein aggregation markers in raw meat and finished products. *Foods and Raw Materials,* 7(1), 118–123. DOI: 10.21603/2308–4057–2019–1–118–123
48. Chernukha I. M., Mashentseva N. G., Afanasiev D. A., Laptev G. U., Ilina L. A. (2019). Study of the effect of cholesterol-lowering starter cultures in smoked sausages on the formation of bioactive peptides and lipid profile in triton-induced hyperlipidemic rats. *The 60th International Meat Industry Conference MEATCON2019,* 333,012049. DOI:10.1088/1755–1315/333/1/012049

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Review paper

PRODUCTS OF CHEMICAL REACTIONS THAT OCCUR DURING HIGH-TEMPERATURE HEAT TREATMENT OF THE MEAT PRODUCTS

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Key words *heterocyclic aromatic amines, carcinogens, meat products*

Abstract

Recently the actively active studies have begun devoted to the accumulation of «harmful» substances in food products, which are supposedly accumulated in the body of a person who often consumes these products. Meat, as a source of full-featured animal protein, is especially popular in this aspect. For the preparation of meat products various types of heat treatment are used, almost each of which will inevitably lead to the destruction of some of the chemical compounds originally present in the product, and the formation of completely new chemical compounds, which can often be harmful to the human body. During high-temperature heat treatment (mainly frying), some chemical reactions in meat products occur, which lead to the formation of heterocyclic aromatic amines (HAA) in it. Due to the great variety of raw meat and cooking recipes, during the heat treatment HAA's of various classes are formed, each of them will be peculiar for the particular type of raw material or recipe components (with the exception of MeIQx and PhIP, which always form during frying). The more complete understanding of the HAA's formation mechanism will help study the products of Maillard reactions and Strecker degradation. In this work we studied the formation of HAA's as a result of the cyclization of creatine and the detaching of water (dehydration) from it during temperature exposure. The classification of the compounds formed as a result of these reactions is presented and the main classes of the HAA obtained in result are considered. The questions of the influence of various factors on amount of HAA formed, such as the fat content, the introduction of Fe^{2+}, Fe^{3+}, are raised. In the future it is necessary to conduct studies of the quantitative content of HAA in meat products to complement the already actively ongoing work on the study of xenobiotics consumed by humans with food, which will give a more comprehensive picture of the carcinogens content in food products.

Introduction

The modern outlook of the customer is formed currently by the mass media, the majority of «near-scientific (pseudo-scientific)» data — are the European researches based on data extracted from various statistical publications (meta-data), that associate meat products with «oncology» risks. The International Agency Research on Cancer (IARC) released the monograph of meta-analysis performed in 2015, which results showed, that meat products have carcinogenic properties in certain conditions, which are explained by a wide range of xenobiotics trapped in the product from outside sources and directly formed in it during the heat treatment [1, 2].

Besides this, the National Cancer Institute informed, that during the heat treatment of food products with high protein content (meat, poultry, fish), it is produced the heterocyclic amino acids (HAA) — these substances are known ~~of~~ for their expressed carcinogenic and mutagenic properties [3, 4], which explains the need of conducting research of the chemical nature of their formation. Their mutagenic and carcinogenic properties have been proven both by the Ames test, and by the course of long-term experiments on the rodents and monkeys [5].

Main part

Based on the results [6] it can be concluded, that the main factors, contributing to the formation of the HAA in the product are the temperature (150 °C) and the duration of heat treatment, and also the contact of the product and the heating surface. Regarding this, the HAA will mainly be formed in products cooked at home, and also in public catering enterprises. The range of the HAA values content in food products varies from 1 μg / kg to 100 μg / kg. The quantitative content of the HAA in food products is directly proportional to the temperature of heat treatment. The profile of the substances related to the HAA depends on the temperature in the same way [7,8,9,10,11,12,13,14, 15,16,17,18,19].

Raw materials and product recipe formulation, including ~~to~~ secondary factors, influence the quantitative content of the HAA in the meat products.

Firstly, the HAA were discovered in 1977 by the professor Takashi Sigimura and his collaborators, as a result of conventional home cooking processes. The beginning of the work on the HAA study was put by suspicions that the smoke, which is formed during the heat treatment of meat products, can be carcinogenic. Due to this there were discovered 20 different compounds, not registered as recipe components, which are actually formed during heat treatment, and they fell into the category of the HAA [7,9].

In the 80s of the last century, on the basis of the assumption on formation of mutagenic HAA groups of imidazoquinolines and imidazoquinoxalines during the Maillard reaction, a mechanism for the formation of the HAA was proposed [19] (Figure 1).

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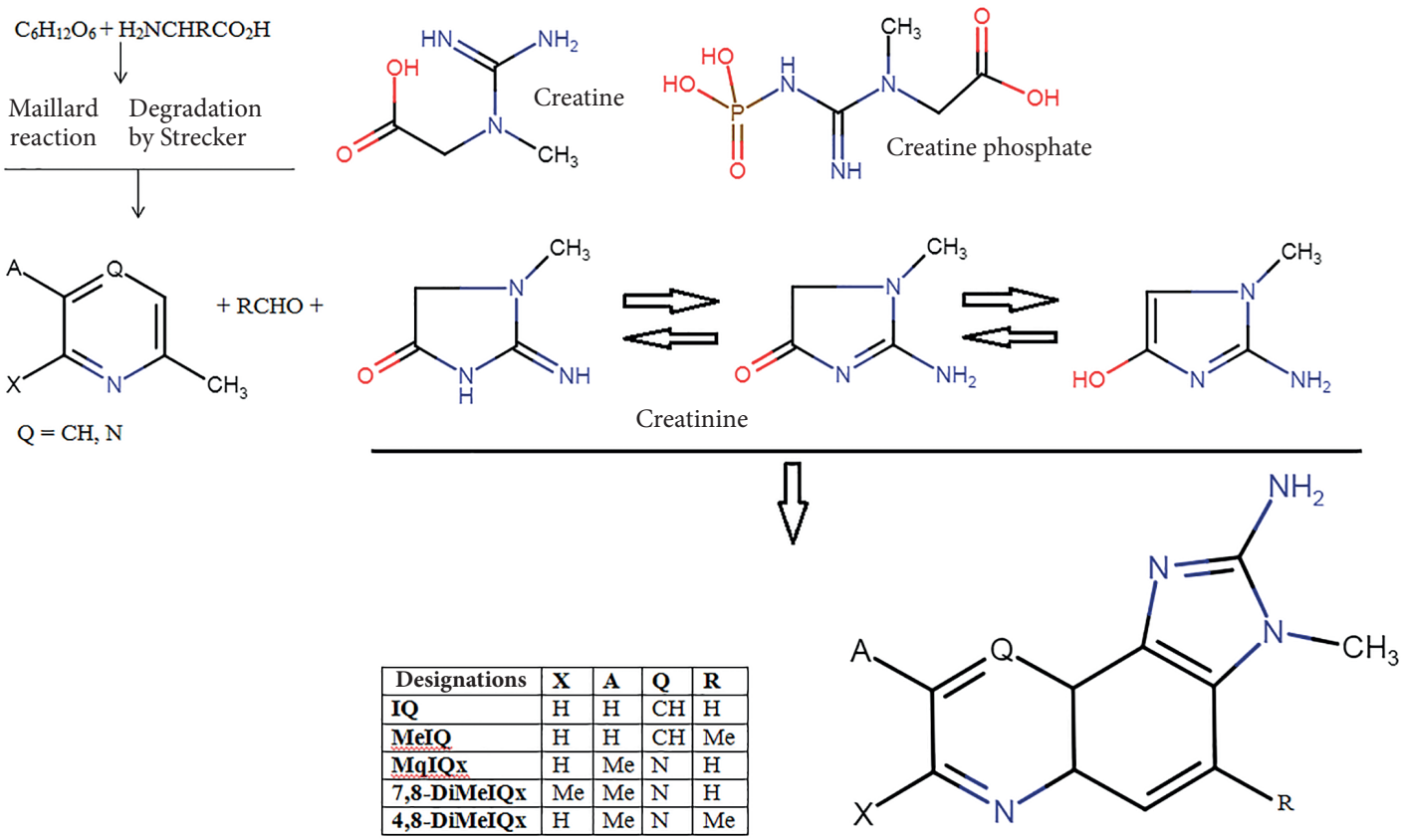

Designations	X	A	Q	R
IQ	H	H	CH	H
MeIQ	H	H	CH	Me
MqIQx	H	Me	N	H
7,8-DiMeIQx	Me	Me	N	H
4,8-DiMeIQx	H	Me	N	Me

Figure 1. Mechanism of formation of HAA

In result of cyclization and detaching of water, according to the proposed scheme, creatine turns into creatinine and form forms the amino-imidazole part of the HAA, and the Strecker degradation products, such as pyridine derivatives or pyrazines, formed in result of the Maillard reaction between hexoses and free amino-acids, «completing» the chemical structure of the HAA. The important participant in the «completion» of the HAA molecule is the so-called Strecker aldehyde (or the corresponding base of Schiff), which is also formed in result of Strecker degradation.

As we can see from the name, the HAA have at least one heterocyclic ring and the amino group. Heterocyclic ring is a cyclic hydrocarbon, in which one or more carbon atoms are replaced by other atoms (with oxygen heteroatom). In most cases the heteroatom is nitrogen and less often it is oxygen, and still less often it is sulfur. In cases, where the heteroatom is nitrogen, the compound is an amine itself, in other cases the aminogroup is attached to the heterocycle. The majority of the HAA, except the group of β-carbolines (such as harman and norharman, Figure 2 and 3 respectively), the aminogroup is located out of the cycles.

The natural content of the HAA can be found, ranging from plants and algae to vitamins and antibiotics.

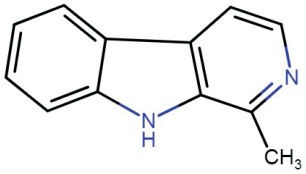

Figure 2. Harman (1-methyl-9H-pyrido[3,4-b]indole)

The HAA, which are formed in result of heat treatment of high-protein foods, being the carcinogens, can be divided into two major groups.

The first group includes aminoimidazoarenes (AIA) or «thermal» HAA, which have in their molecule N-methyl-2-aminoimidazole part. These HAA are usually formed during the «home» cooking temperatures of 150 °C primarily as a result of the reaction of aminoacids pyrolysis products (such as pyridine and pyrizine) with creatine and carbohydrates, forming imidazoquinolines (IQ), imidazoquinoxalines (IQx), and imidazopyridines (Fig. 4) [10,18,19].

Figure 5 shows the presumed reaction of IQ and IQx formation. Depending on the polarity, aminoimidazoarenas can be divided to polar and nonpolar.

The second group — «the pyrolytic HAA» or aminocarbolins are formed by higher temperatures (over 300 °C) by thermal destruction of tryptophan, phenylalanine, ornithine and glutamic acid. At high temperatures, these amino acids form desaminated and decarboxylic reaction products with reactive radical fragments, the condensation of which produces heterocyclic ring structures. This group mainly includes one of the fragments of 5 structurally different groups of pyridoindole, pyridoimidazole, phenylpyridine, tetraazaflurantene or benzimidazole (Figure 6) [11,12,19].

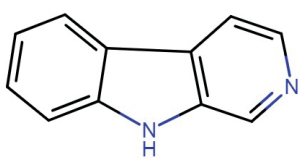

Figure 3. Norharman (9H-pyrido[3,4-b]indole)

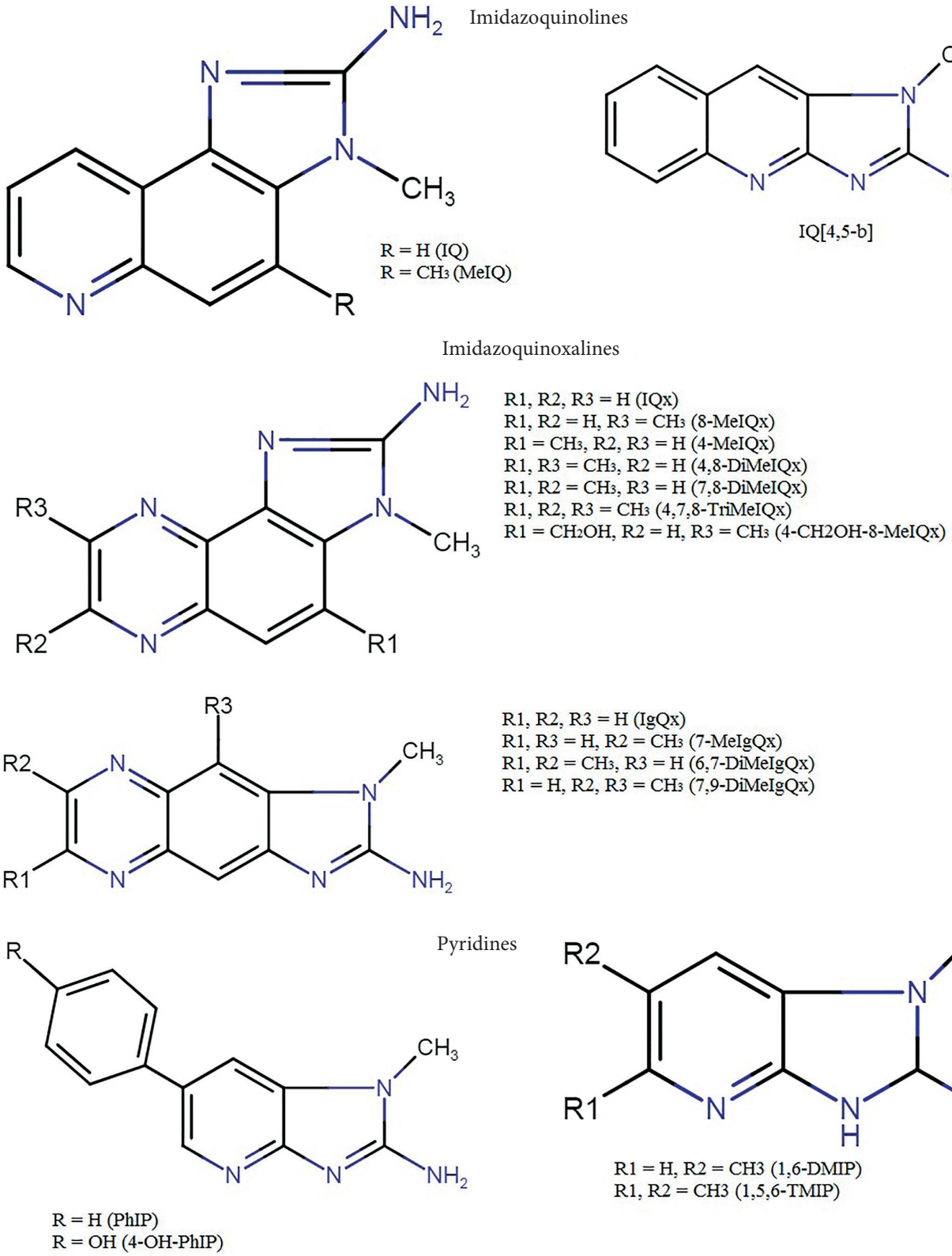

Figure 4. Graphic formulas of imidazoquinolines, imidazoquinoxalines and pyridines

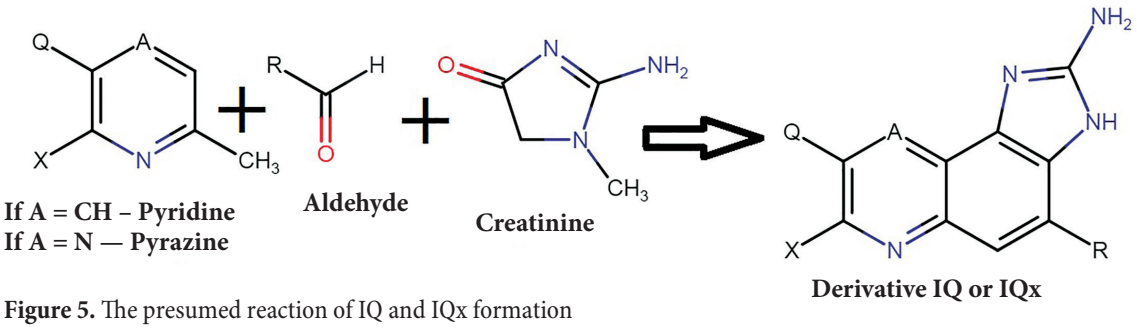

Figure 5. The presumed reaction of IQ and IQx formation

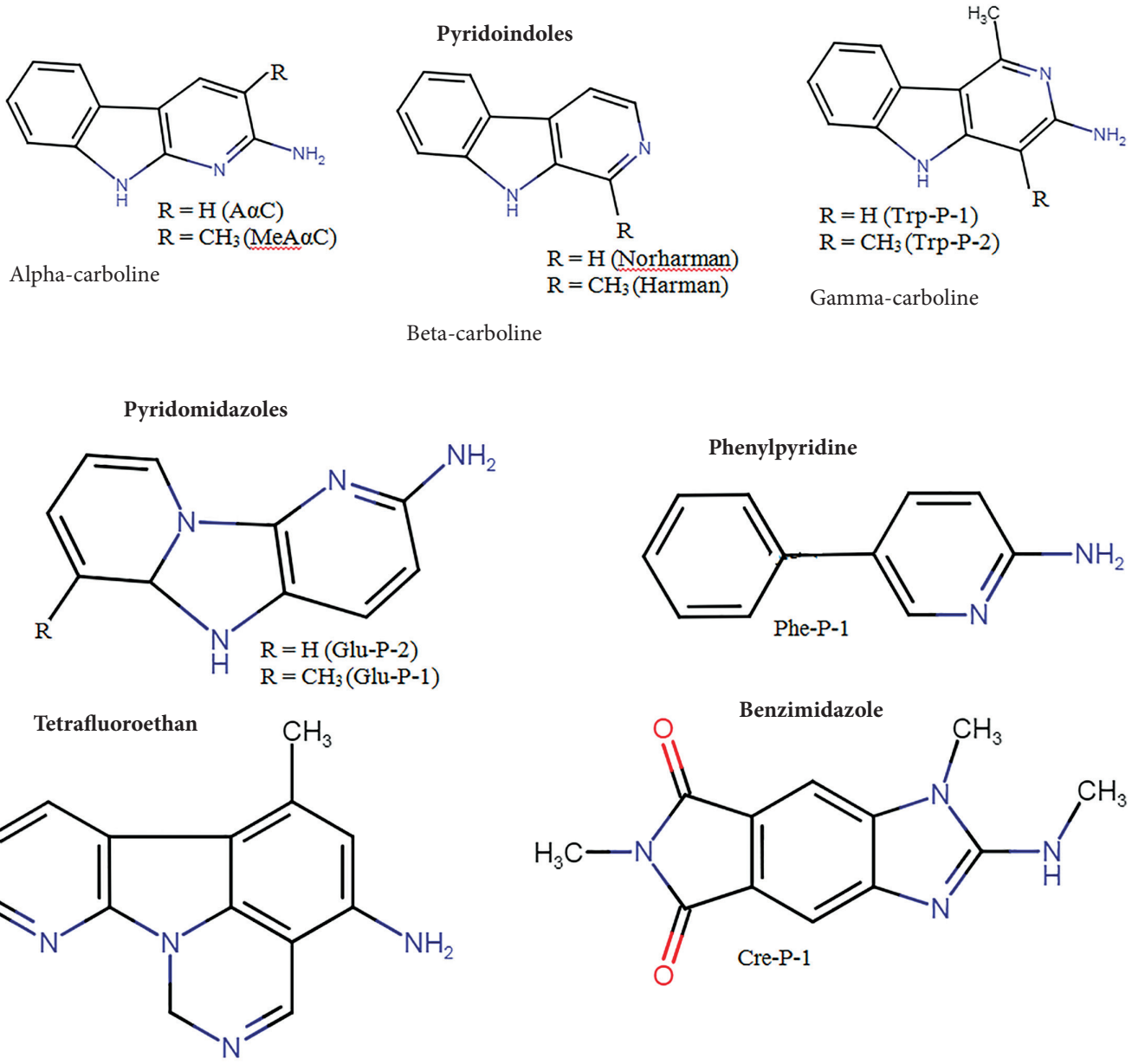

Figure 6. Graphic formulas of 2 groups of the HAA

Due to the fact that AIA are formed at lower temperatures, they are more common and, accordingly, are mutagenic and carcinogenic HAA studied in a better way. Also, alpha- and gamma-carbolines are mainly formed as a result of pyrolysis of plant proteins in concentrations 10–100 times less than the number of the formed beta-carbolines, such as harman and norharman [18].

In 1993 the IARC considered 8 Heterocyclic aromatic amines (HAA), (MeIQ, 8-MeIQx, PhIP, AαC, MeAαC, Trp-P-1, Trp-P-2 and Glu-P-1) as possible carcinogens for humans (class 2B) and 1 (IQ) as probable carcinogen for humans (class 2A) and recommended reducing exposure to these compounds. So in 2004, IQ, MeIQ, 8-MeIQx and PhIP were listed in the National toxicology program, because they are carcinogenic to humans [20]. These results are based on the results of long-term animal experiments. Although the epidemiological evidence suggests that the roasted meat consumption is associated with an increased risk of cancer in humans, data were insufficient to confirm that this risk is caused due to the presence of the HAA (MeIQ, 8-MeIQx, or PhIP). Sample studies show very contradictory results.

The HAA, a family of mutagenic compounds, are formed during the cooking process of many animal products, including chicken, beef, pork, and fish. Meat, cooked with moderate temperatures but grilled or fried, can contain significant amounts of these mutagens. [8,19].

The longer the meat is cooked and the higher is the temperature, the more these compounds are formed [6,7,8,9,10]. Studies have shown that during the cooking of the grilled chicken the higher concentration of the HAA were found than in other types of meat [19].

The main classes of heterocyclic amines include aminoimidazoquinolines or aminoimidazoquinoxalines (collectively called type IQ compounds) and aminoimidazopyridines such as PhIP. Compounds such as IQ and PhIP are formed from creatine or creatinine, specific amino acids and sugars [13]. All meat (including fish) contains a large

amount of creatine, because of what the maximum formation of the HAA occurs during the cooking of meat at high temperatures (grilled or fried). Eating deep-fried meat and PhIP is associated with an increased risk of breast cancer and colon cancer [20,21,22,23,24,25,26,27].

In addition to the type and duration of heat treatment, the amount of the HAA produced is influenced by the content of antioxidants, precursors, and the HAA inhibitors in the product. One of the inhibitors, but at the same time a catalyst in the reaction of the HAA formation in the product is fat. In research [6] the experiment was carried out, where the semi-finished meat products with different mass fraction of fat were subjected to frying. The results showed that with a relatively low fat content in the product (no more than 20 %), the amount of the formed HAA grew in direct proportion to the amount of fat, and with an amount of fat over 20 %, the amount formed during heat treatment of the HAA was less in comparison with the samples where the fat content was less than 20 %, but at the same time it remained relatively stable and did not change with further changes in the amount of fat in the recipe formulations of the products. Most likely, this is due to the fact that a large amount of fat in the product acts as a wall between the product and the heating surface, and with smaller amounts of fat this fat is involved in the reaction to form the HAA. An important role is played by the type of the fats used — the greatest mutagenic activity was found while using butter, and the use of vegetable oils leads to the formation of smaller amounts of food mutagens. It was found that the introduction of iron (Fe2+, Fe3+) increases the number of formed IQ and IQx almost ~~in~~ two times. The amount of moisture in the recipe formulation components also influences the concentration of the HAA, possibly because of its role as a transporter of water-soluble HAA precursors.

Conclusion

Currently there is a need for experimental studies of the HAA content in food products, which will be another block of great work on the study of xenobiotics entering the human body with food products, which will complement the picture of the chemical carcinogens content in the food products.

In world practice the meat products are considered as high-risk products, which are characterized by both biological and chemical risks. The Codex Alimentarius and the World Organisation for Animal Health (OIE) documents provide guidance on the application of a risk-based approach to the analysis of products of animal origin. However, in Codex Alimentarius they refer to the finished product and are considered in relation to a human health, and in OIE documents they refer mostly to the veterinary welfare of farm animals. The technological component is not highlighted in these documents, but it serves as an essential mechanism for managing both individual risks and their aggregate combination, which allows producing a product of guaranteed safety during its shelf life.

In many ways the harm from red meat is determined not so much by the properties of the meat, as by the methods of its cooking and combination with other foods. Often the product becomes harmful because of ignorance of elementary rules of its use. In general, this review emphasizes that species differences and data on the mechanisms of toxic action must be taken into account when transferring data on carcinogenic risks obtained from the use of large doses of the tested substance in experimental animals, to humans receiving small doses of this substance.

The primary objective of the study of the HAA accumulation is to select an indicator of the presence of carcinogens of this type. A range of researchers have found that the most common HAA are PhIP (2-amino-1-methyl-6phenylimidazo[4,5 b]pyridine) and MeIQx (2-amino-3,8-dimethylimidazo[4,5 f]quinoxaline) [8]. Therefore these substances were selected as the main markers for further monitoring of the quantitative content of the HAA in the food products, carried out within the framework of the State task on the basis of the laboratory of the Scientific and methodological works, biological and analytical researches of the V. M. Gorbatov's Federal Research Center for Food Systems.

REFERENCES

1. IARC Monographs evaluate consumption of red meat and processed meat. [Electronic resource: http://scienceblog.cancerresearchuk.org/2015/10/26/processed-meat-and-cancer-what-you-need-to-know Access date 14.11.2019]
2. Carcinogenicity of consumption of red and processed meat. Published early online October 26, 2015 in The Lancet Oncology. First author Veronique Bouvard, International Agency for Research on Cancer Monograph Working Group, Lyon, France. [Electronic resource: https://www.cancer.org/latest-news/world-health-organization-says-processed-meat-causes-cancer Access date 24.10.2019]
3. Vostrikova N. L., Kuznetsova O. A., Kulikovskii A. V., Minaev M. Y. (2017). Formation of the scientific basis of meta-data associated with estimates of «onco-» risks linked to meat products. *Theory and practice of meat processing,* 2(4), 96–113. DOI: 10.21323/2414–438X-2017–2–4–96–113 (In Russian)
4. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. (1993). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 56. Lyon: World Health Organization. Intl. Agency for Research on Cancer. ISBN 978–92–832–1256–0
5. Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN2012. *International Journal of Cancer*, 136, E359–E386. DOI: 10.1002/ijc.29210
6. Solaykov, A.A. (1998). The influence of the thermal treatment and preparation methods of semifinished products for the content of heterocyclic aromatic amines in fried meat food products. Author's abstract of the thesis work applied for the scientific degree of Candidate of Technical Sciences. M: G. V. Plekhanov's Russian University of Economics. — 27 p. (In Russian)
7. Gibis, M. (2007). Occurrence of carcinogenic heterocyclic aromatic amines in fried patties of different animal species. *Proceedings of the 53th International Congress of Meat Science and Technology, Beijing, China,* P. 13.
8. Puangsombat, K., Gadgil, P., Houser, T.A., Hunt, M.C., Smith, J.S. (2012). Occurrence of heterocyclic amines in cooked meat products. *Meat Science,* 90(3), 739–746. DOI: 10.1016/j.meatsci.2011.11.005

9. Alaejos, M.S., Afonso, A.M. (2011). Factors that affect the content of heterocyclic aromatic amines in foods. *Comprehensive reviews in food science and food safety*, 10(2), 52–108. DOI: 10.1111/j.1541–4337.2010.00141.x
10. Buła, M., Przybylski, W., Jaworska, D., Kajak-Siemaszko, K. (2018). Formation of heterocyclic aromatic amines in relation to pork quality and heat treatment parameters. *Food Chemistry*, 276, 511–519. DOI: 10.1016/j.foodchem.2018.10.073
11. Jägerstad, M., Skog, K., Grivas, S., Olsson, K. (1991). Formation of heterocyclic amines using model systems. *Mutation Research/Genetic Toxicology*, 259(3–4), 219–233. DOI: 10.1016/0165–1218(91)90119–7
12. Messner, C., Murkovic, M. (2004). Formation of heterocyclic aromatic amines in model systems. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 802(1), 3–10. DOI: 10.1016/j.jchromb.2003.09.026
13. Kataoka, H., Miyake, M., Saito, K., Mitani, K. Formation of heterocyclic amine–amino acid adducts by heating in a model system *Food Chemistry,*130(3), 725–729. DOI: 10.1016/j.foodchem.2011.07.094
14. Skog, K.I., Johansson, M.A.E., Jägerstad, M.I. (1998). Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence, and intake. *Food and Chemical Toxicology,* 36, 879–896. DOI: 10.1016/s0278–6915(98)00061–1
15. Teng, H., Chen, Y., Lin, X., Lv, Q., Chai, T.-T., Wong, F.-C. Chen, L., Xiao, J.(2019). Inhibitory effect of the extract from Sonchus olearleu on the formation of carcinogenic heterocyclic aromatic amines during the pork cooking. *Food and Chemical Toxicology,*129, 138–143. DOI: 10.1016/j.fct.2019.04.043
16. Yang, D., He, Z., Gao, D., Qin, F., Deng, S., Wang, P., Xu, X., Chen, J., Zeng, M. (2019).Effects of smoking or baking procedures during sausage processing on the formation of heterocyclic amines measured using UPLC–MS/MS. *Food Chemistry,* 276, 195–201. DOI: 10.1016/j.foodchem.2018.09.160
17. Soladoye, O.P., Shand, P., Dugan, M.E.R., Gariépy, C., Aalhus, J.L., Estévez, M., Juárez, M. (2017). Influence of cooking methods and storage time on lipid and protein oxidation and heterocyclic aromatic amines production in bacon. *Food Research International*, 99, 660–669. DOI: 10.1016/j.foodres.2017.06.029
18. Sinha, R., Rothman, N., Salmon, C.P., Mark, S.D., Brown, E.D., Levander, O.A., Knize, M.G., Swanson, C. A. Felton, J.S., Rossi, S.C. (1995). High Concentrations of the Carcinogen 2-Amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) Occur in Chicken but Are Dependent on the Cooking Method. *Cancer Research*, 55(20), 4516–4519
19. Jägerstad, M., Reuterswärd, A.L., Olsson, R., Grivas, S., Nyhammar, T, Olsson, K., Dahlqvist, A. (1983). Creatin(in)e and Maillard reaction products as precursors of mutagenic compounds: Effects of various amino acids. *Food Chemistry,* 12(4), 255–264. DOI: 10.1016/0308–8146(83)90014–6
20. NTP Report on Carcinogens. Selected heterocyclic amines. U. S. Department of Health and Human Services. Public Health Service. Natl. Toxicology Program. Eleventh Edition, 2004, P.135.
21. Robbana-Barnat, S., Rabache, M., Rialland, E., Fradin, J. (1996). Heterocyclic amines: occurrence and prevention in cooked. *Environmental Health Perspectives*, 104(3), 280–288. DOI: 10.1289/ehp.96104280
22. Chan, D.S.M., Lau, R., Aune, D., Vieira, R., Greenwood, D.C., Kampman, E., Norat, N. (2011). Red and Processed Meat and Colorectal Cancer Incidence: Meta-Analysis of Prospective Studies. *PLoS ONE*, 6(6), e20456. DOI: 10.1371/journal.pone.0020456
23. Bruce, W.R. (1987). Recent hypotheses for the origin of colon cancer. *Cancer Research,* 47(16), 4237–4242.
24. Thiebaud, H.P., Knize, M.G., Kuzmicky, P.A., Hsieh, D. P. Felton, J.S. (1995). Airborne mutagens produced by frying beef, pork, and a soy-based food. *Food and Chemical Toxicology*, 33(10), 821–828. DOI: 10.1016/0278–6915(95)00057–9
25. Murtaugh, M.A., Ma, K.N., Sweeney, C., vCaan, B.J., Slattery, M.L. (2004). Meat Consumption patterns and preparation, genetic variants of metabolic enzymes, and their association with rectal cancer in men and women. *The Journal of Nutrition,* 134(4), 776–784. DOI: 10.1093/jn/134.4.776
26. García-Closas, R., García-Closas, M., Kogevinas, M., Malats, N., Silverman, D., Serra, C., et al. (2007). Food, nutrient and heterocyclic amine intake and the risk of bladder cancer. *European Journal of Cancer,* 43(11), 1731–1740. DOI: 10.1016/j.ejca.2007.05.007
27. Rahman, U.U., Sahar, A., Khan, M.I., Nadeem, M. (2014). Production of heterocyclic aromatic amines in meat: Chemistry, health risks and inhibition. A review. *LWT — Food Science and Technology*, 59(1), 229–233. DOI: 10.1016/j.lwt.2014.06.005

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Original scientific paper

DETECTION OF SOYBEAN BY REAL-TIME PCR IN THE SAMPLES SUBJECTED TO DEEP TECHNOLOGICAL PROCESSING

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Key words: *real-time PCR, Glycine max, species identification, food processing, DNA degradation*

Abstract

During deep technological processing, DNA of food product components (specifically, in canned foods) is subjected to strong degradation, which makes the PCR-based food components identification more difficult. In this work, a primer-probe system is proposed, which was selected for the multi-copy region of long terminal repeat (LTR) of soybean (Glycine max). We confirmed its high sensitivity and specificity for soybean detection by real-time PCR. Using the selected system, we successfully analyzed the samples of meat-and-plant canned foods and other food products subjected to deep technological processing — tofu, preserved tofu, soy sauces, confectionary products containing soy lecithin. To compare with these samples, real-time PCR was carried out using the primer-probe system selected for the single-copy le1 gene. In terms of sensitivity, the use of the primer-probe system specific to the single-copy region was significantly inferior to the primer-probe system specific to the LTR region. The difference in the rate of degradation of these genomic DNA regions of Glycine max was found.

Introduction

Soybean and products of its processing are widely used in the food industry. This category of raw materials is often used for meat product falsification (replacing part of meat raw materials or exceeding a quantity specified in TS). It is also necessary to note that soybean is an allergen and one of the main cultures subjected to genetic modification.

At present, several methods have been developed to detect soybean in foods. Methods based on the polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are applied most frequently. Soy proteins are also used in manufacturing products that have undergone deep technological processing (canned foods, fermented products). In this case, both DNA and proteins are subjected to strong degradation, which reduces sensitivity of these methods [1,2].

As soybean is an allergen, several authors choose the lectin gene (*le1*) to develop methods for PCR identification of soybean. The lectin gene is presented by a single copy in genomic DNA. Therefore, sensitivity of the method can be insufficient when analyzing certain samples. This constraint can be overcome by digital PCR with higher sensitivity compared to other PCR methods [3]. Primers specific to the lectin gene are used mainly in multiplex test systems of quantitative detection of GMO by digital PCR [2,4].

To increase PCR sensitivity, multicopy DNA markers are used, in particular, DNA of organelles: mitochondrial [5] or plastid [6]. This approach in combination with highly effective DNA extraction enables identification of a targeted matrix in low concentrations in deep processed samples [7]. However, in this case, time and expenditures for analysis are increased.

Retrotransposons are promising DNA regions for primer design when analyzing products that have undergone deep technological processing. Their main advantage is multiple copies. For example, Ballin *et al.* used the elements called the chicken repeat 1 (CR1) with the copy number of approximately 26,650 in the chicken genome and the trinucleotide repeat containing 5 (TNRC5) with the copy number of approximately 100,000 in the pork genome for comparative quantitative assessment of pork and poultry meat in the model samples [8]. This approach had high theoretical sensitivity of the method. However, the practical limit of detection (LOD) was restricted to 0.01% chicken in pork and 1% pork in chicken. This was explained by nonspecific interspecies amplification at the last cycles. The authors noted that predicted copy numbers of the targeted region in the pig genome were higher than the real ones by the order of magnitude. This was caused by nonspecific annealing of the developed primers on other regions of the genome. Therefore, the use of these regions was necessary for designing a primer system.

Retrotransposons are mobile genetic elements, which are most often represented in the eukaryotic genome by a large number of copies. Due to an absence in their composition of genes that are functionally significant for the organism, retrotransposons rapidly accumulate substitutions and, consequently, diversify. Moreover, part of retrotransposons are endogenous retroviruses, which allows suggesting that its own retrotransposon can appear in a taxon of any level due to insertion of a retrovirus into the genome and the following loss of its ability to develop virus particles. Because of this, an advantage of using these regions is low probability of non-specific annealing of primers for DNA of closely related species. To identify soybean, Yamakawa *et al.* [9] used primers for the retrotransposon. Detection of amplification products was carried out using gel electrophoresis. Specificity was controlled using 11 related (legume) and 16 other species

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of plants. The limit of detection (LOD) of non-degraded DNA was 0.001 %. A critical factor for the development of the PCR test-systems is a length of an amplicon. The longer an amplicon, the higher a probability of breakage at this DNA region under an effect of a technological process [10,11,12]. For real time PCR, a length of 100–150 nucleotide bases is considered optimal.

In the present work, we developed a primer-probe system for identification of soybean in products with its low content and in products that have undergone deep thermal processing. The primer-probe system was complimentary to the long terminal repeat (LTR) 83l18-re-1 [13]. The products that were subjected to deep technological processing were analyzed. For comparison, parallel analyses with primers for the single-copy lectin gene were carried out.

Materials and methods

Objects of the research

Soy flour was used as a positive control. Flour produced from samples of pea, kidney bean and chickpea was a negative control. The samples were taken from the collection of the Laboratory of molecular biology and bioinformatics of the V. M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences. The following food products available in the retail chains were taken as test samples: four articles of canned meat-and-plant pastes (code designation P1-P4), six articles of meat-and-plant canned foods (C1–C6). To compare an effect of other technological processes on DNA degradation, samples of tofu, preserved tofu and three samples of soy sauces (S1–S3) were taken as well. We also analyzed four samples of confectionary products that contained soy lecithin (Con1-Con4) to assess a possibility of the method to detect soybean components that have undergone deep technological processing in food product samples.

As a positive control of soybean degraded DNA, we prepared a sample of soy flour that was diluted in distilled water in a ratio of 1:4 by mass. The obtained paste was treated in an autoclave in a glass vial at a temperature of 120 °C for 20 min.

Sampling and DNA extraction

Food products were minced in a knife mill GRINDOMIX GM 200 (Retsch, Haan, Germany). For DNA extraction, food product samples and control samples of 50 mg each were taken. To extract DNA from the positive control of degraded DNA, 200 mg were taken. After that, lysis and purification with chloroform were performed using the reagent kit Sorb-GMO-B (Syntol, Moscow, Russia) according to the instruction. The following DNA extraction was carried out using the MagNA Pure LC2.0 isolation station (Roche) with MagNa Pure LC DNA Isolation Kit II (tissue) (Roche, Mannheim, Germany).

Primer design

The primer-probe systems were complementary to the regions of le1 and long terminal repeat (LTR) 83l18-re-1 (Table 1) available in the GenBank database [14]. For system design, the programs Primer-BLAST [15] and OligoAnalyzer v. 3 were used [16].

Real-time PCR

Real-time PCR was carried out using an amplifier ANK-32 (Syntol, Moscow, Russia). The reaction mixture with a volume of 30 µl contained primers with a concentration of 300 nM, a probe with a concentration of 150 nM, 2.5 mM MgCl2, dNTPs with a concentration of 0.25 mM each, SynTaq polymerase with a concentration of 2.5 activity units and 5 µL of extracted DNA. The components of the reaction mixture were produced by Syntol. The parameters of PCR were the following: initial denaturation at 95 °C for 7 min and 45 cycles of amplification (60 °C, 40 s and 95 °C, 15 s). All samples were investigated in triplicate. The obtained data were analyzed using the software ANK-32 (Syntol). Statistical analysis was carried out with the use of Microsoft Excel 2016 [17].

Results and discussion

Detection of efficiency, specificity and cut-off cycles

To detect the reaction parameters and detection limit, PCR with selected primer pairs was performed. For analysis of primers for the *le1* gene, soybean DNA and its decimal dilutions up to 0.001 % were used. For analysis of primers for the region 83l18-re-1, we used a dilution of DNA extracted from soy flour with concentration of 10 % to 0.0001 %.

The limit of detection in PCR with primers for le1 was 0.01 % of the targeted matrix in a sample. The calculated coefficient of correlation of PCR with primers for le1 was R2 = 0.99; PCR efficiency was E = 85.5 %. The equation of linear regression is the following:

$$y = -4.295x + 49.01 \quad (1)$$

The calculated coefficient of correlation of PCR with primers for 83l18-re-1 was R2 = 0.99; PCR efficiency was

Table 1. Sequences and positions of the primers used in the study

Primer	Primer sequence (5'→3')	Amplification region	Amplification size, bp
Lec1-F	CTCTACTCCACCCCCATCCA	*le1*	111
Lec1-R	ATCTGCAAGCCTTTTTGTGTCAG		
Lec1-P	(FAM)-TT(C–LNA)AA(C–LNA)TTCA(C–LNA)(C–LNA)TT(C–LNA)TATGCC-(RTQ1)		
Gly_MAX-F	CTCTCTATGGATTGAAGCAAGCTC	83l18-re-1	91
Gly_MAX-R	TCAATTCCTCCCTTCCTATACCCT		
Gly_MAX-P	(FAM)-CTTGGTATGAAAGGCTAACAGAGTTCC-(RTQ1)		

bp — base pairs

E = 98.0 %. The equation of linear regression is the following:

$$y = -3.4267\,x + 50.63 \quad (2)$$

When using Lec1, there was no non-specific annealing with samples of chickpea, kidney beans and pea, as well as in the negative control without DNA. When using primers for 83l18-re-1, the threshold cycle (Cq) for all negative controls was 36.5±1.4 (presented as the mean ± standard deviation, N=18) and was equal to Cq of the reaction without DNA addition (36.3±0.05, N=3). Therefore, the cut-off cycle for the reaction with Gly_MAX was the 34th cycle, and the practical limit of detection was 0.001 % of non-degraded DNA. The obtained values are comparable with the primers used in the work of Yamakawa *et al.* [9].

Analysis of food products

In the world market, as a rule, food products containing soy components are subjected to fermentative (tofu, soy sauces) and thermal treatment (confectionary products, bakery products). For quantitative detection of soybean content in products of deep processing, several authors proposed corresponding models of DNA degradation: a temperature regime [4,10,18], enzyme treatment [19] and combination of an impact of a temperature and pH value [11,19]. The recipe of the model objects and temperature regimes were selected with consideration for the baking technology. For the Russian market, however, a study of canned foods is topical. To assess an effect of sterilization on DNA, DNA of soy flour after autoclaving was taken as a positive control. The Cq values of this sample were lower than in 1% of non-degraded DNA (Table 2). This suggests a significant effect of sterilization regimes on DNA. When using the primer-probe system Lec1, a difference between values Cq (ΔCq) of 1% soybean DNA and 100 % degraded DNA was 8.08 cycles, while ΔCq of the same samples was 2.34 when the primer-probe system Gly_MAX was used (Table 2). Therefore, the primer-probe system Gly_MAX has higher comparative sensitivity in analysis of samples that have undergone technological processing. The calculated sensitivity for this model of degradation ensures the calculated sensitivity of 0.1 % soybean in a sample.

During the investigation, soybean DNA was not revealed in the samples C3–C6, which corresponded to the declared composition. The results of real-time PCR of other samples are presented in Table 2. Soybean was found in the paste samples and sample C1 with the use of both primer-probe systems: Lec1 and Gly_MAX. In these samples, soybean was declared in the product composition, but it was not the main component. The Cq values obtained in real-time PCR were close to the positive control of DNA of 100 % autoclaved soybean. In this semi-quantitative analysis, however, it is necessary to select a model of DNA degradation that corresponds to the studied sample. When studying the sample C2, soybean was detected with the use of the prime-probe system Gly_MAX, while real-time PCR with the use of Lec1 gave a positive result only in two of three replicates. Soybean was not declared in the composition of this product.

Table 2. The result of PCR in the control samples and food samples with the use of the primers specific to the le1 gene (Lec1) and LTR83l18-re-1 (Gly_MAX)

	Result of ampification, Cq		ΔCq between Leq1 and Gly_Max
	Leq1	Gly_Max	
Control samples			
Soybean 1 %	28.14 ± 0.11[a]	18.26 ± 0.03[a]	9.88
Soybean 100 %, 120°/20 min	36.22 ± 0.63[a]	20.6 ± 0.09[a]	15.62
Meat-and-planned canned foods			
P1	32.6 ± 0.37[a]	20.58 ± 0.04[a]	12.02
P2	33.49 ± 0.19[a]	20.68 ± 0.11[a]	12.82
P3	33.63 ± 0.26[a]	21.19 ± 0.05[a]	12.44
P4	34.71 ± 0.04	22.64 ± 0.03[a]	12.07
C1	35.31 ± 0.3[a]	20.27 ± 0.07[a]	15.04
C2	37.63 ± 0.71[b]	29.21 ± 0.07[a]	
Preserved tofu	36.16 ± 1.43[a]	21.8 ± 0.04[a]	14.36
Tofu	26.28 ± 0.04	14.0 ± 0.02	12.28
Soy sauces			
S1	NR	31.9 ± 0.15[a]	
S2	NR	31.37 ± 0.11[a]	
S3	NR	30.25 ± 0.02[a]	
Confectionary products			
Con1	37.55	29.6 ± 0.1[a]	
Con2	28.26 ± 1.43[a]	17.9 ± 0.9[a]	10.32
Con3	37.80 ± 0.02[b]	31.14 ± 0.19	
Con4	NR	33.3 ± 0.38[a]	
Con5	37.37	31.95 ± 0.13[a]	

The Cq values are presented as: the arithmetic mean ± standard deviation (a – N=3, b — N=2), NR — negative result

In production of tofu, soybean raw materials are heated at 85–110 °C in the acidic environment [20]. In this process, DNA is also subjected to significant degradation [11]. During the investigation, a decrease in a quantity of non-degraded DNA was found (table 2). For example, the Cq value for preserved tofu was comparable with the results of the analysis of meat-and-plant canned foods. With that, Yamakawa et al. failed to detect specific DNA in thermally treated (fried) tofu [9].

Soy sauces are produced by long fermentation. This leads to a significant degradation of DNA and proteins. A majority of developed PCR and ELISA methods do not detect the presence of soybeans in a soy sauce. With that, this product retains its allergenicity [21]. In our work, PCR with the primers Gly_MAX gave a positive result. This is in agreement with the data of Yamakawa et al., who used primers for the retrotransposon region in their work [9].

In the samples of the confectionary product Con2, soy flour was declared in the composition. Its presence was successfully confirmed in PCR with both primer pairs. In the samples Con1, Con2 and Con5, soy lecithin was declared

in the composition. In the sample Con4, soy components were not indicated on the label. In analysis with the primers Gly_MAX, soybean was found in all samples. In analysis with the primers Lec1, a positive result was observed in the samples Con1 and Con5 only in one of three replicates, in the sample Con3 in two. The result of PCR with the primers Lec1 was negative in the sample Con4.

An effect of temperature regimes on a degree of degradation of different genome regions.

DNA degradation has a non-linear character depending on a technological process, for example, with an increase in time of exposure to a certain temperature [10]. Therefore, for quantitative assessment, it is necessary to know precise parameters of a technological process, which is impossible in practice. Mano et al. developed an index of DNA fragmentation based on the results of PCR of a targeted product with several primer pairs giving different length of amplicons [10]. However, the authors applied this index only for calculating the detection limit of the reaction for a certain sample and not for quantitative assessment of targeted DNA.

It was found during the investigation of control samples and products that a rate of degradation of the *le1* gene region was higher than those of LTR83l18-re-1. It is well seen when comparing the difference in values Cq (ΔCq) obtained in PCR of the sample with the primers Lec1 and Gly_MAX (Table 2). For example, in analysis of positive controls, ΔCq for the control sample of non-degraded 1% soybean was 9.88; while for the control sample of degraded DNA, it was 15.62.

The obtained value ΔCq of the control 1% soybean DNA (9.88) was close to ΔCq of the sample Con2 (10.32), in which composition soy flour was declared. In the sample C1, ΔCq (15.04) was close to the control sample of degraded DNA (15.62). It is necessary to note that exposure time of the control sample (20 min.) was lower than in production of meat-and-plant canned foods. The comparable degree of DNA degradation can be explained, most likely, by the low volume and container type, in which the sample was sterilized. ΔCq of meat-and-plant canned foods was lower and varied in a range of 12.02 to 12.82, which is linked with different sterilization regimes for this product category. ΔCq of tofu and preserved tofu also expectedly differed from each other. ΔCq was not calculated for the samples C2, Con1, Con3-Con5 as the positive reaction with the primers Leq1 was not observed in all replicates.

Therefore, ΔCq between the values obtained with the primers for single-copy and multi-copy regions was higher when the technological impact on DNA was larger.

Conclusion

We developed a highly sensitive method for soybean detection in food samples based on selection of a multi-copy region of genomic DNA for primer design. The samples of meat-and-plant canned foods and other samples that underwent deep technological processing were successfully detected by this method. The method showed high sensitivity compared to the use of the primers selected for the single-copy DNA region: 0.001% non-degraded soybean DNA for the primers Gly_MAX vs. 0.01% for Leq1.

During the investigation, a difference in the rate of degradation of the *le1* gene region and LTR83l18-re-1 was found. This effect can be used for the development of a method for assessing DNA degradation in food products underwent deep technological processing and the following quantitative assessment of the composition.

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REFERENCES

1. Scharf, A., Kasel, U., Wichmann, G., Besler, M. (2013). Performance of ELISA and PCR methods for the determination of allergens in food: an evaluation of six years of proficiency testing for soy (Glycine max L.) and wheat gluten (Triticum aestivum L.). *Journal of agricultural and food chemistry,* 61(43), 10261–10272. DOI: 10.1021/jf402619d
2. Köppel, R., Dvorak, V., Zimmerli, F., Breitenmoser, A., Eugster, A., Waiblinger, H. U. (2010). Two tetraplex real-time PCR for the detection and quantification of DNA from eight allergens in food. *European Food Research and Technology,* 230(3), 367–374. DOI: 10.1007/s00217–009–1164–3
3. Costa, J., Amaral, J. S., Grazina, L., Oliveira, M. B. P. P, Mafra, I. (2017). Matrix-normalised real-time PCR approach to quantify soybean as a potential food allergen as affected by thermal processing. *Food chemistry*, 221, 1843–1850. DOI: 10.1016/j.foodchem.2016.10.091
4. Murray, S. R., Butler, R. C., Timmerman-Vaughan, G. M. (2009). Quantitative real-time PCR assays to detect DNA degradation in soy-based food products. *Journal of the Science of Food and Agriculture,* 89(7), 1137–1144. DOI: 10.1002/jsfa.3563
5. Ladenburger, E. M., Dehmer, M., Grünberg, R., Waiblinger, H. U., Stoll, D., Bergemann, J. (2018). Highly sensitive matrix-independent quantification of major food allergens peanut and soy by competitive real-time PCR targeting mitochondrial DNA. *Journal of AOAC International*, 101(1), 170–184. DOI: 10.5740/jaoacint.17–0406
6. Mayer, W., Schuller, M., Viehauser, M. C., Hochegger, R. (2019). Quantification of the allergen soy (Glycine max) in food using digital droplet PCR (ddPCR). *European Food Research and Technology,* 245(2), 499–509. DOI: 10.1007/s00217–018–3182–5
7. Pegels, N., González, I., López-Calleja, I., Fernández, S., García, T., Martín, R. (2012). Evaluation of a TaqMan real-time PCR assay for detection of chicken, turkey, duck, and goose material in highly processed industrial feed samples. *Poultry Science,* 91(7), 1709–1719. DOI: 10.3382/ps.2011–01954
8. Ballin, N. Z., Vogensen, F. K., Karlsson, A. H. (2012). PCR amplification of repetitive sequences as a possible approach in relative species quantification. *Meat science*, 90(2), 438–443. DOI: 10.1016/j.meatsci.2011.09.002
9. Yamakawa, H., Akiyama, H., Endo, Y., Miyatake, K., Sakata, K., Sakai, S., Moriyama, T., Urisu, A., Maitani, T (2007). Specific detection of soybean residues in processed foods by the polymerase chain reaction. *Bioscience, biotechnology, and biochemistry*, 71(1), 269–272. DOI: 10.1271/bbb.60485
10. Mano, J., Nishitsuji, Y., Kikuchi, Y., Fukudome, S.-I., Hayashida, T., Kawakami, H., Kurimoto, Y., Noguchi, A., Kondo, K., Teshima, R. Takabatake, R., Kitta, K. (2017). Quantification of DNA frag-

mentation in processed foods using real-time PCR. *Food chemistry*, 226, 149–155. DOI: 10.1016/j.foodchem.2017.01.064
11. Bauer, T., Weller, P., Hammes, W. P., Hertel, C. (2003). The effect of processing parameters on DNA degradation in food. *European Food Research and Technology*, 217(4), 338–343 DOI: 10.1007/s00217–003–0743-y
12. Caldwell, J.M., Pérez-Díaz, I.M., Sandeep, K.P., Simunovic, J., Harris, K., Osborne, J.A., Hassan, H.M. (2015). Mitochondrial DNA Fragmentation as a Molecular Tool to Monitor Thermal Processing of Plant-Derived, Low-Acid Foods, and Biomaterials. *Journal of food science,* 80(8), M1804-M1814. DOI: 10.1111/1750–3841.12937
13. Wawrzynski, A., Ashfield, T., Chen, N.W.G., Young, N.D., Innes, R.W. (2008). Replication of nonautonomous retroelements in soybean appears to be both recent and common. *Plant Physiology,* 148(4), 1760–1771. DOI: 10.1104/pp.108.127910
14. GenBank®. Bethesda, MD, USA: National Center for Biotechnology Information (NCBI), US National Library of Medicine; 2017. Available from: http://www.ncbi.nlm.nih.gov/
15. Primer-BLAST. Bethesda, MD, USA: National Center for Biotechnology Information, U. S. National Library of Medicine; 2017. Available from: https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi
16. OligoAnalyzer 3.1, Integrated DNA Technologies, Inc., Coralville, IA, USA; 2017. Available from: http://eu.idtdna.com/calc/analyzer.
17. Microsoft Excel 2016, Microsoft, Redmond, WA, USA; 2016.
18. Arun, Ö. Ö., Muratoğlu, K., Eker, F. Y. (2016). The effect of heat processing on pcr detection of genetically modified soy in bakery products. *Journal of Food and Health,* 2(3), 130–139. DOI: 10.3153/jfhs16014
19. Bauer, T., Hammes, W. P., Haase, N. U., Hertel, C. (2004). Effect of food components and processing parameters on DNA degradation in food. *Environmental Biosafety Research,* 3(4), 215–223. DOI: 10.1051/ebr:2005005
20. Kostenko, A.A (2015). Methods of production of cheese tofu. *Scientific works of Dalrybvtuz,* 35, 143–148. (in Russian)
21. Hefle, S. L., Lambrecht, D. M., Nordlee, J. A. (2005). Soy sauce retains allergenicity through the fermentation/production process. *Journal of Allergy and Clinical Immunology,* 115(2), S32. DOI: 10.1016/j.jaci.2004.12.143

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Original scientific paper

THAWING PROCESS CALCULATION OF EGG INGREDIENTS PARTICLES IN MINCED MEAT

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Key words: *egg products, meat and egg products, pasteurized liquid egg, egg white, yolk, minced meat for sausages, thawing, energy balance*

Abstract

Sausages from poultry meat are present on the domestic market. In this regard, the problem of improving the competitiveness of these products is urgent. One way to solve this problem is to replace part of the raw meat in the minced meat for sausages with chicken egg products (pasteurized liquid egg, egg white, yolk). Using egg products as ingredients of minced meat will help stabilize minced meat for sausages before and after heat treatment, increase the nutritional value of the finished product, and reduce its cost. However, currently, the mass fraction of egg ingredients in meat and egg products formulations does not exceed 5–10 %. This is due to the appearance of a specific taste, if it is exceeded, and because it is impossible to obtain a pattern of egg fragments in the section of the finished products, if necessary. This fact restricts the use of egg products as ingredients of meat and egg products. A technological method to eliminate these problems is freezing egg ingredients before adding to minced meat. In order to control the application of this method, the mechanism of changes in frozen egg ingredients during the preparation and heat treatment of minced meat for sausages is revealed. It was found that at the stage of minced meat mixing, the liquid part of the egg ingredients resulting from the thawing of frozen particles surface mixes with meat ingredients. Moreover, when unmoved relative to the surrounding minced meat, the frozen particles of egg ingredients are caught by the minced meat, and then locally coagulate in the process of meat and egg product heat treatment. The weight of the liquid phase resulting from the thawing of frozen egg ingredient particle and the weight of its remaining local part depend on the duration of the minced meat mixing process, its temperature and particle weight. Based on the knowledge about this mechanism, analytical equations are obtained using the energy balance method. They describe the duration of egg ingredients thawing in meat and egg products depending on the particle weight and the temperature of minced meat. The experimental data of the authors are used as a basis for calculating the process of egg ingredients thawing. The proposed calculation method will allow purposeful controlling the process of change in frozen egg ingredients aggregative state in minced meat for sausages, under production conditions.

Introduction

Currently, the volume of poultry meat on the Russian market exceeds that of slaughtered animals, which are traditional raw materials for the production of sausages [1].

For this reason, in recent years, various types of sausages from poultry meat, including cooked ones, have been widely represented on the domestic market [2].

In this regard, it should be noted that each change in the technology of sausage production may require the introduction of appropriate additional technological methods.

In particular, some researchers note the need to replace fat in the composition of minced meat based on poultry meat, since chicken fat, due to the low melting point, easily melts during heat treatment forming oily «pockets» in minced meat for sausages, thereby reducing the quality of products [3, 4].

The stabilizing function in an unstable heterogeneous system of minced meat for cooked sausages is performed by emulsifiers and gelling agents that provide binding and retention of water and fat in a single system before and after heat treatment [5].

One of the best natural emulsifiers and gelling agents are products chicken egg processing, i. e. pasteurized liquid egg and yolk (emulsifiers), egg white (gelling agent) [6].

In addition, the introduction of these egg products into minced meat for sausages will significantly increase the nutritional value of the finished product, because, for example, pasteurized liquid egg almost completely corresponds to human milk in terms of essential amino acids content (g/100 g of protein), which is the highest standard of nutritional value [7].

At the same time, the use of egg products as ingredients of minced meat for cooked sausages will reduce their cost because the cost of chicken eggs is lower than the equivalent weight of poultry meat.

Thus, the use of egg products in minced meat for cooked sausages will solve the following important problems: stabilize minced meat for sausages before and after heat treatment, increase the nutritional value of the finished product and reduce its cost.

However, currently, egg products are used in the production of sausages in liquid form. This fact determines their low mass fraction in the formulations of combined meat and egg products (on average 5–10 %) [8], which restricts their use as ingredients of meat and egg products.

In addition, the liquid state of egg ingredients does not allow obtaining a pattern of egg fragments in the section of finished products, if necessary.

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In this regard, the search for technological methods that can eliminate these limitations is urgent.

One of such methods is to freeze egg ingredients before adding to minced meat.

The use of frozen egg ingredients during the mixing of minced meat for sausages will allow to use them in the process of finished product manufacturing for two purposes. The thawed liquid part mixed with meat ingredients of minced meat will act as an emulsifier (pasteurized liquid egg and yolk) or as a gelling agent (egg white). Moreover, the part of egg ingredients remaining in the frozen state will be transformed into pattern in the section of finished products as a result of coagulation at the heat treatment stage.

In general, this technological approach will allow to better stabilize of minced meat for sausages, increase the mass fraction of egg ingredients in combined meat and egg products and the nutritional value of finished products without giving them a specific egg flavor, cool down minced meat in the process of its preparation and reduce the cost of finished products.

The practical implementation of the above technological method requires the possibility of calculating the changes in frozen egg ingredients during the preparation and heat treatment of minced meat for sausages.

The aim of this work was to obtain analytical equations describing the duration of egg ingredients thawing in meat and egg products depending on the particle weights and ambient (minced meat) temperature.

Materials and methods

Objects of study: egg ingredients of meat and egg products, i. e. pasteurized liquid egg, yolk and egg white, cylindrical (D = H), where D is the diameter of the cylinder and H is its height.

Subject of study: egg ingredients thawing process.

The calculation of food products thawing duration is a complex problem, since it requires consideration of many factors, including changes in the thermophysical properties of the product during this process.

There are number of mathematical models developed for this purpose, for example [9,10,11,12, 13,14,15].

These calculation methods may be divided into two groups: relatively simple analytical dependencies based on a number of assumptions, and therefore, the results of calculations using them contain unacceptably large errors; and complex mathematical models that require unreasonably complicated calculations to solve practical problems.

We have chosen an intermediate analytical and empirical model that optimally combines their advantages (simplicity and acceptable accuracy).

In order to obtain engineering equations for calculating the process of egg ingredients thawing convenient for use in production conditions, kinetic indicators (the rate of egg products thawing — pasteurized liquid egg, yolk and egg white, in relative units) obtained empirically by the authors were used as the basis for the calculations [16].

The experiments to obtain the base for calculations were performed as follows.

Egg white, yolk, and pasteurized liquid egg were transferred into cylindrical metal containers with an inner diameter and height of 43 mm. The temperature sensor was fixed in the thermal center of the sample (on the axis of the cylinder, at the same distance from the bottom of the container and the surface of the sample). In order to ensure the identical conditions of the experiment for all objects of study, three containers with prepared samples (protein, yolk and pasteurized liquid egg) were simultaneously placed into freezer and frozen.

After freezing, product samples were removed from the freezer and thawed at room temperature. During freezing and thawing, changes in temperature of the samples over time were recorded.

The temperature was measured using IS203.4 temperature recorder (Russia). The sensor readings were recorded automatically, with an interval of 2 seconds. The absolute measurement error was ± 0.2 °C. Overall, five experiments were performed at the same air temperature in the freezer, i. e. minus (15 ± 0.5) °C.

The coefficient of variation for the set of measurements did not exceed 4 %.

The relationship between the temperature of the study objects and their aggregative state was established qualitatively (visually) and quantitatively (by determining the penetration using KP-3 penetrometer (Russia). The difference between the results of three parallel measurements did not exceed 3 %.

Analytical equations describing the duration of egg ingredients thawing in meat and egg products depending on particle weight and ambient temperature were obtained based on the energy balance of egg ingredients thawing process [17,18]. In such a case, the results were used of studies on the mechanism of change in egg ingredients during mixing and heat treatment of minced meat for sausages.

Comparison of calculated and experimental data confirmed the validity of this assumption, i. e. the relative error of the calculation results with the proposed equations did not exceed 5 %, which is acceptable for technical calculations in sausage production.

Results and discussion

The surface of egg ingredient frozen particle located in minced meat with a positive temperature thaws. During minced meat mixing, the resulting liquid is mixed with meat ingredients. If the frozen particle (or its frozen part remaining after thawing) is unmoved relative to the surrounding minced meat, then it is caught by the minced meat and locally coagulates during the subsequent heat treatment forming the pattern in the section the meat and egg products [16].

The weight of the liquid phase resulting from the thawing of egg ingredient frozen particle and the weight of its remaining local part depend on the duration of the minced meat mixing process, its temperature and the weight of the egg particle.

In the production environment, purposeful control of the mechanism for changing the aggregative state of egg ingredients frozen particles in the minced meat for sausages is possible only with the help of analytical equations that describe the dependence of duration of egg ingredients thawing on particle weight and ambient (minced meat) temperature.

The indicated analytical dependences are as follows.

Calculation of the duration of egg ingredient particle thawing depending on its weight

The energy balance equations for the thawing process of two cylindrical frozen particles of egg ingredient of different weights are as follows. The first particle of egg ingredient (equation members are indicated by index 1):

$$W \times \omega \times M_1 \times r = q \times F_1 \times \tau_1, \quad (1)$$

where:

W is the humidity of egg ingredient, %;

ω is the fraction of frozen water in egg ingredient, %;

M_1 is the weight of egg ingredient, kg;

r is the specific heat of ice thawing, J/kg;

q is the specific heat flux that enters the surface F1, m^2 of frozen egg ingredient, J/m^2s;

τ_1 is the duration of thawing process, s.

By analogy with equation (1), the energy balance equation for the second particle of egg ingredient (equation members are indicated by index 2) is as follows:

$$W \times \omega \times M_2 \times r = q \times F_2 \times \tau_2. \quad (2)$$

As a result of transformations of equations (1) and (2) (i. e. expressing the areas F and weights M of the egg ingredient particles through their radii: $F = 4\pi R^2$; $M = \rho^*\pi^*R^3$, where ρ is the specific gravity of the egg ingredient frozen particle, kg/m^3) the following equations are obtained:

$$\tau_1 = W \times \omega \times r \times \rho \times \pi \times R_1^3 / q \times 4\pi R_1^2. \quad (3)$$

$$\tau_2 = W \times \omega \times r \times \rho \times \pi \times R_2^3 / q \times 4\pi R_2^2. \quad (4)$$

Dividing equation (3) by (4) gives the following equation:

$$\tau_2 = \tau_1 \times R_2/R_1, \quad (5)$$

where:

R_1 and R_2 are the radii of the first and second particles, m.

By expressing the radii of the particles through their weights ($R^3 = M/\rho \times \pi \times$), we obtain the desired equation:

$$\tau_2 = \tau_1 \times \sqrt[3]{M_2/M_1}. \quad (6)$$

Calculation of the duration of egg ingredient particle thawing depending on ambient temperature

The energy balance equations for the thawing process of two frozen particles of egg ingredient of the same weight at different ambient temperatures are as follows.

The particle of egg ingredient at the ambient temperature T1 (equation members are indicated by index 1):

$$W\omega M \times r = q_1 \times F \times \tau_1, \quad (7)$$

where:

M is the weight of egg ingredient;

q_1 is the specific heat flux that is transmitted to the surface F of frozen egg ingredient under the influence of temperature difference between the environment and the frozen product surface ΔT_1;

By analogy with equation (7), the energy balance equation for the particle of egg ingredient at the ambient temperature T_2 (equation members are indicated by index 2) is as follows:

$$W\omega M \times r = q_2 \times F \times \tau_2, \quad (8)$$

where: q_2 is the specific heat flux that is transmitted to the surface F is the specific heat flux that is transmitted to the surface ΔT_2;

From equations (7) and (8), we express τ_1 and τ_2

$$\tau_1 = W\omega M \times r/q_1 \times F, \quad (9)$$

$$\tau_2 = W\omega M \times r/q_2 \times F, \quad (10)$$

Dividing equation (9) by equation (10) gives the following equations:

$$\tau_2 = \tau_1 \times q_1/q_2 \quad (11)$$

Based on the Newton's law of cooling ($q = \alpha^*\Delta T$)), we can write equation (11) in the following form:

$$\tau_2 = \tau_1^* \Delta T_1/\Delta T_2 \quad (12)$$

The change in the temperature difference between the environment (minced meat) and the surface of the egg ingredient frozen particle, under the influence of which the heat flux is transferred for thawing, depends only on the changes in the ambient temperature. This is because the surface temperature of the frozen object that receives heat flux from the environment is constant and equal to the cryoscopic temperature of egg ingredient.

For this reason, to perform the calculations with convenience, we replace ΔT in equation (12) with the ambient temperature T.

As a result, the desired equation is as follows:

$$\tau_2 = \tau_1 \times T_1/T_2, \quad (13)$$

where T_1 and T_2 are the ambient temperatures.

Here is an example of calculating the parameters of the meat and egg product manufacturing process regarding the use of frozen egg ingredients using equations (6) and (13).

Statement. It is necessary to produce meat and egg product with a total content of egg ingredients equal to 25 %. The part of egg ingredients, which should be turned into a liquid state and mixed with meat ingredients, is 10 % of the whole product weight. The remaining part is 15 % of the whole product weight. It forms a pattern on the product section, i. e. local coagulated inclusions of cylindrical shape $H = D = 1.10$ cm (weight 1.0 g).

Unknown. The initial sizes of the egg ingredients frozen particles ($H = D$) and the duration of minced meat (with a temperature of 12 °C) mixing with added egg ingredients.

Solution. According to the statement, the total weight of the egg ingredients particles forming the pattern on the product section should be 15 % of the whole product weight. The total weight of thawed egg ingredients

should be 10 % of the whole product weight, i. e. 1.5 times less than the total weight of the particles forming the pattern. As for one particle, the weight of its thawed part is 1.0 g/1.5 = 0.67 g. Hence, the initial weight of the frozen particle is 1.0 g + 0.67 g = 1.67 g.

To determine the size of the initial frozen particle, the following well-known equation should be used:

$$M = \rho \times V = \rho \times \pi \times H^3/4 \quad (14)$$

where:

M is the initial weight of the frozen particle;

ρ is the specific gravity of the egg ingredient frozen particle;

V is the volume of the egg ingredient frozen particle;

H is the height of the cylinder equal to its diameter (D).

Expressing H from equation (10):

$$H = \sqrt[3]{4 \times M/\rho \times \pi}. \quad (15)$$

By substituting the numerical values of the symbols in equation (11) (for the convenience of calculations, we approximate the numerical values of M to 1.70 g, and ρ to 1.0 g/cm^3), we obtain the size of the initial egg ingredient frozen particle: H = D = 1.30 cm.

Next, using equations (6) and (13), we can determine the duration of the minced meat mixing (we consider the temperature of minced meat equal to 12 °C) with added frozen egg ingredients (e. g. pasteurized liquid egg). This duration must provide the specified values of the mass fractions of liquid (10 %) and coagulated (15 %) phases of egg ingredients in the finished product.

First, using equation (6), we determine the duration of thawing for pasteurized liquid egg with weight of 0.7 g at the ambient temperature of T_2 = 23 °C.

For this purpose, by substituting in this equation the numerical values of the symbols: τ_1 = 16 min and M_1 = 60 g (determined experimentally [16]), and the given value M_2 = 0.7 g (according to the statement, this weight should be turned into the liquid state), we obtain:

$$\tau_2 = \tau_1 \times \sqrt[3]{M_2/M_1} = 16 \times \sqrt[3]{0{,}7/60} = 16 \times 0{,}23 = 3{,}7 \text{ min}$$

Then, using equation (13), we determine the duration of thawing for pasteurized liquid egg with weight of 0.7 g at the ambient temperature of T_2 = 12 °C.

By substituting the numerical values of the symbols in equation (13), we obtain the following value:

pasteurized liquid egg:

$$\tau_2 = \tau_1 \times T_1/T_2 = 3{,}7 \times 23/12 = 7{,}1 \text{ min}$$

Using kinetic indicators of the egg ingredients thawing process expressed in relative units (yolk — 1.3; pasteurized liquid egg — 1.1; egg white — 1.0) [16], we calculate the duration of thawing for particles with weight of 0.7 g for yolk and egg white at the ambient temperature of T_2 = 12 °C. We can make it using as a base the obtained value of a similar indicator for pasteurized liquid egg:

yolk: $\tau_2 = 7{,}1 \times 1.1/1{,}3 = 6{,}0$ min

egg white: $\tau_2 = 7{,}1 \times 1.1/1{,}0 = 7{,}8$ min

Result of the solution. As a result of solving this problem, it was established that the fulfillment of the above statement requires the following process parameters:

Initial frozen cylindrical particles of egg ingredients should have the dimensions: D = H = 1.30 cm, and the duration of minced meat mixing with added particles (at the minced meat temperature T_2 = 12 °C) should have the following values for various egg ingredients (min) — pasteurized liquid egg, 7.1; egg white, 7.8; yolk, 6.0.

Conclusion

The obtained analytical equations allow carrying out calculations of changes in the aggregative state and the geometric dimensions of the egg ingredients frozen particles in the minced meat for sausages, when egg ingredients are used to develop the technology of meat and egg products.

The simple structure of the obtained equations, which contains only physical parameters, ensures their convenient application in a production environment.

REFERENCES

1. Expert and analytical center of agribusiness. [Electronic resource: http://ab-centre.ru/news/rynok-myasa-pticy—klyuchevye-tendencii-i-prognozy/ Access date 20.08.2019Ъ
2. Sausage reaches a poultry. [Electronic resource: http://vestnikapk.ru/articles/importozameshchenie/kolbasa-dokatilas-do-ptitsy/ Access date 10.08.2019]
3. Choe, J.-H., Kim, H.-Y., Lee, J.-M., Kim, Y.-J., Kim, C.-J. (2013). Quality of frankfurter-type sausages with added pig skin and wheat fiber mixture as fat replacers. *Meat Science,* 93(4), 849–854. DOI: 10.1016/j.meatsci.2012.11.054
4. Campagnol, P.C.B., dos Santos, B.A., Wagner, R., Terra, N.N., Rodrigues Pollonio, M.A. (2012). Amorphous cellulose gel as a fat substitute in fermented sausages. *Meat Science,* 90(1), 36–42. DOI: 10.1016/j.meatsci.2011.05.026
5. Murashev, S.V., Sherzody, S, (2016). Peculiarities of physics-chemical and mechanical processes of making the filling for cooked sausages. *Scientific journal NRU ITMO Series «Processes and Food Production Equipment»,* 2, 54–62.
6. Agafonychev V. P., Petrova, T.I., Makhonina, V.N., Dmitrienko, I.S. (2016). Functional properties of egg products. *Poultry and Poultry Processing,* 6, 52–54.
7. Lysikov, Yu.A. (2012). Amino acids in human nutrition. *Experimental & clinical gastroenterology,* 2, 88–105.
8. Stefanova, I.L., Klimenkova, A. Yu. New chicken egg products. [Electronic resource: https://sfera.fm/articles/pticeprom/novye-produkty-iz-kurinykh-yaits Access date 18.09.2019]
9. Góral, D., Kluza, F., Spiess, W.E.L., Kozłowicz, K. (2016). Review of Thawing Time Prediction Models Depending on Process Conditions and Product Characteristics. *Food Technology and Biotechnology*, 54(1), 3–12. DOI: 10.17113/ftb.54.01.16.4108
10. Bergman, T.L., Lavine, A.S., Incropera, F.P., DeWitt D.P. (2011). Fundamentals of heat and mass transfer. New York: John Wiley & Sons Inc. —1076 p. ISBN: ES8–1–119–32042–5
11. Wang, J., Pham, Q.T., Cleland, D.J. (2010). Freezing, thawing, and chilling of foods. Chapter 3 in book: Mathematical modeling of food processing. Boca Raton: CRC Press. pp. 375–398. DOI: 10.1201/9781420053548
12. Mascheroni, R.H., (2012). Chilling and Freezing by Cryogenic Gases and Liquids (Static and Continuous Equipment). Chapter 12 in book: Operations in food refrigeration. Boca Raton: CRC Press, Taylor & Francis Group. pp. 331–352. DOI: 10.1201/b12137

13. Cao, W.R., Sun, Z.Z. (2010). Maximum norm error estimates of the Crank–Nicolson scheme for solving a linear moving boundary problem. *Journal of Computational and Applied Mathematics,* 234(8), 2578–2586. DOI: 10.1016/j.cam.2010.03.024
14. Christoph, J. B., Baoki, C.J. (2015). Modeling, Estimation and Control of Freezing and Thawing Processes: Theory and Applications. PhD Thesis, Norwegian University of Science and Technology.
15. Bredihin, S.A., Maksimov, D.A., Yakushev, A.O. (2011). Mathematical models of the defrosting process of raw meat. *Izvestiya Tula State University. Technical science,* 1, 151–161.
16. Agafonychev V. P., Makhonina, V.N., Dmitrienko, I.S., Korenev, V.V., Roslikov, D.A., Tsvetkov, A.I. (2018). Changes in the aggregative state of egg products during freezing and process kinetics. *Poultry and Poultry Processing,* 6, 21–23.
17. Grosheva, L.P. (2006). The principles of the energy (thermal) balance and thermal calculations of chemical-technological processes. Novgorod: Yaroslav-the-Wise Novgorod State University. — 14 p.
18. Chizhov, G.B. (1979). Thermophysical processes in food refrigeration technology. Moscow: Food industry. — 271 p.

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