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FOCUS AND SCOPE

The top priority goal of the Journal "Teoriâ i praktika pererabotki mâsa" (Theory and practice of meat processing) is to distribute in the world scientific community the results of the research in the field of meat science performed by the scientists from scientific centers, scientific-research institutes and institutions of higher education from Russia and the CIS countries, increase the level of presentation of the achievements of the respective science in the international arena, inform the Russian scientists about the research carried out abroad, highlight the results of the prospect directions of the research activities in the meat and poultry processing industries.

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Original scientific article

POLYMORPHISM OF THE GENE *GDF9* IN SHEEP OF PRIKATUN TYPE OF ALTAI MOUNTAINS BREED AND ITS CORRELATION WITH INDICES OF MEAT RATE PRODUCTIVITY

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Abstract
In recent decades in many countries of the world the development of sheep farming is determined by the efficiency of mutton production. One of the promising areas of selection improvement of sheep breeds of meat and meat & wool productivity is an application of molecular-genetic methods. To obtain high-quality mutton it is advisable to use the Altai Mountains sheep breed, which features by high energy of growth of young animals and their adaptability to all-year-round pasture management. One of the candidate genes responsible for sheep meat productivity is the gene of growth differentiation factor -GDF9 (growth differentiation factor 9). The article presents the results of research of polymorphism of the gene GDF9 and its relation with the parameters of meat productivity in young sheep of Altai Mountains breed. It was found that the rams-carriers of AA genotype outperformed their peers of AG and GG genotypes in terms of pre-slaughter weight, carcass weight, meat yield and meat content ratio by 1.62 and 7.01 kg, 1.34 and 3.98 kg ($P<0.05$), 1.21 and 1.86 abs. percent, 0.16 and 0.39 units ($P<0.05$) accordingly. The muscle tissue of rams of the desired genotype featured the highest content of protein, fat and, accordingly, energy value in comparison with AG and GG genotypes. The animals of AA genotype had the largest area of a rib eye and diameter of muscle fibers. The superiority over other genotypes achieved 1.55 cm^2 and 1.98 microns in average. In addition the higher number of inter-fiber and inter-bundle inclusions of fat and lower content of connective tissue stipulated higher marbling score (MB)–higher by 3.32 points. The obtained data indicate that it is promising methods to increase the number of homozygotic animals in term of gene GDF9 by intended selection of parent animals whose genotype contains a desirable element for further improvement of quantitative and qualitative parameters of meat rate productivity in sheep of Altai Mountains breed.

Introduction

One of the relevant objectives of modern animal husbandry is an application of new methods for assessing the productivity characteristics of farm animals, directly based on genome analysis [1]. All over the world the large-scale studies are conducted, which studies are aimed to identify genes associated with economically useful features of animals [2]. Genetic differences are usually presented in the form of single-nucleotide polymorphisms (SNP), which allows using them as a molecular genetic marker, on which basis the animals can be taken for further selection [3].

It is known that both in breeding of farm animals as a whole and in a single herd, some individual animals have a low rate of productivity, some animals feature medium rate, and some — high, and a very small number of animals have the highest rate of productivity. It is these very animals which are of the greatest interest for the selection process. The development of molecular genetic methods, the possibility of simultaneous genotyping of dozens of loci in a single genome — together with the phenotypic assessment allows the breeder to select with much higher accuracy the valuable animals, which shall transmit their best economically valuable features to their offspring, thereby providing a significant acceleration of selection breeding progress. In addition, the molecular-genetic approach to genotyping avoids the manifestation of genetic regression, when an animal highly valuable for its own rate of productivity does not transmit its meat qualities to subsequent generations.

To increase meat productivity is important for sheep breeding, since in recent decades in many countries of the world the development of sheep breeding is determined by efficiency of mutton production. In its turn it leads to a steady increase of specialized meat breeds share and increasing demands on the very meat rate productivity for sheep of meat and wool breeds [4]. However, the marker-associated approach in selection of this type of productivity in sheep should be recognized as an insufficiently developed topic [5].

There are reports of associations of the myostatin (myostatin, MSTN), calpain-calpastatin cascade (calpain, *CAPN*, calpastatin, *CAST*), leptin (lepnin, LEP) genes with characteristics that determine the number and size of muscle fibers, features of hemolytic processes during meat maturation, and determine the degree of its tenderness and juiciness [5,6,7,8,9,10]. These genes are considered to be

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promising for their use in breeding programs to improve the meat rate productivity of sheep [11].

The genes of growth hormone (GH) and growth differentiation factor 9 (*GDF9*) can be considered as candidate genes associated with the general development of muscle mass and, accordingly, with the meat rate productivity of sheep.

The growth hormone gene, or somatotropic hormone, is a key regulator of somatic growth throughout the life of an animal. Range of studies has demonstrated the relation of genetic variants of the gene with characteristics of meat rate production in sheep. Thus, in young animals of Salsk, Kalmyk and Edilbaevskaya breeds, the AB genotype stipulated the significantly higher parameters of carcass mass and its slaughter yield [12, 13]. The nucleotide substitution at the point rs400358099 of the *GH* gene was associated with the growth energy of Texel breed lambs [14]. Different genotypes in the growth hormone (*GH*) gene determined differences in live weight of young sheep of the dark-headed sheep breeds, as well as the breeds of Poll Dorset, Beluchi, Santa Yves and seven breeds of sheep from China [15,16,17,18,19].

The majority of studies witness a link between the growth differentiation factor gene and the reproductive qualities and fertile multiplicity of sheep [20,21,22]. It has been shown that GDF9 expression is significantly higher in ovarian tissues than in other body tissues, while the protein product of the gene plays an important role in maintaining the normal folliculogenesis [23,24]. At the same time there are data on influence of different genetic variants of GDF9 on the live weight of young Salsk sheep, the dairy productivity of Avassi sheep, which indicates the feasibility of studying its polymophism and its relation with meat rate productivity indices [25,26].

The Altai Mountains breed of sheep seems to be promising to obtain high-quality mutton. It belongs to the wool-meat type of productivity, it was bred by the method of complex long-term reproductive crossing in the 30–40s of the last century, it is perfectly adapted to all-year-round pasture management in cold climate and the highlands of Altai Republic. In the last decade the main direction of breeding activity has been aimed to improve the meat rate productivity of sheep while maintaining the quality characteristics of semi fine wool. The result of this work was the development of a new intra-breed type — Prikatun sheep, which belongs to the meat-wool type of productivity and currently seems to be the most promising one for breeding. The live weight of rams and ewes of Prikatun type of Altai Mountains breed in breeding farms is 94 kg and 58 kg respectively. The milk yield of ewes is more than 100 kg for four months period of lactation, the average daily weight gain of lambs during this period varies from 240 g to 260 g, the live weight by the moment of lambs weaning from ewes is 30–32 kg [27].

For further improvement of the type it is quite appropriate to include genotyping by candidate genes of meat rate productivity into breeding programs. To determine the desired genotype it is necessary to apply comprehensive approach, including analysis of the quantitative and qualitative indices of meat, its nutritional and energy value, and consumer characteristics determined by chemical, biochemical, and microstructural indices [28].

The above was the rationale for the objective of this research — this is to study the indices of meat rate productivity, biochemical parameters of meat and histological parameters of rib eye muscle (*m. longissimus dorsi*) of sheep of Prikatun type of Altai Mountains breed in cases of different genotypes for gene GDF9.

Materials and methods

40 rams of Prikatun type of the Gorno-Altaisk (Altai Mountains) region were put under the research (private farm "Usoltsev N. A." of the Ust-Kansky district of Altai Republic). The studied material was DNA extracted from blood samples using the *Diatom^{tm} DNAPrep* kit (Isogen, Moscow) in amount of 3–5 micrograms/100 mcl with an OD of 260/280 from 1.6 to 2.0. Amplification of the site (exon) of the gene GDF9 (NCBI-National Center for Bio-technology Information ID is *rs410123449*, position *46547268* in the Oar_rambouillet_v1.0 assembly, *41770341* — in the Oar_v4 assembly.0) on a programmable thermal cycler "Tertsik" made by the company "DNA-technology" (Russia) with kits of "genePakPCRCore" (Isogen, Moscow) with the direct primer — 5'-gaa-gac-tgg-tat-ggg-gaa-atg-3' and the reverse primer 5'-cca-atc-tgc-tcc-tac-aca-cct-3'. The presence of amplicons and their quality were preliminarily evaluated after electrophoresis in 2.0% agarose gel. The samples were restricted with BstHH1 endonuclease and the following genotypes were identified: AA, AG, and GG (Figure 1).

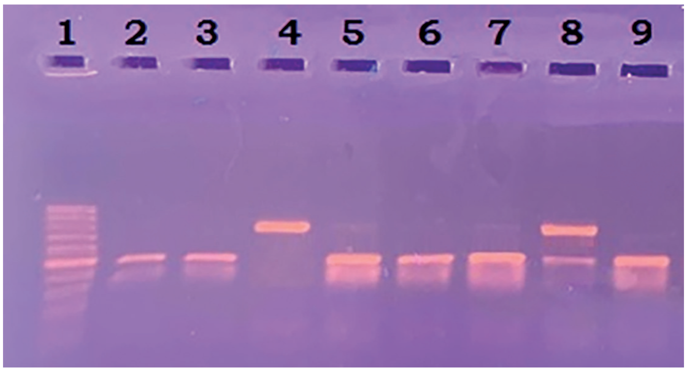

Figure 1. Electrophoregram of the results of PCR-RFLP analysis of the gene *GDF9* in V 2.0% agarose gel: 1-DNA marker 50 bp (Isogenic) — 4-AA genotype; 8-AG genotype; 2, 3, 5, 6, 7, 9 — genotype GG

The occurrence frequency of genotypes and alleles was determined in accordance with the Methodological Recommendations "System of integrated assessment of the genetic potential of breeding animals" [29].

The meat rate productivity was studied on the basis of results of the rams slaughter parameters at the age of 7 months. In each genetic variant — AA, AG, GG — three animals were slaughtered, which animals corresponded to the average live weight in their group.

The pre-slaughter live weight was measured by weighing the animals after 24 hours of starvation, with an accuracy of 0.1 kg. The mass of the carcass was determined by weighing the carcass together with kidneys and perinephral fat. The carcass yield was determined by the ratio of the carcass mass to the pre-slaughter live weight after 24 hours of starvation, expressed as a percentage. Slaughter masswas determined by summing the mass of an exsanguinated carcass without a head, skin, tail, internal organs, limbs cut off at the hock and wrist joints, and without internal fat. The mass of internal fat was determined by the total amount of pelvic, renal, intestinal, gastric, and diaphragmatic fat. The slaughter yield was calculated as the ratio of the slaughter mass to the pre-slaughter mass, expressed as a percentage. The meat content coefficient was determined by the ratio of the mass of the boneless meat to the mass of bones, expressed as percentage. The boneless meat coefficient was determined by separating the muscle tissue from the bone tissue and their weighing with an accuracy of 0.01 kg.

Some indices of meat rate productivity of young sheep of Altai Mountains breed of different genotypes were used from the experiment, where these indices were studied when comparing animals for two genes-GDF9 and CAST [30] simultaneously.

During the control slaughter the rams meat samples were taken from the main topographic areas of the carcass for the preparation of an average sample and chemical analysis, as well as samples of the rib eye muscle (*m. longissimus dorsi*) were prepared for histological studies.

The amount of moisture in the average sample of minced meat was determined according to GOST 33319–2015[1] "Meat and meat products. Method for determination of moisture content». The sample was minced and mixed by meat homogenizer, dried in a drying chamber till a constant air-dry mass at a temperature of 103 ± 2 °C. The mass fraction of moisture, expressed as a percentage, was determined by the difference in the weight before and after drying. The amount of fat was determined according to GOST 23042–2015[2] "Meat and meat products. Methods of fat determination", by extracting the fat from the sample with petroleum ether in a Soxlet device, followed by its drying to a constant mass and determining the percentage difference. The amount of protein was determined according to GOST 25011–2017[3] "Meat and meat products. Protein determination methods", using the method of Kjeldahl, by the amount of nitrogen formed during the mineralization of organic substances and its conversion to protein applying coefficient of 6.25. The caloric content was determined by calculation according to the formula of V. M. Alexandrov:

$$C = [D - (F + A)] \times 4,1 + (F \times 9,3),$$

where

C — caloric content, kcal;

D, A, F — respectively, the amount of dry matter, ash, fat.

[1] GOST 33319–2015. "Meat and meat products. Method for determination of moisture content". Moscow: Standartinform, 2018. — 14 p. (In Russian)

[2] GOST 23042–2015 "Meat and meat products. Methods of fat determination". Moscow: Standartinform, 2019. — 8 p. (In Russian)

[3] GOST 25011–2017 "Meat and meat products. Protein determination methods". Moscow: Standartinform, 2018. — 14 p. (In Russian)

For histological analysis, the samples were fixed in a 10% solution of neutral formalin, then compacted by gelatin pouring. The slices of 7–8 microns thick were obtained by freezing microtome. Structural components of the muscle tissue were defined by hematoxylin staining by Caracci with Sudan III according to Ehrlich method. The area of the muscle bundle, the quantity of muscle fibers in the bundle, the diameter of the muscle fiber and the bundle, the ratio of muscle and connective tissue were defined according to GOST 19496–2013[4] "Meat and meat products. Method of histological study".

The resulting numerical material was biometrically processed using the software Microsoft Office and BIOSTAT. Based on the average values and standard errors, the reliability of the difference between the average values was calculated using the Student's criterion.

Results and discussion

Genotyping of sheep of Altai Mountains breed of Prikatun type revealed that the polymorphism of the gene *GDF9* is represented by A and G alleles. At the same time, the G allele and the GG genotype featured high prevalence, their occurrence frequency was 0.73 and 0.55, respectively. Whereas the occurrence frequency of allele A and genotypes AG and AA was 0.27; 0.35 and 0.10 (Table 1).

Table 1. Occurrence of frequency of genotypes and alleles of the gene *GDF9* in sheep of Altai Mountains breed of Prikatun type

Genotype	Number of animals	Occurrence frequency	
		Of genotype	Of allele
AA	4	0.10	A 0.27 G 0.73
AG	14	0.35	
GG	22	0.55	

The results obtained are similar to the data on the *GDF9* polymorphism obtained by other researchers in the study of sheep breeds of Russian and foreign selection breed. Thus, in sheep of the Salsk and Romanov breeds, the *G* allele and the *GG* genotype also had a high frequency of occurrence — 0.95 and 0.90, 0.80 and 0.61, respectively. At the same time the occurrence of heterozygous AG genotype was detected with a low and medium frequency of 0.10 and 0.39 respectively [31]. In Hissar sheep the *GG* genotype was found in 96.3% of cases, and in Avassi breed — in 80.0% [26,32].

The comparative analysis of the slaughter parameters of young sheep of Altai Mountains breed of different genotypes for the gene GDF9 at 7 months of age revealed that the AA-genotype rams were superior in comparison with their peers of the AG, GG-genotypes in terms of pre-slaughter weight, carcass weight, slaughter yield and meat ratio, respectively by 1.62 and 7.01 kg; 1.34 and 3.98 kg ($P<0.05$); 1.86 and 1.21 abs. percent, 0.16 and 0.39 units ($P<0.05$) (Table 2).

[4] GOST 19496–2013 "Meat and meat products. The method of histological study" Moscow: Standartinform, 2019. — 12 p. (In Russian)

Table 2. Slaughter parameters of Altai Mountains rams of Prikatun type with different genotypes according to the gene GDF9, in the age of 7 months

Parameters	Genotype		
	AA	AG	GG
Pre-slaughter live weight, kg	46.33 ± 2.18*1	44.71 ± 2.99*2	39,32 ± 2.78
Weight of a carcass, kg	22.17 ± 0.94*1	20.83 ± 1.37	18.19 ± 0.84
Carcass yield, %	47.86 ± 0.17*1	46.65 ± 0.12	46.00 ± 0.26
Weight of internal fat, g	744.0 ± 112.62	619.2 ± 85.17	709.3 ± 65.43
Slaughter weight, kg	22.91 ± 2.76	21.45 ± 1.30	18.89 ± 1.20
Meat rate productivity	4.10 ± 0.01	3.94 ± 1.19	3.71 ± 0.26
Slaughter yield, %	49.44 ± 0.28	48.04 ± 0.22	47.97 ± 0.24

* — P < 0,05, [1] — AA-GG; [2] — AG-GG

The obtained results allow to conclude that in order to increase meat rate productivity, it is advisable to select the animals which carry the A allele. This will allow obtaining a greater number of homozygous AA individuals and, accordingly, their specific weight in the herd, which will ultimately affect its overall level of meat productivity.

The results of the comparative analysis of chemical analysis indices of average sample of minced meat obtained from animals of different genotypes also confirm the above information. The superiority of the AA genotype was established in protein, fat, and caloric content over the AG and GG genotypes by 1.19 and 1.30%; 1.24 and 0.81%; 164.16 and 128.63 kcal respectively (Table 3).

Table 3. Chemical analysis of mutton from rams of Altai Mountains of Prikatun type of different genotypes by the gene *GDF9*

Parameter	Genotype		
	AA	AG	GG
Moisture, %	68.80 ± 0.31	71.22 ± 0.29	70.90 ± 0.24
Dry matter, %	31.20 ± 0.19	28.78 ± 0.16	29.10 ± 0.15
Protein, %	15.42 ± 0.19	14.23 ± 0.17	14.12 ± 0.14
Fat, %	14.82 ± 0.18	13.58 ± 0.14	14.01 ± 0.19
Ash, %	0.96 ± 0.01	0.97 ± 0.02	0.97 ± 0.01
Calories, kcal	2010.46 ± 8.13	1846.30 ± 7.12	1881.83 ± 6.89

Microstructural analysis of *m. longissimus dorsi* also confirmed the advantage of the AA genotype in terms of morphometric parameters. Thus, the rams had larger diameter of muscle fibers: the difference from AG and GG genotypes was 2.52 and 1.44 microns. In addition, there was a greater number of inter-fiber and inter-bundle inclusions of fat and a lower content of connective tissue, which gave the higher marbling score of 3.13 and 3.52 points respectively. The carriers of the genotype AA featured a larger area of the rib eye. Their superiority over the AG and GG genotypes was 1.36 cm^2 and 1.74 cm^2 (Table 4, Figure 2).

Table 4. Microstructural analysis of *m. longissimus dorsi* in rams of Altai Mountains breed of Prikatun type of different genotypes by the gene *GDF9*

Genotype	Indices				
	Number of muscle fibers, pcs. per mm^2	Diameter of muscle fiber, µm	general "marbling score", points	Content of connective tissue, %	Meatiness (muscle eye area), cm^2
AA	441.12 ± 8.31	28.67 ± 1.55	33.27 ± 2.82	8.45 ± 0.31	17.84 ± 2,31
AG	448.39 ± 9.67	27.23 ± 2.03	30.14 ± 0.76	9.26 ± 0.44	16.48 ± 1.79
GG	452.44 ± 10.01	26.15 ± 1.88	29.75 ± 0.14	9.41 ± 0.37	16.10 ± 2.23

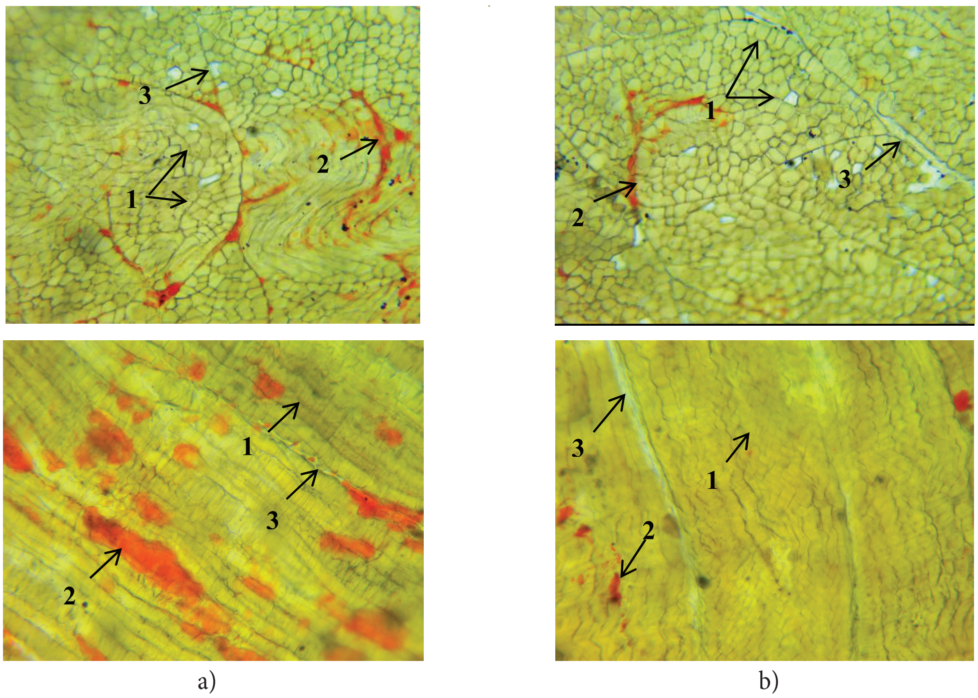

Figure 2. Histological slices of *m. longissimus dorsi* of the rams of Altai Mountains breed of Prikatun type of different genotypes for the gene *GDF9* a) AA-genotype b) GG-genotype (top — transverse slices; bottom — longitudinal slices) 1 — muscle fibers; 2 — fat tissue; 3 — connective tissue (color: hematoxylin Carracci and sudan III, magnified 10 × 40)

Conclusion

In result of DNA typing, the polymorphism of the gene *GDF9* and its relation with the indices of meat rate productivity in young sheep of Altai Mountains breed of Prikatun type were proven.

The most widespread in the breed is G allele and the GG genotype respectively, but the results of our own research and the data of other authors defined that A allele is desirable for breeding to increase the reproductive qualities and productivity of sheep.

The study of the influence of different genotypes in the gene GDF9 on the indices of sheep meat rate productivity also allowed to confirming the positive effect of A allele.

It was revealed that the rams-carriers of AA genotype were superior to their peers of AG and GG genotypes in terms of pre-slaughter weight, carcass weight and yield, meat content ratio respectively by 1.62 and 7.01 kg; 1.34 and 3.98 kg ($P < 0.05$); 1.21 and 1.86 abs. percent, 0.16 and 0.39 units ($P < 0.05$).

The muscle tissue of rams of the desired genotype featured the highest content of protein, fat and, accordingly, energy value in comparison with the sheep of AG and GG genotypes. Animals of the AA genotype had the largest area of the rib eye and the diameter of the muscle fibers. The superiority over other genotypes achieved 1.55 cm^2 and 1.98 microns in average. In addition a bigger number of inter-fiber and inter-bundle inclusions of fat and lower content of connective tissue stipulated the higher marbling score — by 3.32 points.

The obtained data prove the feasibility of studying the influence of the gene *GDF9* on the meat rate productivity of sheep of other breeds, in particular, A alleles and AA and AG genotypes. This is also promising from the point of view that a range of researches have demonstrated that the AG genotype has a positive effect on fertile multiplicity of ewes [20, 21, 22]. All thing being equal, getting more lambs provides increase of mutton rate production, which largely determines the economic efficiency of meat sheep farming.

The results of this research allow to conclude that the genotyping of sheep of Altai Mountains of Prikatun type by the gene *GDF9*, the purposeful selection of parents in order to obtain more desirable genotypes, will not only increase the meat rate productivity of a herd as a whole, but also will contribute to the production of mutton of high quality parameters.

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OBTAINING AND ESTIMATING THE POTENTIAL OF PROTEIN NUTRACEUTICALS FROM HIGHLY MINERALIZED COLLAGEN-CONTAINING BEEF RAW MATERIALS

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Abstract
Highly mineralized collagen-containing beef raw materials (tibia, fibula and costal bones) are a source of valuable protein nutraceuticals. They include high molecular weight proteins, oligopeptides and amino acids, which anabolic and physiological potential is used insufficiently. Protein nutraceuticals were obtained by high-temperature hydrolysis of beef raw materials in combination with enzymolysis by proteolytic enzyme preparations Alcalase 2,5 L, Protamex, Protosubtilin G3x. The water-soluble fraction of hydrolysates was studied after its separation and freeze-drying on the content of nitrogenous compounds, fats, minerals, formol-titrated nitrogen, fractional molecular composition. The mathematical dependencies of accumulation of low molecular weight products of protein hydrolysis on enzymolysis duration and doses of different enzyme preparations were obtained. The rational technological scheme of complex processing of beef raw materials with production of protein, fat and mineral-protein additives was proposed. The protein weight fraction in the freeze-dried protein hydrolysates was 69.5–89.6%. All studied protein additives contained peptides with a molecular weight of not more than 100 kDa. The content of low-molecular weight oligopeptides with a molecular weight of less than 10 kDa in the protein additives obtained by enzymatic thermal hydrolysis was more than 90%. The amino acid composition of protein additives produced by different hydrolytic methods was analyzed. Sensory and functional-technological properties of freeze-dried protein compositions were studied. The amino acid potential, high assimilability and physiological activity of protein nutraceuticals from collagen-containing beef raw materials were established. It is recommended to use them in the composition of specialized biologically active additives (BAAs) to food of the osteotropic direction in recipes of specialized and personalized products as a source of amino acids and active peptides.

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Introduction

The Russian meat industry produces about one million tons of secondary meat and bone raw materials annually. About 20% of this amount is industrially processed, while bone waste is practically not used for production of value-added products[1]. Today, more than 150 enterprises have been working in the Kaliningrad region in the sphere of beef husbandry accumulating from 20 to 50% of secondary material weight (bones, skin, viscera), which use is also problematic. The most acute problem is the processing of cattle bone raw materials characterized by an increased content of highly mineralized collagen tissues (tibia, fibula and costal bones). This raw material is often utilized by different methods including incineration causing ecological damage to nature[2].

An increased use of the potential of collagen-containing waste from meat raw material processing is a topical task in many slaughterhouses, poultry factories and enterprises of processing and food industries of many countries in the world [1,2]. The experience in the processing of collagen-containing waste from poultry factories shows that feeding meal and different additives, nitrogen-containing media for microbial biotechnology, organic fertilizers, polymer compositions for textile production, collagen dispersions used in feed and food industries are produced today from poultry feather raw materials [3,4,5,6,7]. A rational way for solving the problem of using fish bone raw materials including those that are unacceptable for feeding purposes (scales, smoked fish heads) is producing food additives of the protein, fat and protein-mineral composition by deep hydrolysis [8,9]. Today, highly mineralized meat and bone raw materials (waste from cattle processing), which differ from poultry and fish waste by the increased strength and specific amino acid composition of collagen proteins, are mainly used for animal feed [10].

It is important that all types of collagen-containing animal raw materials are a valuable source of natural proteins, amino acids, lipids, macro- and microelements, nucleic materials and other natural biologically active substances (BASs). Isolation of these BAAs and the fullest use of their potential for humans are an aim of many scientific stud-

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ies. At present, many scientific developments suggest using the products of secondary raw material processing in the food technology as food additives and BAAs to food, in the composition of multicomponent premixes, protein concentrates [1,2,3,4,5,6,7,8,9,10]. Protein compositions obtained by deep hydrolysis of bone and meat raw materials are constituents of pharmacological compositions, microbiological media, constructive tensides [11,12]. It is promising to use a potential of collagen-containing resources in aquaculture as sources of amino acid and fatty nutrition [10]. It is important that nowadays domestic feedstuff for industrial fish production is critically deficient and this is the main problem in the development of domestic fish farming [13]. Today, many farming enterprises engaged in poultry and pig breeding suffer from a deficiency of the highly assimilable natural protein feedstuff. The task of using the potential of collagen-containing raw materials in the food industry is not less important. With guaranteed food safety and proved biological effectiveness of recovered biologically active substances, it seems expedient and rational to use them in a composition of new generation products — specialized and functional products intended for strengthening the locomotor system of the body, in sports nutrition, gerodietetic products, curative nutrition for patients suffering from collagenosis and so on [14,15,16].

Recovery of valuable organic components from secondary meat and bone raw materials is based on their destruction, which is performed by different methods. The main methods for degradation of highly mineralized raw materials are based on tough hydrolysis, which is carried out using chemical reagents (acids, alkali) as well as by enzymatic, thermal or combined ways [2,17,18,19,20,21,22,23]. However, acids and alkali destroy several valuable amino acids, reduce the biological value of proteins and peptides, hydrolyse lipids. With that, the process is accompanied by formation of salts and chemical pollution of effluents, excludes the use of other fractions of organic raw materials (lipids, minerals) for food purposes [12,18,22].

It is also necessary to note that the investigated enzymatic methods for protein production from meat and bone raw materials are not effective enough for industrial use of end products of hydrolysis in food systems [17,18,19]. It was established that enzymatic hydrolysates of animal raw material waste often contain emulsion-type protein-fat mixtures, which are often suitable only as a biodiesel fuel, as well as in the composition of feedstuff for animals, fertilizers for soil, as a carbohydrate and nitrogen source for microbial synthesis. The latter is promising for the development of industrial microbial biotechnology, since a complex process that includes enzymatic biodegradation of waste and production of enzymes at a single stage is possible in this case [1,2,10,13]. It should be noted that today even the most active proteolytic enzymes with the collagenase activity hydrolyze strong framework structures of meat and bone raw materials only by 30–40%; then, the process attenuates. With that, in its entirety, the process is characterized by long duration, a necessity to inhibit enzymes and often is accompanied by formation of non-protein components that cause unpleasant odor and increased microbial contamination, which excludes using end products of hydrolysis for food purposes [21]. Another problem of enzymolysis is a necessity to take into account specificity, activity and also costs of enzymes, which price is steadily rising in the modern market [13].

Despite available scientific technological developments, the question of the full and effective use of the biological potential of meat and bone raw materials has no economically efficient solution up to now. Proposed processing methods allow recovering the most valuable protein fraction from different collagen raw materials at a level of 50–60% of the weight of contained protein; with that, the complex and non-waste processing is not achieved [13,21,23].

To solve the problem of the complex and rational use of secondary meat and bone raw materials and to increase sustainability of meat processing productions, it is expedient to use modern biotechnologies that allow processing raw materials using the principles of their deep molecular conversion based on combination of enzymatic and thermal destruction. This principle of raw material processing positively showed itself in comparative investigations of different methods for destruction of mineralized collagen-containing fish scales [8]. The method is based on the preliminary action of specific proteolytic enzymes of new generation on raw materials with the following high temperature processing. This allows deep destruction of highly mineralized protein components (collagen, elastin, reticulin), hydrolysis of connective tissues liberating all organic fractions including fatty and mineral [9]. With that, valuable amino acids of protein fractions are preserved, including essential (lysine, valine, tryptophan, cystine, cysteine and others), as well as amino acids of the osteotropic action (proline, oxyproline, glycine, alanine); high molecular weight complexes are destroyed with formation of protein-mineral compositions (calcium, phosphorous, magnesium, potassium). The following recovery and purification of each fraction enable their use as natural food and feed additives. Finally, it is possible to obtain three compositions of natural biologically active components (protein, fatty and mineral-protein) with the high physiological potential from problematic meat and bone raw materials.

The most important fraction produced by combined hydrolysis of meat and bone raw materials is the protein fraction, which according to its potential can be assigned to natural functional nutriceuticals having the biological activity and required by the body, first of all, for the anabolic and energy purposes. Deep enzymatic thermal hydrolysis enables obtaining low molecular weight peptides (1–100 kDa) from collagen-containing animal tissues. The most valuable is the fraction with a molecular weight of 1–10 kDa, which is assigned to the so-called active peptides and comprises di-, tri-, and tetra-peptides with novel physiological properties [23]. It was established that low molecular weight peptides of animal origin are effective antioxidants, antiseptics, have the immunomodulatory, hypotensive, regenerative, reproductive activities, exert the cytotoxic effect on several cancer cell lines, inhibit angiotensin-converting enzymes [11,12,25,26,27].

The valuable protein potential of collagen tissues from meat and bone raw materials and their use in food technologies were confirmed by results of multiple studies [1,2,10,11,13,14,17,18,19,20,22,27]. Low molecular weight products of collagen protein destruction are a promising bio-material for food, pharmaceutical, medical, microbiological, feed industries as they have increased assimilability and can influence effectiveness of many biological processes. It was proved that di- and tri-peptides with the amino acid sequence -Pro-Hyp- and -Pro-Hyp-Gly- have notable stimulating ability of collagen synthesis, which is important for wound healing processes [11,12,25,26,27]. It is important that oral and enteric consumption of collagen peptides favorably affect joints, rate of wound and ulcer healing [1,26]. When consuming peptides of collagen origin, patients with joint degenerative diseases showed the growth in the mineral density of bone tissues [27]. It was established that food with "collagen pieces" improves the structure and composition of hair and fingernails, facilitates an increase in the size of collagen fibrils in the Achilles tendons, induces the strength of fibroblasts and collagen fibrils in derma of human skin [1].

To recover and use proteins from highly mineralized collagen-containing beef raw material, it is necessary to take into consideration peculiarities of its composition, namely, high structural strength conditioned by the high content of calcium and the procollagen form of proteins. For tissue degradation, therefore, it is topical to use high temperature hydrolysis in the aqueous medium carried out at elevated pressure and with the preliminary action of specific proteolytic enzymes (endo- and exopeptidases). This method of hydrolysis proved its effectiveness in processing highly mineralized fish heads, scales and backbones that are accumulated in fish canning enterprises as waste [8,9].

The aim of the work was production of protein components from highly mineralized beef meat and bone raw materials by the extraction hydrolytic method with enzymatic thermal hydrolysis and assessment of their potential for using as food and biologically active additives — protein nutriceuticals.

The main tasks of the study were to substantiate rational regimes for deep hydrolysis of beef tibia, fibula and costal bones for obtaining protein products and determine their targeted quality indicators that suggest their biological and physiological value, functionality and processability.

Objects and methods

The experiments were carried out in the Center for Advanced Technologies of Protein Use of the Department of Food Biotechnology of the Kaliningrad State Technical University.

Beef tibia and fibula with heads provided by the Golubevsky meat processing plant "LEAR" and beef costal bones from the slaughterhouse in the Dobroye settlement (Kaliningrad region) were used as raw materials.

Proteolytic enzyme preparations with the collagenase activity Alcalase 2,5 L and Protamex (Novozymes, Denmark), Protosubtilin G3x and pancreatin (Sibbiopharm Ltd., Russia) were used for enzymolysis.

The generally accepted, traditional, standard and authors' methods were used in the experiments. The protein content was determined by GOST 25011–2017[3], fat by GOST 23042–2015[4], ash by GOST 31727–2012 (ISO 936:1998)[5]. The depth of hydrolysis was assessed by accumulation of formol-titrated nitrogen (FTN) or amino-ammonia nitrogen (AAN) according to GOST R55479–2013[6]. The amino acid content was determined by GOST 32195–2013 (ISO 13903:2005)[7]. The fractional molecular composition of peptides was determined by the methods of high performance liquid chromatography (HPLC) using an exclusion chromatographic column Yarra from Phenomenex (Yarra 3u SEC-2000).

When grinding, hard meat and bone raw materials were restructured step-by-step. Initially, a press was used, which crushed bones into 20–50 mm size pieces. Then, this mass was transformed into ground mixtures consisted of 3–5 mm size particles in special containers using the "rotating knife" method.

In the experiments on hydrolysis, 100 g of ground raw materials were initially placed into hermetical glass jars with lids, mixed in warmed water in a ratio of 1:1 by weight at a temperature of 50–55 °C. Then, the samples were introduced into a shaking apparatus for enzymolysis during a preset time period. After the end of enzymolysis, recovered fat was removed adding hot water into the ground mass. After holding for 30 min., fat was separated by centrifugation. Thermal hydrolysis of the fermented mass was carried out in a thermal reactor with a rotating mixer at a temperature of 130–160 °C within the mass for one hour; then, the content was cooled and fractionated by centrifugation. After centrifugation, three fractions were obtained — fatty (upper), water soluble (medium) and mineral-protein (sedimentary, lower). The complex scheme of processing highly mineralized collagen-containing beef raw materials with production of protein nutraceuticals and accompanying fatty and protein-mineral additives is presented in Figure 1.

The water soluble protein fraction, which was freeze-dried with preliminary thickening by the vacuum evaporation method, was analyzed.

Results and discussion

Initially, the most effective enzyme preparation and rational regimes of its use for enzymolysis of meat and bone raw materials were selected. This would allow maximum transformation of highly mineralized high molecular

[3] GOST 25011-2017 "Meat and meat products. Protein determination methods". Moscow: Standartinform, 2018. — 14 p. (In Russian)

[4] GOST 23042-2015 "Meat and meat products. Methods of fat determination". Moscow: Standartinform, 2019. — 8 p. (In Russian)

[5] GOST 31727-2012 (ISO 936:1998) "Meat and meat products. Determination of total ash". Moscow: Standartinform, 2019. — 11 p. (In Russian)

[6] GOST R 55479-2013 "Meat and meat products. Method for determination of amino-ammonia nitrogen". Moscow: Standartinform, 2019. — 8 p. (In Russian)

[7] GOST 32195-2013(ISO 13903:2005) "Feeds, compound feeds. Method for determination of amino acids". Moscow: Standartinform, 2020. — 23 p. (In Russian)

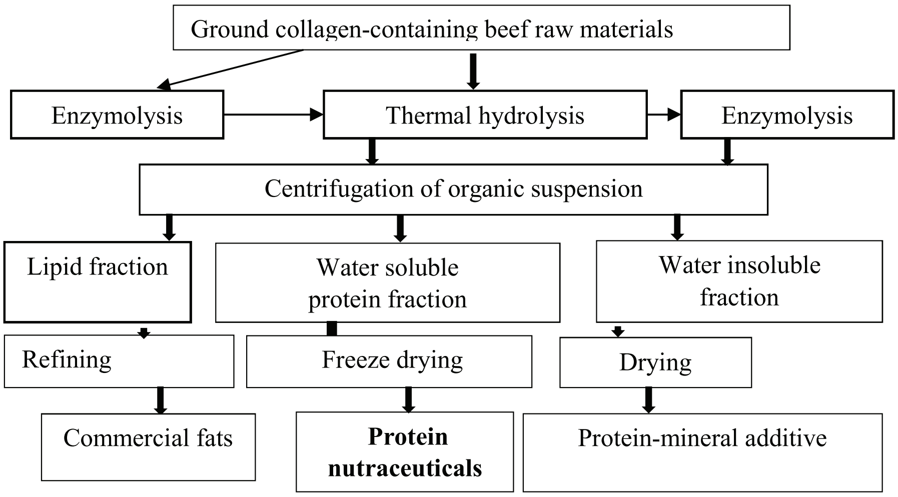

Figure 1. The scheme of collagen-containing raw material processing

weight proteins from beef bones into the dissolved state [12,13,14,17,19,20,21,22]. The results of the experiments carried out using various enzyme preparations with varied doses are presented in Table 1.

The obtained data (Table 1) indicate that enzymolysis performed after thermolysis allows a 2–2.5-fold increase in the hydrolysis depth as demonstrated by the growth in FTN. With that, the following regimes can be considered rational: EP doses of 0.75% and 1% for Alcalase 2.5 and Protosubtilin G3x with process duration of 4 hours.

The experimental data obtained earlier [13] were used for objective optimization of enzymatic processing of meat and bone raw materials. The data were processed by methods of mathematical statistics to establish significant dependencies between the quantity of generated products of protein enzymatic degradation, doses of enzyme preparations, temperature and enzymolysis duration.

As an optimization parameter, FTN was used, which value demonstrates the concentration of amino acids, di-tri- and other oligopetides with the free amino group in a hydrolysate. Creation of enzymolysis models allows regulating the process depth depending on a raw material type, enzyme preparation and process duration.

To this end, initially, the experimental values of FTN obtained in the beef samples at different EP doses and duration were analyzed (Figure 2, [13]). It is seen from the data that this dependence can be described by exponential function (1):

$$FTN(t, EP) = FTP_0 + (FTN_\infty(EP) - FTN_0) \times \times (1 - \exp(-\psi(EP) \cdot t)), \qquad (1)$$

where $FTN_\infty(EP)$ and $\psi(EP)$ are empirical functions that are to be determined using the experimental data.

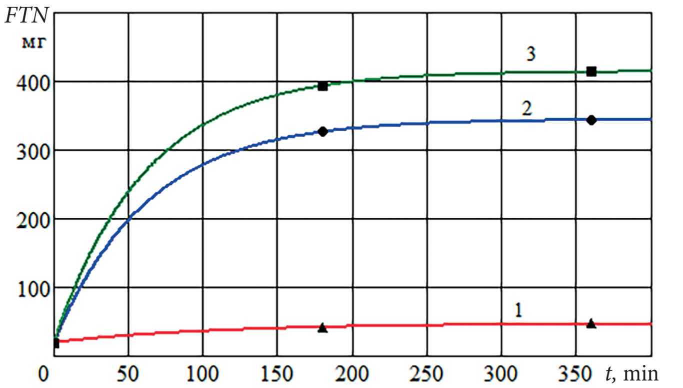

Figure 2. Changes in FTN depending on duration of beef rib enzymolysis at different doses of EP Alcalase 2,5 L: 1–0%, 2–1%, 3–2% (points are experimental data, lines are points of calculation by equation (1)

Table 1. Results of the assessment of the hydrolysis depth of beef rib proteins using various enzymatic preparations (EP) in hydrolysates obtained by thermal hydrolysis at 130 °C over 1 hour

Hydrolysate samples at 130 °C	Dose of EP, % of hysrolysate mass	Enzymolysis duration, hours	FTN, mg/100 g	Samples	Dose of EP, % of hysrolysate mass	Enzymolysis duration, hours	FTN, mg/100 g
Control (without EP)	0	0	212.8	Control (without EP)	0	0	193.2
With EP Alcalase 2.5 L	0.25	0	238.7	with EP Protosubtilin G3x	0.5	0	224.0
		2	322.0			2	299.6
		4	351.4			4	305.2
		6	371.0			6	316.4
	0.5	0	357.4		0.75	0	243.6
		2	337.4			2	347.2
		4	403.6			4	408.8
		6	410.2			6	411.6
	0.75	0	290.3		1.0	0	268.8
		2	351.4			2	414.4
		4	406.0			4	495.6
		6	417.2			6	498.4

The value of the coefficient ψ was found from equation (1):

$$\psi_i = -\frac{1}{180} \cdot \ln(1 - \left(\frac{FTN_{i180} - FTN_0}{FTN_{i360} + FTN_0}\right) \quad (2)$$

The values of coefficient ψ found by the equation (2) were also changed according to the exponential dependence and can be presented by empirical functions (3) and (4), which can illustrate the enzymolysis process using EP Alcalase 2,5 L by means of equation (1):

$$FTN_{\infty}(EP) = 47.4 + 367 \cdot (1 - \exp(-1.657 \cdot EP)), \quad (3)$$

$$\psi(EP) = 0.0910 + 0.0720 \cdot (1 - \exp(-3.214 \cdot EP)). \quad (4)$$

The use of the regression model (1) in respect to the experimental data on enzymolysis with EP Protosubtilin G3x is reflected in Figure 3.

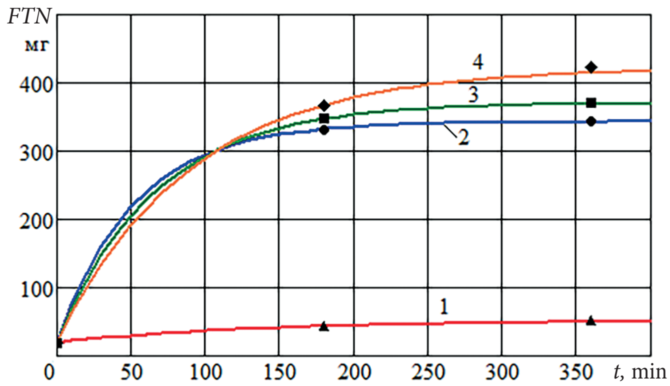

Figure 3. Dependence of FTN in beef rib enzymolysis on process duration at different doses of EP Protosubtilin G3x: 1–0%, 2–1%, 3–2%, 4–3%. Points are experimental data, lines are points of calculation by (1), (3), (4)

Obtained dependencies that link the quantity of enzymolysis products of collagen-containing beef raw materials with regulated process factors allow optimizing its duration with the use of the given EP, above which it is inexpedient to carry out the process as the indicators of protein destruction depth (FTN) change insignificantly. It was established with their use that all enzyme preparations were close in terms of effectiveness for beef rib enzymolysis. To increase quality of protein degradation, it is recommended to use EP compositions (endo- and exopeptidases) combining EP Alcalase 2,5 L, Protamex and Protosubtilin G3x. With that, rational duration is 240–250 min., total dose of enzyme preparations should be close to 3% of raw material weight.

As a result of experiments on the established recommended values of enzymolysis of preliminarily defatted meat and bone raw materials, the samples of freeze-dried protein hydrolysates were obtained, which overall chemical composition is presented in Table 2.

Analysis of the obtained data (Table 2) shows that in the combined method of hydrolysis (enzymolysis + thermolysis) from beef meat and bone raw materials, it is possible to obtain protein compositions in a dry form with the protein content from 69.45% to 89.57%, fat from 0.78% to 9.2% and minerals from 3.62% to 15.8%. The highest protein content (about 90%) was in the samples obtained in combined hydrolysis of beef ribs with preliminary processing with EP Alcalase 1% over 1 hour with the following enzymolysis with EP Protosubtilin 1% over 2 hours and thermal treatment at 140 °C.

The obtained data allow proposing a processing technology for highly mineralized collagen containing meat raw materials based on regulated hydrolysis, which enables production of water soluble powders containing over 70% of highly assimilable proteins — protein nutraceuticals potentially suitable for food purposes. The principle technological scheme of processing collagen-containing meat raw materials and balances of the main chemical substances at the main operations upon production of protein food additives (the main product), fatty and mineral-protein compositions (additional products) are presented in Figure 4.

The technological scheme (Figure 4) presents the chemical composition and calculated values of a recovery degree for the main raw material components (dry matter, protein, fat and minerals) at the key operations of the hydrolytic technology (moist semi-finished products, dry finished products). The technological scheme illustrates the chemical transformation of raw materials into food additives by the example of the enzymatic-thermal hydrolysis of the beef bone mixture (tibia, fibula and costal bones). It is seen that when processing using these scheme, 85.2 kg of the freeze-dried protein powder can be obtained from 1000 kg of raw materials without waste. This protein powder contains 83.3% of protein (yield of proteins from raw

Table 2. Chemical composition of freeze-dried protein hydrolysates obtained using different EP by various regimes of hydrolysis of collagen-containing beef raw materials

No. Characteristics of raw materials and hydrolysis conditions	Protein hydrolysate, indicators, % wt				
	Water	Dry substances	Fat	Minerals	Protein
1. Beef tibia and fibula: enzymolysis, EP Alcalase 1% (2 hours) + EP Protosubtilin 1% (2 hours)	7.55	92.45	3.24	13.48	75.73
2. Beef tibia and fibula: enzymolysis, EP Alcalase 0.5% (2 hours) + EP Protosubtilin 0.5% (2 hours) + thermolysis 140 °C (1 hour)	5.48	94.52	9.24	15.83	69.45
3.Beef rib: enzymolysis, EP Alcalase 0.5% (2 hours) + EP Protosubtilin 0.5% (2 hours) + thermolysis 140 °C (1 hour)	5.43	94.57	4.02	4.41	86.14
4. Beef rib: enzymolysis EP Alcalase 1% (2 hours) + EP Protosubtilin 1% (2 hours) + thermolysis 140 °C (1 hour)	6.03	93.97	0.78	3.62	89.57

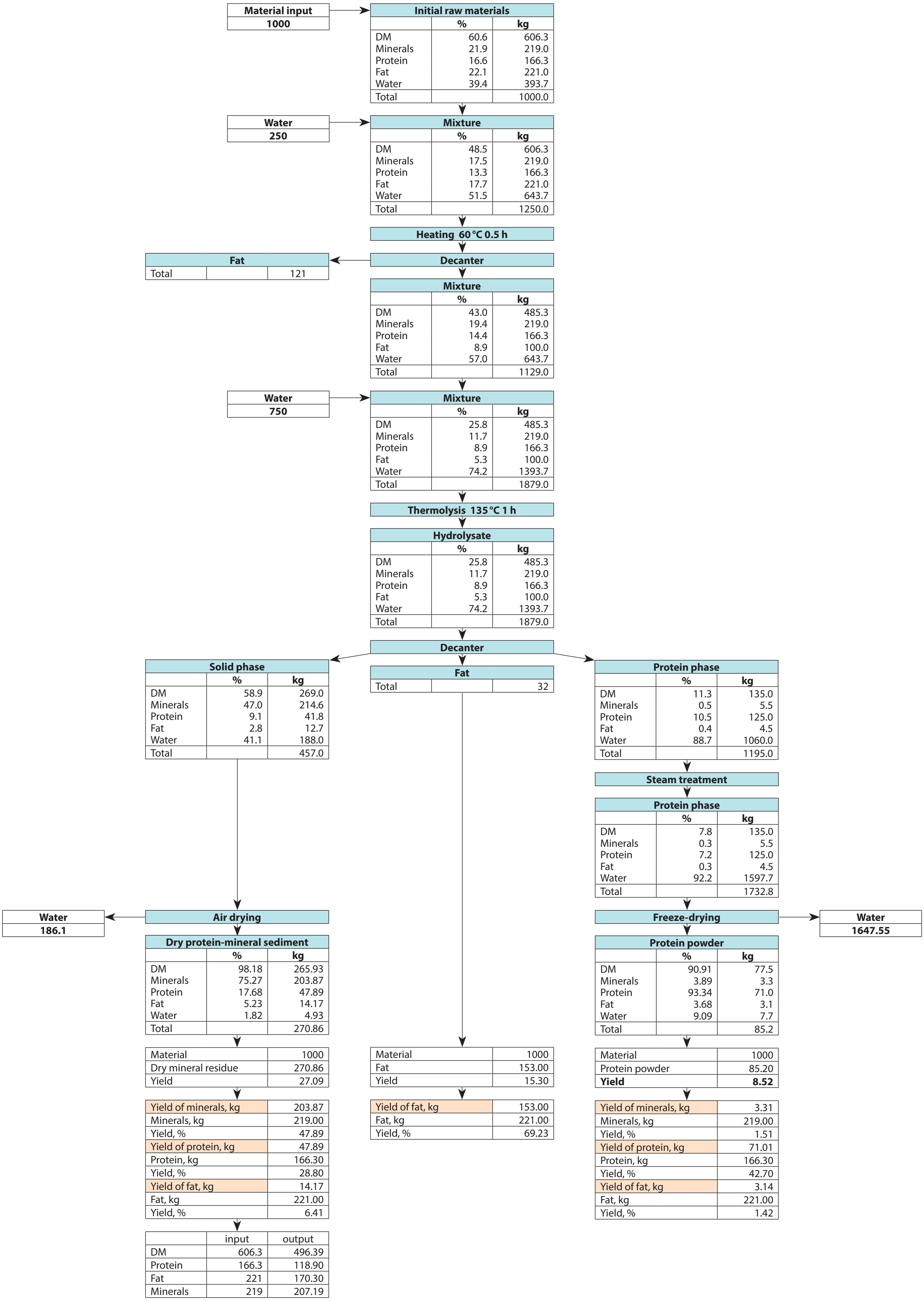

Figure 4. Technological scheme and mass post-operational balances of the main chemical substances in enzymatic thermal hydrolysis of the beef bone mixture (tibia, fibula and costal bones)

materials 71%), 221 kg of fat (yield of fat from raw materials 69.2%) and 270 kg of the dry mineral-protein additive containing 75.3% of minerals, 17.7% of proteins, 5.2% of fat. Therefore, the rationality and high workability of processing highly mineralized collagen-containing beef raw materials according to the proposed hydrolytic technology was demonstrated.

To assess the nutritional value of the protein fraction of the obtained water soluble beef hydrolysates, its quality was assessed by determining the amino acid composition, molecular weight and fractional composition of peptides, as well as their functional-technological properties by standard indicators.

The results of the amino acid composition of the protein formulations obtained by different hydrolytic methods and illustrating their anabolic potential are presented in Table 3.

As shown in Table 3, the amino acid composition in all samples was close irrespective of their production method, and corresponds to collagen meat raw materials by its amino acid composition [1,2,4,7,9,10,14,16]. Glycine accounts for the main quantity of amino acids in the hydrolysates (23.1–23.5 g/100 g protein), which indicates a possibility to use them in the composition of biologically active products of the neuroprotective direction as glycine is a functional neurotransmitter and is recommended for the use in the composition of neuroprotective BAAs [1,10,24]. The obtained hydrolysates are of conditionally full-value by the amino acid composition as they contain all essential amino acids (without regard for tryptophan); although, in significantly lower amounts compared to ideal protein. The crucial essential amino acid in hydrolysates is lysine (3.55–4.20 g/100 g protein), which has many important functions in the body (anabolic material for muscles, immunomodulator, facilitates calcium assimilation, has a sedative effect). The lysine content in these hydrolysates is also important because its content in plant proteins (from cereals, legumes) is, as a rule, lowered. This means that it is rational to use the obtained hydrolysates in the composition of combined products with plant components. In regard to the absolute content, high amounts of non-essential amino acids are present in the hydrolysates of beef meat and bone raw materials. After glycine, proline stands out among quantitatively prevalent amino acids (11.6–13.1 g/100 g protein) being a peculiar kind of the collagen tissue indicator. Also, increased amounts of alanine (9.6–10.2 g/100 g protein), glutamic and aspartic acids, arginine (10.6–11. 6 g/ 100 g protein and 5.8–6.3 g/ 100 g protein; 7.6–7.9 g/100 g protein) were established in the hydrolysates. These amino acids also have important physiological effects in the body [1, 24,26]; with that, it is important that they are in the assimilable form.

The indicators of the amino acid balance of protein hydrolysates obtained by different methods that were calculated by conventional equations [9] are presented in Table 4.

Table 3. Comparative amino acid composition of protein hydrolysates of beef ribs obtained by different regimes

Amino acids (AA)	Amino acid content in protein hydrolysates of beef ribs, g / 100 g of protein		
	Thermolysis: 140 °C, 1 hour	Enzymolysis: EP Alcalase 0.5% (2 hours) + EP Protosubtilin 0.5% (2 hours) + thermolysis 140 °C, 1 hour	Enzymolysis: EP Alcalase 1% (2 hours) + thermolysis 135 °C, 1 hour
Aspartic acid (Asp) + asparagine (Asn)	6.1 ± 0.80	6.3 ± 0.8	5.8 ± 0.8
Glutamic acid (Glu) + glutamine (Gln)	10.9 ± 1.20	11.6 ± 1.3	10.6 ± 1.2
Histidine (His)	1.09 ± 0.30	1.18 ± 0.32	1.20 ± 0.33
Serine (Ser)	3.16 ± 0.28	3.39 ± 0.31	3.11 ± 0.28
Arginine (Arg)	7.9 ± 0.80	7.7 ± 0.70	7.6 ± 0.70
Glycine (Gly)	23.1 ± 2.30	23.1 ± 2.30	21.5 ± 2.20
Threonine (Thr)	1.88 ± 0.15	2.19 ± 0.18	2.05 ± 0.17
Alanine (Ala)	9.6 ± 1.00	10.2 ± 1.10	9.9 ± 1.10
Tyrosine (Tyr)	0.8 ± 0.23	1.01 ± 0.29	0.97 ± 0.28
Valine (Val)	2.66 ± 0.35	3.30 ± 0.40	3.05 ± 0.40
Methionine (Met)	0.70 ± 0.08	0.75 ± 0.09	0.84 ± 0.10
Proline (Pro)	13.1 ± 2.00	11.6 ± 1.70	12.2 ± 1.80
Isoleucine (Ile)	1.59 ± 0.13	1.79 ± 0.14	1.70 ± 0.13
Phenylalanine (Phe)	2.16 ± 0.29	2.52 ± 0.34	2.45 ± 0.33
Leucine (Leu)	3.44 ± 0.32	4.27 ± 0.39	4.12 ± 0.38
Lysine (Lys)	3.55 ± 0.34	4.20 ± 0.40	3.95 ± 0.38
Cystine (Cys)	< 0.035	0.091 ± 0.019	0.082 ± 0.017
Tryptophan (Trp)	was destroyed upon sample preparation		

Table 4. Comparative assessment of biological value indicators in protein hydrolysates from beef ribs obtained by different methods

Essential amino acids (AA)	AA content in protein FAO WHO, g / 100 g	Hydrolysates from beef ribs obtained by different methods					
		Thermolysis: 140 °C, 1 hour (4/73)		Enzymolysis: EP Alcalase 0.5% (2 hours) + EP Protosubtilin 0.5% (2 hours) + thermolysis 140 °C, 1 hour		Enzymolysis: EP Alcalase 1% (2 hours) + thermolysis 135 °C, 1 hour	
		AA content, g / 100 g protein	AA score, %	AA content, g / 100 g protein	AA score, %	AA content, g / 100 g protein	AA score, %
Ile + Leu	9.10	4.99	54.83	6.06	66.59	5.82	63.96
Lys	4.80	3.55	73.96	4.20	87.5	3.95	82.29
Val	4.00	2.66	66.50	3.30	82.5	3.05	76.25
Met + Cys	2.30	0.73	31.96	0.84	36.52	0.92	40.00
Thr	2.50	1.88	75.20	2.19	87.6	2.05	82.00
Phe + Tyr	4.10	2.96	72.19	3.53	86.10	3.42	83.41
Trp	0.60	—	—	—	—	—	—
Total:	27.40	16.77		20.12		19.21	
Coefficient of amino-acid score difference (CAASD), %		26.12		32.52		26.84	
Biological value (BV), %		73.88		67.47		73.16	
Coefficient of utility, U, unit fractions		0.51		0.49		0.56	

It is seen from Table 4 that despite a low content of essential amino acids in the hydrolysates, they are quite balanced relative to ideal protein, which is indicated by the values of the biological value (BV) in a range of 67.47% to 73.88%. With that, the best calculated amino acid balance was noticed in hydrolysates obtained with thermolysis (73.88%). On the other hand, the best value in terms of the utility (U) indicator was in the hydrolysates obtained by the combined method using EP Alcalase with preliminary enzymolysis with the following thermal hydrolysis.

The obtained data demonstrate the prospects of using obtained protein hydrolysates of meat and bone raw materials as food additives both in the composition of multicomponent food products and as biologically active additives. In the latter case, the nutritional effect will be conditioned by the presence of the amino acids glycine, proline, alanine, aspartic acid and glutamic acid having the physiological action, including the osteotropic effect [1, 25].

The physiological functionality of the obtained protein hydrolysates was assessed by detection of the molecular weight and fractional composition of the contained low molecular weight peptides with the molecular weight of less than 100 kDa [10]. In these investigations, a special attention was paid at the quantitative and qualitative identification of the low molecular weight peptide fractions with the molecular weight less than 10 kDa that are assigned to active functional peptides with proved functional effects [11,12].

The solubility index of protein hydrolysates indicating their transition into the dissociated state and, thus, assimilability in the body was studied in the preparation operations. The summarized results of the fractional molecular composition of the freeze-dried hydrolysates by peptide groups with a molecular weight of up to 100 kDa are presented in Table 5, Figure 5 and Figure 6.

Table 5. Characteristics of solubility of freeze-dried protein hydrolysates and their differentiation by the fractional molecular composition of low molecular weight peptides

No., Characteristics of raw materials and hydrolysis conditions (see Table 2)	Soluble part of hydrolysate, %	Number of peptides with molecular weight of ≤10 kDa, %	Number of peptides with molecular weight of 10–50 kDa, %	Number of peptides with molecular weight of 50–100 kDa, %	Number of peptides with molecular weight of more than 100 kDa, %
1	90.4	94.18	4.99	0.83	0
2	95.4	93.78	5.27	0.95	0
3	99.7	47.38	36.95	9.19	6.48
4	100.0	43.61	38.02	10.60	7.77

It can be seen from Table 5 that solubility of the obtained protein products is high (more than 90%) and is determined by a degree of raw material grinding. In case of using tubular bones, when fine grinding was difficult, solubility of obtained protein hydrolysates was lower (90.4–95.4%). When grinding less strong costal bones, protein hydrolysates with increased solubility (99.7–100%) were obtained. It is necessary to note that the quantity of low molecular weight peptides with a molecular weight of less than 10 kDa in the soluble part of the hydrolysates was significantly higher than in the similar samples from costal bones (93.78–94.18%).

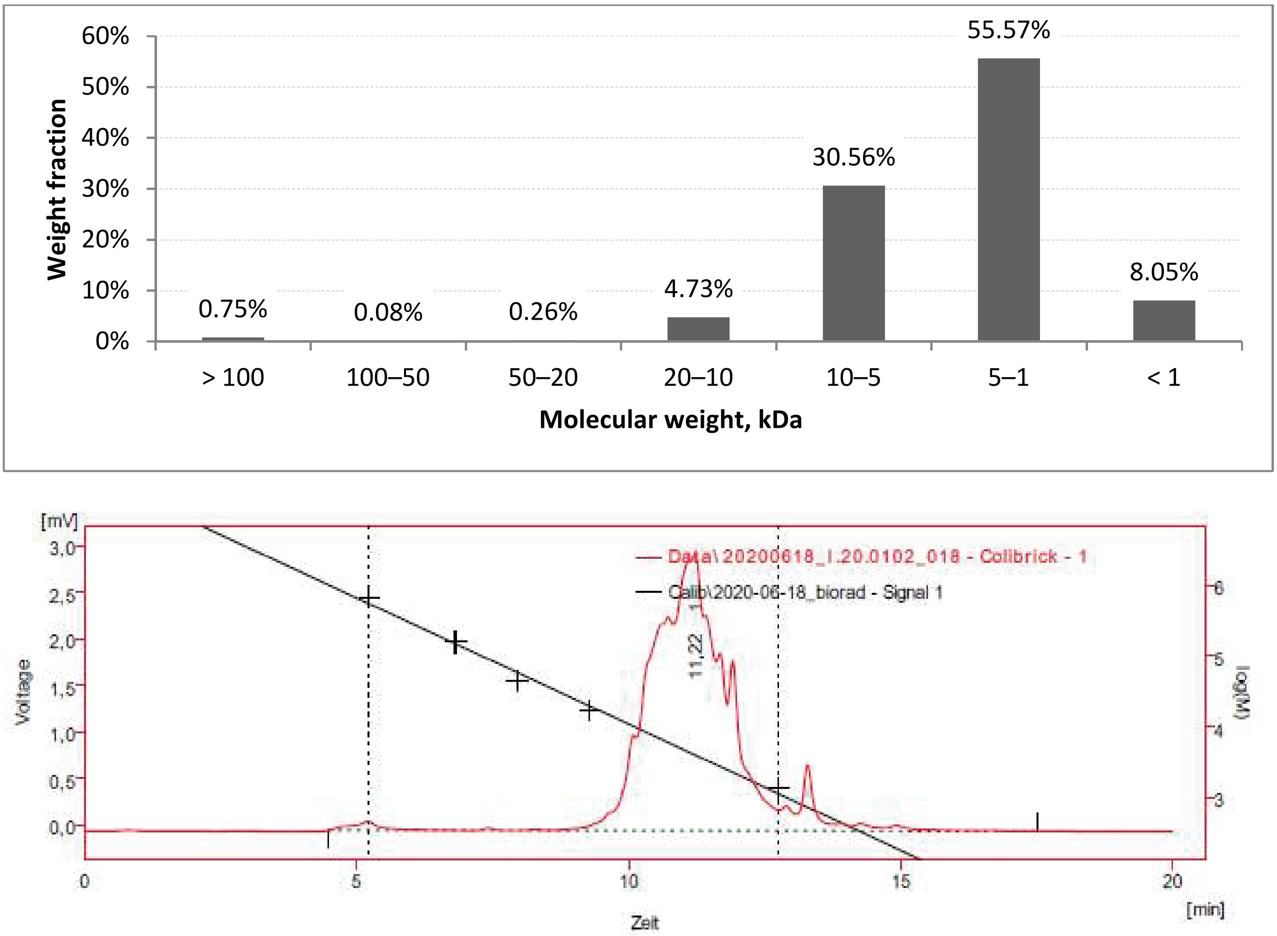

Figure 5. Fractional distribution of peptides by molecular weight in the freeze-dried protein hydrolysate produced by enzymolysis of beef ribs (Alcalase 1% (2 hours) + Protosubtilin 1% (2 hours)

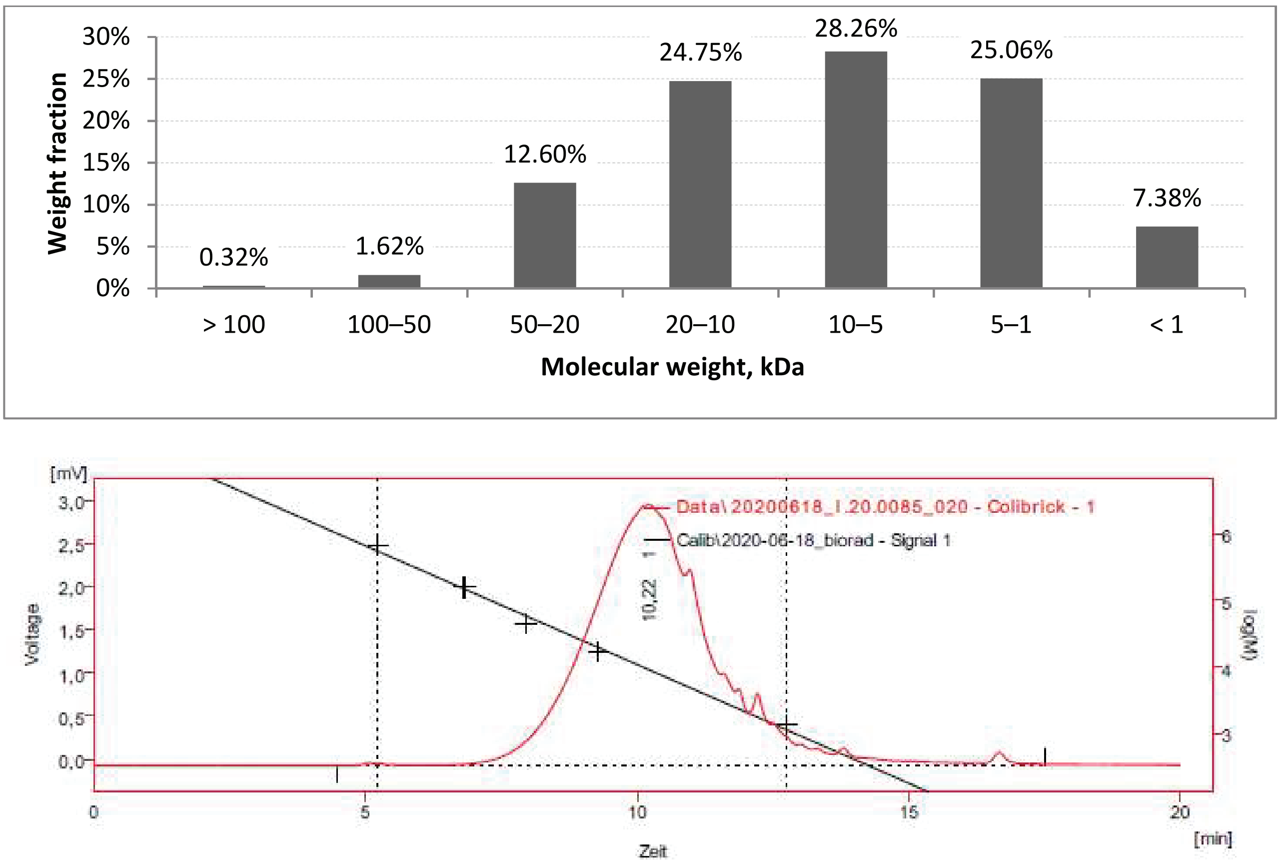

Figure 6. Fractional distribution of peptides by molecular weight in the freeze-dried protein hydrolysate produced by thermolysis (160 °C, 1 hour)

Table 6. Results of assessing the emulsifying capacity (EC) of freeze-dried protein hydrolysates produced from different collagen-containing beef raw materials at different processing regimes

Raw materials	Regime of hydrolysis	Volume of emulsion, ml	EC, %	Stability of emulsion, %
Beef tibia and fibula	Thermolysis: 140 °C, 1 hour	110	220	100
Beef tibia and fibula	Thermolysis: 150 °C, 1 hour	100	200	100
Beef tibia and fibula	Thermolysis: 160 °C, 1 hour	110	220	100
Beef tibia and fibula	enzymolysis, EP Alcalase 1% (2 hours) + EP Protosubtilin 1% (2 hours) + thermolysis: 140 °C, 1 hour	115	230	Emulsion was separated upon centrifugation and settling
Beef ribs	Enzymolysis, EP Alcalase 0.5% (2 hours) + EP Protosubtilin 0.5% (2 hours) + thermolysis 140 °C (1 hour)	106	212	Emulsion was stable upon settling, but was separated upon centrifugation
Beef ribs	Enzymolysis, EP Alcalase 0.1% (2 hours) + thermolysis 130 °C (1 hour) + enzymolysis EP Alcalase 1% (2 hours) + thermolysis 130 °C (1 hour)	115	230	Emulsion was separated upon centrifugation and settling
Beef ribs	Enzymolysis, EP pancreatin: 0.5%; 0.75%; 1% (2 hours)	95 in all three samples	190 in all three samples	Emulsion was separated upon centrifugation and settling in all three samples

Analysis of the obtained data on molecular fractional distribution of peptides in the water soluble parts of freeze-dried hydrolysates of meat and bone beef raw materials (Table 5, Figure 5 and Figure 6) allows making a conclusion that these protein products can be considered concentrates of low molecular weight active peptides. It is known that these peptides are highly functional in the body, and by a degree of assimilability, they can be assigned to protein nutraceuticals that exert positive physiological effects. It is important that the number of peptides with the molecular weight of less than 100 kDa was close to 100% in all samples. With that, the content of the especially important low molecular weight fraction with the molecular weight of less than 10 kDa (active peptides) was 43.61 to 94.18% depending on a hydrolysis method. Maximum quantity of active peptides was in protein products obtained by the enzymolysis method without thermal treatment (Figure 5). This hydrolysate is basically a mixture of pure amino acids, di- and tripetides that have the immunomodulating, antioxidant, anabolic and other physiological effects [1,2,24,27]. These peptide nutraceuticals can be recommended for using in the composition of food BAAs that increase the immune status of the body.

To substantiate recommendations on the use of freeze-dried protein nutraceuticals in the food systems, their functional technological properties were assessed by indicators that demonstrate their structure-forming abilities in the multicomponent systems. The emulsifying capacity (EC), water holding capacity (WHC), water binding capacity (WBC) and fat binding capacity (FBC) were measured in the test samples. The obtained results are presented in Table 6 and Table 7.

Results of the analysis of the data in Table 6 and Table 7 show that freeze-dried protein hydrolysates produced by thermolysis of tubular bones during one hour at temperatures of 140–160 °C, have the high emulsifying capacity (200–220% of the mass of the used oil). With that, the developed emulsion was very stable, was not separated upon centrifugation at 2700 rpm, and retained its stability (was not separated into layers) up to 4 days of holding at a temperature of about 20 °C.

Protein hydrolysates produced by the combined method (enzymolysis with different enzyme preparations + thermal hydrolysis) as well as enzymolysis with pancreatin did not have the high emulsion stability upon the high emulsifying capacity (190–230%). Emulsions obtained with their use were separated into the oil and water fractions in all cases upon centrifugation at 2700 rpm and settling.

It is obvious that enzymatic treatment by a complex of enzymes alcalase and protosubtilin in different combinations allows obtaining protein hydrolysates from both types of raw materials. These protein hydrolysates have the high quantity of low molecular weight peptides and free amino acids, and possess the emulsifying capacity due to an increased number of opened functional groups. With that, however, their decreased molecular weight does not allow formation of a stable structure in the emulsion and it is quickly separated into the fatty and aqueous fractions.

Table 7. Water holding capacity (WHC), water binding capacity (WBC) and fat holding capacity (FHC) of freeze-dried protein hydrolysates from beef tibia and fibula obtained at different regimes of thermolysis

Raw materials	Regime of hydrolysis	WHC, %	WBC, g water/g hydrolysate	FHC, g fat/g hydrolysate
Beef tubular bones (tibia and fibula)	Thermolysis 1 hour, 140 °C	66.6	0.89	82.3
Beef tubular bones (tibia and fibula)	Thermolysis 1 hour, 150 °C	43.3	0.97	118
Beef tubular bones (tibia and fibula)	Thermolysis 1 hour, 160 °C	33.3	0.98	197

Analysis of these hydrolysates regarding functional-technological properties (Table 6) shows that high temperature thermolysis enables obtaining protein products with quite high structure-forming capacities. For example, WHC of hydrolysates at a level of 33.3% — 66.6% points to a potential possibility to retain 33% — 66% of water by these hydrolysates, while WBC of these samples was close to one (0.89–0.98) indicating high strength capacities of bound water retention. However, the hydrolysates showed the highest indicators of technological functionality in fat sorption. It is known that FHC at a level of 100g/g is typical for pure protein preparations [3], while protein products obtained by thermal hydrolysis at 150–160 °C are superior to them by fat holding capacity (118–197 g fat/g).

Analysis of functional-technological properties of protein hydrolysates (Table 7) also shows that with an increase in a thermolysis temperature from 140 to 160 °C, a decrease in the water holding capacity (WHC) and an increase in water binding (WBC) and fat holding (FHC) capacities were observed. This dependence can be explained by intensification of protein molecule destruction reflected in the values of molecular weights of obtained peptides. The higher the hydrolysis temperature, the lower the molecular weight of generated peptides and the higher the weight fraction of low molecular weight peptides in the protein mixture (Table 4, Figure 5). With that, the WHC values in hydrolysates dropped from 66.6% (thermolysis at 140 °C) to 33.3% (thermolysis at 160 °C), and WCC and FHC in the same samples grew from 0.89 to 0.98 g water/g and from 82.3 to 197 g fat/g hydrolysate. It is necessary to note the positive effect of the thermolysis temperature growth on an increase in the fat holding capacity (FHC) of the hydrolysates, which rose more than twofold in the protein products obtained at 160 °C compared to hydrolysates generated in thermolysis at 140 °C. This property can be applied when using freeze-dried hydrolysates in food systems with the increased fat content.

The obtained results indicate prospects and rationality of using dry protein nutraceuticals in different food systems. They correspond to the requirements for food additives in terms of sensory properties, chemical composition and safety. All protein nutraceuticals have a natural color from beige to light-brown, weak meat odor and meat flavor with mild gelatin tint, do not bring off-flavor or unpleasant flavor to other products. When consumed orally, protein compositions with prevalence of peptides with the molecular weight of less than 10 kDa have insignificant bitter off-flavor, which is necessary to take into account in developing corresponding food systems. In this case, it is recommended to introduce them in the encapsulated form as food BAAs or consume them with food ingredients having compensatory favor characteristics.

The normative documentation "Food technological additive. Collagen hydrolysate" was developed for produced protein nutraceuticals. In the special experiments, several recipes were developed using these additives. In particular, consumers gave high scores to specialized restructured meat products (souse, wiener-type sausages) with the osteotropic direction. Protein additives obtained from beef bones by combined hydrolysis were additionally introduced into the recipes of these products in an amount of 2–3% of food weight. Enriched products had high sensory properties and were recommended to the elderly as gerodietetic nutrition intended for strengthening the locomotor apparatus.

Based on the obtained results, recommendations on the use of the obtained protein nutraceuticas in food production were developed. It is recommended to use protein additives from collagen-containing beef raw materials as a source of functional amino acids with the osteotropic direction in the composition of specialized and personalized enriched food products designed for specific population groups (for example, patients suffering from collagenosis). These products will be beneficial for broad layers of the population for prophylaxis and strengthening of the locomotor apparatus. In the latter case, they can be especially in demand among the elderly, sportspersons, workers with increased physical loads and others. The use of low molecular weight protein nutraceuticals is promising in the composition of several protein multicomponent BAAs as a source of active peptides intended for compensation of deficiency in the amino acids — neurotransmitters (glycine, alanine) and others increasing physiological and immune functions of the body [1,2,13,17,18,24,27].

Conclusion

As a result of the experimental study, the rational ways of obtaining low molecular weight protein components (peptides, oligopeptides and amino acids) from highly mineralized collagen-containing beef raw materials (beef tibia, fibula and costal bones) by enzymatic, thermal and enzymatic-thermal methods. These protein components are recommended for using as food and biologically active additives — protein nutraceuticals.

The rational technological scheme and optimal regimes of deep hydrolysis of beef raw materials were established for producing low molecular weight protein nutraceuticals. The molecular fractional composition, amino acid composition and indicators of amino acid balance were determined; emulsifying, water holding, water binding and fat binding capacities were established in the protein compositions. The obtained results indicate the high biological value, functionality and processability of protein nutraceuticals from meat and bone beef raw materials.

The recommendations on the use of protein nutraceuticals from beef raw materials in the composition of food BAAs were substantiated for increasing physiological and immune status of the body and as a source of multifunctional amino acids. Protein nutraceuticals are promising enriching agents in specialized and personalized food products with the osteotropic direction.

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Review article

EDIBLE INSECTS AS A SOURCE OF ALTERNATIVE PROTEIN. A REVIEW

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Keywords: *food resource, entomophagy, environmental protection, public perception, non-animal protein, meat alternatives*

Abstract
The current state and research priorities in the field of using insects as foods and their components are examined. At present, entomophagy is practiced in Africa, South America and Asia. It is shown that the growing world population, which is increasingly limited in resources upon the rising demand for animal protein, has stimulated the interest to new food sources that can include insects as future alternative sources of animal protein. In the forming global model based on the growing share of renewable energy sources, entomophagy fits in as a renewable source of food energy. Over the last decade, the potential of edible insects as a new ingredient has been studied. It is noted that edible insects can be produced with less environmental impact compared to cattle. Insects have a huge potential at all life cycle stages as a source of nutritional and active substances and are a rich source of animal protein, contain essential amino acids, minerals (K, Na, Ca, Cu, Fe, Zn, Mn and P), vitamins (B-group, A, D, E, K and C) and unsaturated fatty acids. Assimilability of insect protein is 76–98%. Insect carbohydrates are represented mainly by chitin contained in a range from 2.7 mg to 49.8 mg/kg of fresh matter. There are data that different insect species can have immune stimulating, sugar reducing, antioxidant and anti-genotoxic activities, as well as the positive effect in cardiovascular and nervous disorders. In the western countries, different methods of insect processing were developed. The review summarizes advantages and risks of eating insects and legal practices of their consumption. Possible ways and strategies of stimulating edible insect consumption are analyzed taking into account that the majority of population in western countries reject the idea of eating insects. The review of performed studies notes the necessity to eliminate emotional and psychological barriers on the way of accepting edible insect consumption.

Introduction

According to forecasts, the world population will exceed nine billion people by 2050 [1, 2, 3]. It is expected that the demand for meat products will increase by more than 75% in 2050 compared to the present level. The growth in per capita meat consumption will be greater in developing countries (from 28 kg in 2005/2007 to 42 kg in 2050) than in developed countries (from 80 to 91 kg). At present, developing countries mainly account for this increase in demand (113%), while it is less in developed ones (27%). It is estimated that the growth in meat consumption will be more than 150% in several world regions from 2010 to 2050. For example, it will be 187% in Middle East and North Africa, 202% in Sub-Saharan Africa and 272% in South Asia [4, 5].

Developed countries have higher per capita protein consumption than developing countries (about 96 g/capita/day); however, a significant proportion (65%) of this amount is meat. On the contrary, protein consumption in developing countries is significantly lower (about 56 g/capita/day) and animal protein accounts only for 15%. With that, animal husbandry, including production of forage crops, occupies about 70% of world agricultural lands (or 30% of Earth's land surface) and uses 77 million tons of plant or animal protein to produce only 58 million tons of protein for human consumption annually [6].

The growth in the global demand for meat and scarcity of land resources stimulate searching for alternative protein sources [4, 7].

This will require an almost twofold increase in food output using existing agroecosystems taking into consideration the fact that global warming is gradually reducing areas used for food production worldwide [8]. The climate change, enhancement of the technogenic impact on the environment, agricultural areas, water resources, forests, fish supply and biodiversity as a result of the industrial development also negatively influence the food sector [7, 9]. With that, it is noted that limitation of the amount of agricultural land causes the necessity to search for an alternative to meat and meat products with regard to the fact that animal husbandry is one of the leading causes of the anthropogenic climate change as it is one of the main sources of greenhouse gas emissions. Therefore, sustainable diets with reduced amount of meat or the use of alternative protein sources are needed. Insects are this alternative and can be regarded as available food for humans or feed for livestock [1, 10, 11, 12, 13].

Insects are accepted as animal food in many Asian, African, Oceanian and Latin American countries, where insects are historically consumed and used as the main protein source [14] ensuring the sufficient nutritional value for humans. However, the rapid growth in food production due to the technological progress to a large extent excluded insects from the human diet [1].

It is believed that insects emit less greenhouse gases and ammonia than conventional livestock (cattle, pigs and poultry), which potentially make them more environmentally friendly [15].

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One of the main reasons of expediency of using insects for nutrition is the fact that they have positive ecological properties: they are cold blooded and do not use energy for maintenance of their body temperature contrary to mammalians and birds; therefore, their feed conversion is much more efficient than in conventional livestock [16].

Insects are poikilothermic. In other words, insects spend much less food energy and nutrients than warm blooded livestock. Insects are much more efficient in transformation of phytomass into zoomass (that is, plant biomass into animal biomass) than conventional livestock. Therefore, much more animal protein is produced per kilogram of phytomass consumed by insects than by conventional livestock. Insects are much more fecund and grow much faster. For example, each individual produces thousands of offspring compared to several ones produced by conventional livestock [17].

Use of insects as a food source. Historical aspects

FAO has considered insects as food since 2003 [16, 18] and encourages consumption of insects (entomophagy) in the Western world due to a possibility of sustainable food production.

Entomophagy plays an important role in assurance of food security and improvement of living conditions in many world nations. Eating insects is a part of the common diet like meat or fish consumption for about 2.5 billion people, mainly in Africa, Asia and Latin America. Insects are eaten in 29 Asian, 36 African and 23 American countries. In some places, they are considered a delicacy, while in others, they are the main diet. Insects represent high quality food for humans and animals, and according to various data, 1600–2100 insect species are consumed worldwide [7, 11, 19].

Entomophagy can be regarded as potentially more sustainable source of animal protein than red and white meat, on which food security depends now in the most parts of the world. In the currently forming global model based on the growing share of renewable energy sources, entomophagy fits in as a future renewable source of food energy [17].

In ancient times, eating insects was quite a common event. In the first century AD, Roman historian Pliny the Elder described cossus, which is the larva of the longhorn beetle. Li Shizhen wrote a comprehensive book about Chinese medicine and food during the Ming Dynasty in China, which included many insects [17,19].

With appearance of organized religions, the number of people avoiding entomophagy began to increase as in several religions, the followers are recommended to eat only certain types of red or white meat (which completely exclude insects), while in some other religions, consumption of any form of animal protein is not recommended to prevent animal sacrifice [17].

In 1737 in France, René Antoine Ferchault de Réaumur indicated the inconsistency of the fact that frogs, snakes, and lizards were eaten across France, but entomophagy caused disgust [19].

In 1885 in England, Vincent Holt wrote "Why not eat insects?". He stated that "it is hard… to overcome the feelings that have been instilled into us from our youth" and that "the general abhorrence of insects seems almost to have increased of late years, rather than diminished, owing, no doubt, to the fact of their being no longer familiar as medicines." A growing taboo from childhood is described but he goes further, "there is not such a very strong prejudice among the poorer classes against the swallowing of insects" and "there cannot be said to be any really strong objection, among the upper classes, to making any new departure in the direction of foods, if it once becomes the fashion to do so" [19].

Nowadays, several countries continue using insects as common food resources.

Over hundreds of years, national cultures in Asia, South America, Africa and Europe included consumption of different insect species [20]. For example, a survey of markets in Bangkok, Thailand, revealed 164 insect species sold for consumption as food. The most commonly eaten insects are beetles, caterpillars, bees, ants, crickets, grasshoppers and locusts. In Zimbabwe, Zambia, and Nigeria, edible insects are usually sold in school cafeterias and open markets. Some insects are appreciated for their sensory characteristics and are consumed in high-class restaurants. For example, escamoles (ant eggs) are considered a delicate gourmet dish in Mexico, Laos, Cambodia, and Europe [21].

Entomophagy has been in existence in China for more than 2000 years; presumably, about 324 species from 11 orders are eaten there. India has many developed ways of using insects, including production of silk, fertilizers, food and medicines. Approximately 255 insect species are used as food depending on seasonal or regional differences in culture. In Thailand, insects are an important source of protein, fat and other nutrients and more than 80 species are considered edible food resources. Moreover, Thailand's Ministry of Public Health recommends rural communities to eat insects to intake necessary nutrients [1].

Sago grub (*Rhynchophorus ferrugineus*) is a popular edible insect in Papua New Guinea and the main part of the annual grub festival. Locusts, crickets, mole-crickets, mantises, and even spiders are consumed in local regions. Aboriginal tribes ate a wide variety of insects from Cossidae, Noctuidae, Cerambycide, and bees. In Australia, entomophagy is low among European-derived populations, but the market of edible insects has sharply grown along with an interest in bushfood and insects are now available in restaurant menus [1].

In Mexico, edible insects are traditionally consumed both in rural and urban areas. However, the growing westernization of cities after Spanish conquest finally limited entomophagy mainly to rural regions [22]. Nevertheless, escamol, a dish from insects fried with aromatic spices, is often served in Mexican restaurants. Edible insects are the main protein source for Amazonian tribes in Brasil, in particular, *Rhynchophorus palmarum* and *Atta* ants are quite popular. In Colombia, the Yukpa people prefer insects to meat; however, they had to reduce insect consumption due to massive deforestation [1].

Summarizing, it can be said that appearance of insects as a viable food group can be explained by their nutritional, ecological and economic value. An increased attention to edible insects is a part of the multifaceted strategy for achieving global food security [1].

It is expected that the global edible insect market will exceed 522 million U.S. dollars by 2023 [23] and according to Bloomberg, it will be 1,181.6 million U.S. dollars (Figure 1).

Nutritional value of insects

Despite existence of many edible insects in the world and active consumption of insects by the population in many countries, available data on the nutrient composition of insects are still insufficient [24, 25]. The nutrition value of insects can be influenced by a particular species, the development stage (for example, T. molitor larvae are a source of calcium, zinc and magnesium; pupae are a source of only magnesium; and adult individuals are a source of iron, iodine, magnesium and zinc), location, season, feed and other factors [25].

Insects as food are usually regarded as a healthy, nutritional alternative to conventional meat products such as chicken, pork and beef. They are rich in protein (in general, from 40 to 70% on a dry weight basis), minerals (calcium, iron and zinc) [26] and vitamins; their essential amino acid content is similar to beef and soybean; the unsaturated acid content is 10–30% of dry matter [12, 13, 27].

Novak et al. [25] analyzed the composition of 236 out of more than 2000 edible insect species and showed that insects are usually rich in protein, fat and minerals, but poor in carbohydrates excluding fiber [27]. The average protein content on a dry matter basis is in a range from 34.35% in termites (*Isoptera*) to 61.32% in grasshoppers, crickets and locusts (*Orthoptera*), the fat content is in a range from 13.41% in *Orthoptera* to 33.40% in beetles and grubs (*Coleoptera*), the fiber content is in a range from 5.06% in termites to 13.56% in true bugs (*Hemiptera*), the nitrogen-free extract is between 4.63% in dragonflies and damselflies (*Odonata*) and 22.84% in termites, and the ash content is in a range from 2.94% in cockroaches (*Blattodea*) to 10.31% in flies (*Diptera*) [28].

As a rule, the predominant amino acids in insect protein are phenylalanine, tyrosine, leucine and valine, while methionine, cysteine and tryptophan were less abundant [28].

The amino acid content in insect protein, in general, corresponds to WHO recommendations. All species of edible insects contain a sufficient amount of isoleucine, leucine, lysine, phenylalanine, threonine, valine, arginine, histidine and tyrosine. As a rule, the highest amount of lysine, valine, methionine, arginine and tyrosine is in *Blattodea* compared to other insects. The amount of leucine in *Coleoptera* is higher than that in other animal protein sources including livestock. Likewise, the amount of phenylalanine in *Hemiptera* is usually higher than that in all other known protein sources. Insects at the stage of nymphs (an immature stage of arthropods with incomplete metamorphosis (ticks, *Apterygota*, some *Pterygota*)) are usually the most abundant source of almost all amino acids. They are especially rich in arginine which improves the condition of heart and blood vessels and strengthens the immune sys-

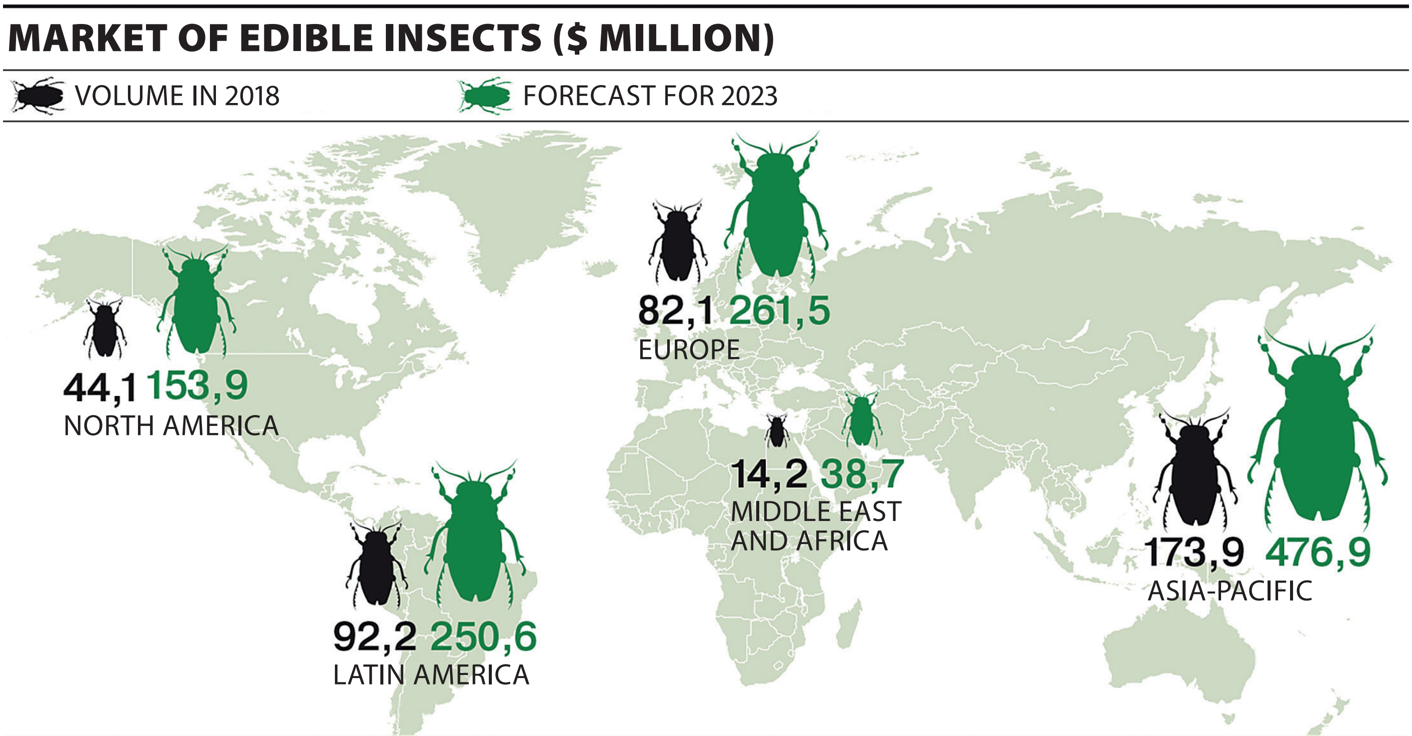

Figure 1. Edible insect market according to Bloomberg

tem. The amount of arginine is more than twice as high in nymphs of cockroaches (*Blatta lateralis*) as in beef and pork [29].

Edible insect proteins correspond to the WHO requirements for the essential amino acid content [28]. Moreover, insect proteins, on average, are more digestible (76–98%) than plant proteins such as peanuts and lentils (52%), and only a little less digestible than animal proteins such as beef and egg white (100%) [12].

The average fat content in insects ranges from 10% to 60% of dry matter; with that, the larval stages have higher fat content than adult ones [28, 30], although these values depend on an insect type and their diet. For example, caterpillars and termites have the highest fat level (from 8.6 to 15.2 g per 100 g of insects), while the fat content in grasshoppers and crickets is lower (from 3.8 g to 5.3 g per 100 g of insects). The lipid fraction of edible insects is rich in mono- (MUFAs) and polyunsaturated fatty acids (PUFAs) with the high content of ω-3 and ω-6 fatty acids [27]. The main MUFAs in edible insects include palmitoleic (C16:1) and oleic acids (**C**18:1n9). The content of palmitic acid is also relatively high. The total content of polyunsaturated fatty acids may be up to 70% of total fatty acids [28, 30].

The fat content in *Lepidopteran* and *Heteropteran* larvae is higher than in other edible insects. Larvae are a rich source of fatty acids compared to insects at other development stages. The adults are the best source of polyunsaturated fatty acids (PUFAs) compared to pork, beef and insects at other stages. Linoleic acid is the main PUFA in insects. Butterflies and moths (*Lepidotera*) having high amounts of PUFAs are especially rich in α-linolenic acid, which was identified as a potential nutraceutical for brain protection against stroke [29].

The results of several studies showed that certain insect species had the high content of omega-6 fats and omega-6: omega-3 ratio in a range from 27 to 17, which is higher than the FAO/WHO recommendations. Another research of insect lipids revealed that the total lipid content in insects varied widely from 24% in cicadas to 0.3% in June beetles [19].

Insect carbohydrates are largely represented by chitin, which content ranged from 2.7 mg to 49.8 mg/ kg of fresh matter. Some species of edible insects contain acceptable amount of minerals (K, Na, Ca, Cu, Fe, Zn, Mn and P), as well as vitamins such as B-group vitamins, vitamins A D, E, K and C [30].

Caterpillars are especially rich in vitamins B1, B2 and B6 [28]. Bee pupae are a source of vitamins A and D, while red palm weevil (*Rhynchophorus ferrugineus*) is a source of vitamin E [29]. A wide range of micronutrients can be found in edible insects, including iron, magnesium, manganese, phosphorous, potassium, selenium, sodium and zinc [28].

The mineral content in various insects is significantly different. The majority of insects contain only a low amount of calcium (less than 100 mg/g on dry matter basis); however, horse fly larvae and adults of melon bugs are rich in it. Pupae of *Polybia occidentalis* can provide only 54 mg of potassium per 100 g, while *Apis mellifera*, which also belongs to *Hymenoptera*, has the high potassium content at all development stages, for example, 1500 mg of potassium per 100 g of mature individual. *Macrotermes nigeriensis* contains only 6.1 mg of magnesium per 100 g, while *Euschistus* egglestoni provides 1910 mg of that substance per 100 g.

The majority of edible insects are particularly rich in iron. The iron content in insects is usually higher than in fresh beef.

The energy efficiency of different insect species has similar and quite high values compared to conventional meat products but depends on their fatness [31].

At the same time, edible insects have the high value in providing calories, which content ranges from 290 to more than 750 kcal/100 g of dry matter [31].

Ramos-Elorduy and Pino [32] calculated energy indicators of 94 insect species used as food and found that among species analyzed, 50% had higher caloricity than soybeans; 87% exceeded corn, 63% were superior to beef and 70% were better than fish, lentils and beans.

Coleopteran and *Lepidopteran* species give significant energy. For example, energy values in meat from farm animals are 165–705 kcal/100 g, in vegetables and potato 308–352 kcal/100g, while edible insects provide 217–777 kcal/100 g, and insects raised on organic waste give 288–575 kcal/100g [33].

As a rule, adult insects contain a high amount of chitin, which is indigestible, and therefore, have the low calorie content. Larvae and pupae usually contain high amounts of proteins and fats, which correspond to high calories. Therefore, products made from insects of different stages can fit people with different needs [29].

Carbohydrates in insects mainly exist in two forms: chitin and glycogen. The first one is a polymer of N-acetyl-D-glucosamine, which is the main component of the exoskeleton [16], while the latter is a source of energy stored in cells and muscle tissues. The mean carbohydrate content in edible insects ranges from 6.71% in stink bug to 15.98% in cicada [34].

An effect on changes in functional properties of meat products when adding components from insects practically was not studied. For example, it was found that when replacing 10% of lean pork in emulsion sausages with *Tenebio molitor* or *Bombyx mori* flour, emulsion sausages had the high added value but the consistency of such sausage was harder regardless of the initial processing and the sausage had lower moisture content compared to the control sausage sample [12].

The use of insects in medicine

Discovery of physiologically important substances contained in edible insects makes their medicinal use a potentially important practical direction. However, taking into account that comprehensive scientific data are absent up to now, further research is necessary to verify benefits of using insects as healthy food or medicines [1].

There are several data indicating that termites (*Macrotermes annandalei*) can have immune stimulating activity. Another insect that historically has been considered beneficial for health is silkworm *(Bombyx mori L.*). Studies revealed a glucose reducing agent in its blood, which led to the development of silkworm powder as an antidiabetic medicine in Korea [1].

The crude extract of mulberry silkworm was effectively used in cardiovascular and nervous disorders as it has a significant effect on hypercholesterolemia and atherosclerosis, possibly, due to its antioxidant and hypolipidemic effect [35].

The methanol extract (1 mg/ml) of muga silkworm (*Antheraea assamensis Helfer*) pupae with the high content of phenolic acid (11.2 mg catechin/g) and flavonoid (5.12 mg quercetin/g) shows the antioxidant and anti-genotoxic activites and provides protection against H2O2-induced DNA damage. The main functional component of the mulberry silkworm extract powder is the alpha-glucosidase inhibitor, which has the blood sugar-lowering effect and a potential in hyperglycemia inhibition in patients with diabetes [36].

Antimicrobial peptides, which are the key elements of the innate immune defense against bacterial and fungal infections, were isolated from insects. The insect metalloproteinase inhibitor and antimicrobial peptides from the greater wax moth (*Galleria mellonella*) may serve as promising templates for the rational design of new medicines as there are data that the combination of antibiotics with inhibitors of pathogen-associated proteolytic enzymes has synergistic therapeutic effects [37].

At the turn of the 20th century, the Russian scientist Ilya Ilyich Mechnikov studied and proved a negative effect of preparations from the greater wax moth on Mycobacterium tuberculosis. When studied the historical experience of folk medicine, he presumed that if the greater wax moth larvae could destroy wax, then they could destroy waxy coating of M. tuberculosis. The extract of the greater wax moth larvae negatively influences M. tuberculosis at any stage of its development destroying its waxy coating. Specific enzymes of the greater wax moth larvae facilitate resorption of local lesions.

The extract of the greater wax moth larvae is a natural immune modulator and non-steroid anabolic, which enhances body defenses and body resistance against different diseases. The pharmacological and therapeutic action of the *Galleria mellonella* extract is quite wide. In addition to the negative effects on *M. tuberculosis*, it has adaptogenic, cardioprotective properties, alleviates myocardial ischemia [38].

Chitin and chitosans of insects are used as immune adjuvants (substances enhancing immunity) and non-allergic drug carriers. In addition, they have the lipid binding function in the human gastrointestinal tract and, therefore, reduce lipid absorption in the intestine. They decrease the level of cholesterol and triglycerides in plasma and improve the HDL/total cholesterol ratio [36].

It is obvious that the above mentioned results as well as other data on the curative effect of insect biologically active compounds require further study to assess the degree of their effectiveness and prove it by clinical trials.

Consumer attitude to entomophagy

Modern models of edible insect consumption

With the agriculture development and livestock domestication, the habits of insect eating disappeared in many regions. As cultural customs changed, insects turned from the main food source into snacks, unusual food ingredients and in several cases, they are consumed as delicacies, for example:

— wasps, bamboo caterpillars, crickets, and locusts are sold as delicacies in the best restaurants and food shops in Thailand.
— annual sales of edible ants in China reach $100 million.
— the rice-field grasshopper, called inago, is a luxury food item in Japan similarly to canned wasps, a 65 g can of which is sold for more than $10. Even more expensive are hornets, which are sold at a price of more than $20 for 100 g.
— there is a sharp growth in tourist interest regarding the native Australian "bush tucker" foods, which include insects such as wichety grubs (Cossidae), bogong moth, and bardee larva.
— in Mexico, high-end restaurants charge more than $25 per plate of escamoles (pupae of an ant species) and gusanos (butterfly larvae). When exported, escamoles have a fantastic price of $50 for a 30 g can (almost two dollar per gram). Escamoles is a national Mexican dish, which is a corn tortilla with unusual filling that on taste resembles a mixture of butter and nuts. The filling is specifically processed larvae and pupae of black ants belonging to the genus *Liometopum*, which lay eggs in the agave roots in Mexico.

An attitude of the Western society and ways of overcoming the consumer negative perception of entomophagy

In the Western culture, eating insects is taboo as the majority of the population consider them disgusting and their eating unacceptable. The persisting negative perception of insects prevents extension of the global market and limits insects as the main nutrition option, which can be linked with the fact that people are skeptical about novel products due to general neophobic trends [20]. Therefore, many consumers regard insects as a source of fear or disgust, have strong aversion to insects as normal food in their diet and fully neglect their high nutritional value [9].

Western consumers are reluctant to eat whole insects and, therefore, insect-based food ingredients included in the composition of other products can be alternative ingredients [13]. In addition, fats, chitin, minerals and vitamins can be extracted from them. The insect gelatin could be used to replace animal gelatin as insects are not associated with Bovine Spongiform Encephalopathy (BSE) and could be an acceptable Halal product.

Hypothesis trying to explain the Western beliefs are varied from psychological associations between insects and diseases, death, dirtiness [36] and food contamination to neophobia. An effect of Western taboo is such that the consumer perception is the main obstacle for the entomophagy development in Western cultures [19].

Nevertheless, insects still enter the human stomach. It is estimated that a person eats up to 0.5 kg of insects during the whole life. We eat mealworms with bread, worms and larvae enter the stomach with jams and tomato paste.

An example of entomophagy in the West is the Italian island of Sardinia, where cheese casu marzu notable for containing live larvae of cheese fly is produced. Casu marzu means 'rotten cheese' in Sardinian language; the term 'maggot cheese' is also used in spoken language. Casu marzu is made from another Sardinian cheese — pecorino. Casu marzu is hold longer than the typical fermentation stage bringing it to the state of decomposition caused by the digestive action of the larvae. The larvae accelerate the breakdown of cheese fats making the product soft. Also, some liquid called lagrima ('teardrop') comes out of the cheese. The cheese is considered edible only when maggots are alive. Due to the health hazard and because 'rotten cheese' is considered a contaminated product its selling was officially forbidden in Italy. However, in 2010, casu marzu was recognized as the cultural heritage of Sardinia and again permitted [40].

An example of indirect entomophagy is the carmine dye obtained from carminic acid produced by female cochineal insects (Dactylopius coccus). Cochineal produces carminic acid in an amount of 17–24% of body weight. Carmine historically has been used as a dye for textile, and also found its use in production of cosmetics and some food products such as processed meats, canned foods, alcoholic beverages, yogurt and bakery products as a replacement for beet. Carmine is registered as food additive E120 [1, 19].

The use of lac insects (*Kerria lacca*) is similar. They are cultivated for production of shellac. The pigment is originally bright red, but can be from violet to red and brown. It is mainly used for dying textile fibers and in cosmetology, but today it is also used in beverage production [29].

However, as was mentioned above, many other cultures include insects as a daily part of their diets, in which insect consumption is not weird. Payne et al. [24] indicate that since the majority of studies on insect acceptance as a food product/ingredient have been carried out in cultures not eating insects, their results show only hypothetical involvement in their consumption.

The consumer perception of food with insects was studied by test food, a vegetable soup with bee larvae included as a visible or non-visible ingredient. It was shown that different soup versions were acceptable from a consumers' point of view; but the soup that contained non-visible bee larvae was accepted to a higher degree than the soups with visible larvae. The results correspond to another study showing that the use of insects in food can be regarded as slightly positive. The results showed that use of 'insect flour' with non-visible insects had higher acceptance than the use of whole and visible insects as food or a food ingredient. Moreover, results from the focus group study showed that consumers preferred food products and dishes with milled or non-visible insects over foods with whole or visible insects [13].

Up to now, the systematic studies of the attitude to insects as food were mainly concentrated on populations not eating insects and had ambiguous results [24]. For example, the preliminary survey of 53 students from the University of Southampton (Southampton, UK) showed that 51% "would not taste" insects mainly due to perception of insect food as "disgusting" and "with bad taste".

Nowadays, edible insect consumption is minimal in developed countries, but people's curiosity is growing. In Europe, edible insects cause different emotions and at the same time huge curiosity. Novel food, especially with insects, can cause anxiety and a sense of insecurity due to ignorance, lack of knowledge and experience in dealing with it. As a result, internal conflicts and barriers linked with eating new unknown food arise. Novel food can cause ambivalent attitudes with components of both positive and negative approaches. Therefore, consumers' prejudice based on visual aspects and their willingness to expand their knowledge about nutrition are contradictory regarding this food [41].

People will accept insect food if it will look and smell familiar and if insects are not served intact. The research carried out in the Universita degli Studi Napoli Frederico II showed that three characteristics increase the consumers' willingness to buy foods from insects: a decrease in the number of insects in meal, addition of the flavoring agent cocoa and organic certification [24].

It is noteworthy that mass media in countries where the tradition of eating insects is absent are prone to present insect food differently as novel, disgusting, healthy and/or ethically preferable compared to meat. Data suggest that this type of information can significantly affect consumer attitudes. The research group from the University of Napoli Federico II (Napoli, NA, Italy) surveyed consumers in two European countries, Denmark and Italy (282 University students) and found that an effect of communication exceeded other factors such as age, gender and nationality influencing a consumer attitude. In the research, information about social benefits was of great influence; data were obtained during investigation of benefits for humans [42]. Therefore, information about social benefits can be a useful marketing strategy; although, taking into consideration the absence of reliable knowledge about consequences of increasing insect consumption, it is, likely, irresponsible.

Finally, product characteristics are also a decisive factor in acceptance formation.

For example, to assess acceptance of the trend towards eating insects promoted by the FAO diet, acceptability of novel edible insect products with mealworms *Tenebrio molitor L.* and house crickets *Acheta domesticus L.* was analyzed among young Polish consumers (101 students; 74 females and 27 males). These products were prepared in the form of four different oatmeal bars: one without insects, one with whole mealworms, one with ground mealworms and one with ground crickets [41].

According to the consumers' opinion, the bars contained whole mealworms were the worst. Moreover, the main problems with acceptance of the product with insects were caused by the color of ground crickets and visible whole pieces of the insects in the edible bars. The study proved that the acceptance of insect bars as food depended on their taste and smell. The acceptance rate for the basic bar was the highest, while the bar with whole mealworms had the lowest acceptance rate. It is necessary to note that a predictor (the smell of the presented bars) was a significant statistical indicator of quality in three out of four cases studied.

Inclusion of edible insects into already known foods can be most acceptable for the insectophobic culture compared to providing insects directly as a food variant, and the use of insects as food ingredients will positively affect formation of sustainable business models [23].

The study performed in the University of Parma (Italy) was aimed at studying the main reasons of the negative perception of insects as food and stimulating consumption of edible insects in the future. It was carried out on the mixed group of Italian individuals (n = 46) with different age and gender. The participants tasted three species of edible insects (cricket, honeycomb moth and grasshopper), and then they were given a questionnaire to reveal their opinions about entomophagy. Analysis of the results shows that curiosity and environmental benefits are the most important factors in motivating insect consumption in the future. However, the majority of respondents stated that entomophagy would not be approved and supported by their family and/or friends. At present, it is difficult to predict whether edible insects will become the "food of the future" [43].

To increase consumer interest in the Western countries, several methods for insect processing were developed. These methods include:

— drying (for example, sun drying, freeze drying, oven drying, microwave drying); extraction by ultrasound, cold atmospheric pressure plasma processing or dry fractionation;
— fractionation mainly aimed toward using insects as ingredients in an unrecognizable form, such as powders or meal [30, 44, 45].

All abovementioned methods can affect sensory properties of edible insects. Aroma and taste are quite different. For example, taste of ants and termites is described by sensory descriptors such as sweet, nutty, fatty, crunchy, notes of cereal and wood; taste of grasshoppers as aroma of cereal, wood and nuttiness, flavor of umami and vegetable, crusty, hard; taste of crickets as popcorn, chicken, creamy, aroma of broth, nuttiness and cereal, flavor of umami and vegetable [46].

Taste and aroma are mainly influenced by pheromones present on the surface of the insect body that depend on the environment, as well as forage and/or methods of processing. For example, scalded insects are practically tasteless, because pheromones are washed off [45]. Moreover, insects take the flavor in added ingredients during cooking [30].

Analysis of new insect product spreading (by the number of records) obtained by BUGSfeed — a website, which is engaged in communication and promotion of entomophagy, shows that the most consumed are crickets (150 records), mealworms (58), grasshoppers (43), ants (28) and silkworms (22). Food products that are the best form for persuading consumers to try novel products are protein bars (41), snacks (37), cookies (25) and sweets (18).

Despite multiple advantages of eating insects, the future of insect industry is unfavorable in Western societies [9] taking into account the fact that existing cultural aversion cannot be changed quickly [47].

To increase consumer acceptability, a special attention should be given to social, practical and contextual factors influencing food consumption. These efforts include continuous education and propaganda of the relative potential of edible insects to solve ecological, demographic and land problems today and in the future.

Quality, safety and legislation in the sphere of using edible insects

The main obstacle in the edible insect industry is the absence of systematic work on assurance of product safety and shelf life [9]. The process of insect growing also requires standardization and quality control and this goal requires the development of state legislation and regulatory acts [7].

At present, the use of insects as food in EU falls within the scope of the Regulation (EU) 2015/2283 of 25 November 2015 on novel foods. This document repealed Regulation (EC) No 258/97. As before, producers are required to assess food safety previous to its placing on the market. In this regard, insects are considered novel food products. This means that insects cannot be grown or sold unless each particular species was recognized as safe and approved by the European Commission. According to the Regulation (EU) 2015/2283, the correspondence of novel foods to the requirements should be assessed in details. Assessment and authorization of novel food have been significantly simplified. Previously, this procedure was carried out in each EU member state. Today, ESFA (European Food Safety Authority) will be engaged in this activity. ESFA published the guiding principles regarding required documentation for submission of an application for official authorization according to the present Regulation. This needs authorization based on risk assessment for a particular use (at a product level), for example: insect meal for use in bread, pasta and snacks, insect protein for use in cocktails, processed meat products and so on.

Food safety is of special importance with regard to new food sources. In the context of edible insects, there are four ways of food safety risk emergence: 1) an insect itself can be toxic; (2) an insect can acquire toxic substances or human pathogens from the environment during its life cycle; (3) an insect can become spoiled after harvest; (4) consumers can have an allergic reaction to an insect [9].

Edible insects are food products of animal origin and usually are eaten as a whole, including the digestive tract. This means that they may contain biological agents with the hazardous potential (for example, bacteria, parasites, viruses, prions, yeasts, molds, mycotoxins, histamine, and antibiotic resistance genes). Therefore, the use of insects as food sources can present a potential hazard regarding pathogen transfer and their safety should be thoroughly controlled [48].

Moreover, the intestinal content of insects can be an important part of their use as a food source as the total biomass content from intestinal microbiota can be 1–10% to the insect body weight and it is quite difficult to remove the intestinal part from edible insects. Moreover, allergic reactions can occur as several edible insects have cross-reactive allergic proteins. Carmine produced from the bodies of female cochineal insects and used as a food dye can cause an allergic reaction in some patients. Taking into account that few studies of allergic reactions to edible insects were carried out, it is necessary to pay attention to unknown potential allergens contained in edible insects. Insects like other food products can cause allergic symptoms even after the first contact. For example, silkworm pupae, which are a rich source of protein and amino acids for humans, are well known for their allergenicity as their eating leads to anaphylactic reactions in more than 1000 patients in China every year. It has been suggested that people suffering from shrimp allergy can also be allergic to other mollusks and arthropods such as cockroaches, grasshoppers, fruit flies and other edible insects [19, 49].

To ensure safe use of edible insects as feed and food resources, it is necessary to study hazardous substances, including insect allergens and toxicants as well as their impact on the development of the pathological symptoms in the human body.

For example, acceptability of three flours for porridges used as complementary foods based on "Winfood Classic" (corn and amaranth fortified with edible termites and small fish) and "Winfood Lite" (multi-micronutrient complementary food fortified with maize and amaranth) compared to corn soy blend plus (CSB+) was assessed among 57 Kenyan infants at the age from six months to 24 months. The results of the study on the frequency of adverse health outcomes such as diarrhea, vomiting, stomach ache, skin rashes and difficult breathing before, during and after the acceptability study did not show adverse health consequences for any of the foods including the "Winfood Classic" containing termites. The revealed cases of adverse health outcomes (9.3 per cent), vomiting in the group of children received the corn soy blend plus, were below the 10 per cent threshold required to state that a product has adverse effects [49].

Although insects actually transfer pathogenic bacteria, they are often not pathogenic to humans and, therefore, many pathogenic bacterial hazards to humans originate from rearing, processing and preservation of insects. Nevertheless, it was found that farmed insects had high levels of aerobic and anaerobic bacteria. Insects may be carriers of both Campylobacter and Salmonella. They may also transfer viruses and have the potential for mycotoxigenic fungal growth, although this is not hazardous to humans upon proper processing and storage. With this, the European Food Safety Authority established that edible insects are unlikely to be of significant safety risk [19]. Edible insects were also studied as carriers of bacteria with transferrable antibiotic resistance genes, and a high frequency of the tetracycline resistance genes was found despite a high variability among samples. It is suggested that these genes may be transferred to human microbiomes because of consuming such insects [48].

The European Food Safety Authority is of the opinion that properly grown insects may be safe with regard to allergenicity and environmental hazards as well as both chemical and biological potential hazards, because their microbiological hazard is comparable to non-processed food of animal origin [50].

Use of edible insects in Russia

In Russia, the question of using insects for human nutrition has not been raised up to now. Few projects on insect growing existing in the country deal with processing waste from animal husbandry by insects and production of animal feed based on insect biomass[1].

Entoprotech (Moscow) founded in 2015 grows black soldier fly and specializes in processing agricultural waste into feed additives for animals.

Daily output is about 700 kg of concentrated flour from black soldier fly with the protein content of up to 62%. The flour is in demand among animal husbandry enterprises. Also, the company sells whole dry larvae of black soldier fly: some customers need the product in this form (for example, for the Moscow zoo), others process them by themselves for their needs.

ZooProtein (Lipetsk region) is engaged in recycling waste from animal husbandry enterprises into protein feeds and fertilizer using maggots of fly Lucilia Caesar. The company has been working since 2016 and initially special-

[1] Karabut T. Protein of the 21 century: crickets, cockroaches and fly larvae. The market of edible insects reached $400 million and will develop with the record rate // Agroinvestor, № 06, June 2019. Retrieved from https://www.agroinvestor.ru/technologies/article/31853-protein-xxi-veka/ Accessed January 20, 2021

ized in growing fishing maggots. Then, they understood the prospects of protein feed production and switched to flies. The company can produce up to 500 kg of protein feeds in a month. Their product is in great demand among poultry farms, animal husbandry enterprises and fish farms.

For InAgroBio (Yaroslavl region), which specializes in aquaculture, production of the housefly (Musca domestica) is more like the secondary activity. This is how the enterprise provides its juvenile fish with feed. The enterprise processes larvae by a special method without drying. At first, necessary biologically active substances are isolated from biomass and, then, remained biomass is dried and used for fish feeds.

Conclusion

It is believed that entomophagy can become a solution to the increasingly urgent global problem of ensuring food security. Several countries have already been using insects as alternative sources of food proteins and feed, as well as with the medical purpose. Available studies confirm the significant nutritional and pharmaceutical value of edible insects. Different strategies were developed to expand the market of edible insects and counteract with existing aversion and hostility of western consumers towards entomophagy. The modern state of technologies for edible insects is still insufficient for replacing traditional animal food worldwide; although, edible insects have a huge potential to be the main source of nutrients.

However, to use insects by people and domestic animals, as well as for other purposes, further research is necessary to confirm their consumer acceptability, sustainability and safety for health. These questions are and will be a subject of research for many scientists from different fields.

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QUALITY PROPERTIES AND STORAGE STABILITY OF BEEF BURGER AS INFLUENCED BY ADDITION OF ORANGE PEELS (ALBEDO)

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Keywords: *citrus fruits, chemical compositions, healthy diet, dietary fibers, shelf life, low fat, sensory analysis*

Abstract
The objective of this study was to investigate the effect of using the orange albedo (OA) on the quality attributes of low fat beef burger. The analysis included: chemical composition, water-holding capacity, cooking loss% and pH values as well as sensory evaluation of low fat beef burger prepared with the introduction of the OA with a concentration of 5% as a partial fat replacer were also studied. Microbiological analysis of frozen minced meat semi-finished products (burger patties, at minus 18 °C) stored for 126 days was included. According to the obtained results, the OA contains 1.47% of lipids, 1.42% of protein and 24.61% of dietary fiber. The result also showed that the usage of OA has a positive impact on organoleptic indicators of beef burger. Nutritional value, water-holding capacity, cooking loss% and pH of treated burger patties were improved. In addition, the caloric value of treatment samples decreased because of replacing the animal fat with the OA in recipe. During the storage of semi-finished products, Thiobarbituric values (TBA) showed that treatment samples substantially developed using OA instead of animal fat had a lower level of lipid oxidation compared to control samples. The shelf life of treated burger patties was significantly increased compared to control sample by decreasing the microbial growth and rate of fatty acids oxidation. Finally, OA could be accepted as a functional component in meat products.

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Introduction

In today's world, processed foods and fast foods have become primary elements of most people's diets. Rapid urbanization, industrialization, globalization, and a rise in the number of women in the workforce have resulted in a rapid tendency toward fast foods in developing countries like Egypt. Many of these processed foods including meat products lack minimum amounts of dietary fiber. Epidemiological research has demonstrated a relationship between a diet containing an excess of energy-dense foods rich in fat and sugar and the emergence of a range of chronic diseases, including colon cancer, obesity, cardiovascular diseases, and several other disorders [1,2,3]. Various reports have revealed that intake of fiber reduces the risk of such diseases [4].

Recently, the food industries are searching to use or produce new functional food components to improve shelf life of food products and to meet consumer needs that represent in food quality and safety. Several types of dietary supplements were used for therapy different types of diseases. Therefore, the manufacturers tending to use different new types of food additives especially from the plant origin [5,6,7,8,9]. Moreover, biologically active additives derived from a variety of plant species found in Africa, South America, and other hot-climate countries have received inadequate attention. Due to the scarcity of raw meat, the challenge is to substitute and enrich the raw meat with plant-based ingredients. Raw materials from plant sources with high protein content, such as isolated soy protein (90% protein) and wheat gluten (80% protein), are used as examples [10,11,12].

Presence of a large amount of animal fat in meat products accelerates oxidation of lipids conducting to more diminutive shelf life. The lipid oxidation in meat products during the time of processing and storage has a negative impact on such important quality characteristics as taste, color and nutritional value. Various types of plant additives that have antioxidant activity affect the shelf life of processed meat. In current years, the application of natural antioxidants in food has been increased [13,14,15]. Much attention paid to food additives derived from nuts, fruits, vegetables, herbs, and spices planned to be used to fortify food products with dietary fiber, micro and macro elements, to increase shelf life, improve taste and extend the range of meat products [16,17].

Albedo is spongy white tissue rich in cellulose, which is the principal citrus peel component and Figure1 shows a cross section in an orange fruit. Moreover, albedo has better qualities than other sources of dietary fiber due to the presence of associated bioactive compounds (flavonoids and vitamin C) with antioxidant properties, which may exert more health promoting effects in addition to those of the dietary fiber. Citrus by-products main components are pectin 7.50%, hemicelluloses 11.0%, cellulose 36.25% and lignin 22.50% [18].

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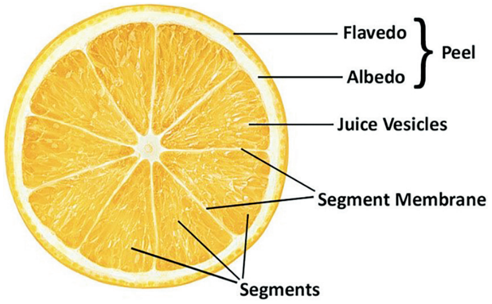

Figure 1. Cross section in an orange fruit

Citrus fiber may be incorporated into a broad range of products such as meat products, fish and dairy product. Dietary fiber is a necessary food component for supporting consumer's health [19]. In meat industry, dietary fiber has a great importance because of its technological properties such as improving textural properties, reducing cooking loss, formulation cost and fat substitution [20]. As for fermented dry sausages, dietary fiber is mostly used for partial fat substitution [21].

Orange fiber usage in sausage product also decreased the bacterial growth and in another hand the growth of lactic acid bacteria was positively affected and decreased the residual nitrite level in sucuk (Turkish sausage) samples [22]. For these reasons, there is an interest in increasing the consumption of low fat meat products and all foods that can supply fiber to daily food intake. Fiber incorporation to food products would help to overcome the current fiber deficit. The source of fiber is important because the differences on the structure and constitution of plant cells can affect fiber properties. Albedo is a white, spongy tissue, which is the principal component of the citrus peel, and could be considered as a potential source of fiber.

Studying the possibility of using of orange albedo as a partial fat replacer and the effect of that on the chemical, physicochemical and sensory characteristics of frozen beef burger was the main objective of this study.

Objects and methods

Chemicals and raw materials

Chemicals and reagents used to conduct methods of analysis obtained from (Sigma-Aldrich Chemical Co., Denmark). Albedo was obtained directly from commercial oranges *(Citrus sinensis)* was obtained from Egypt local market. (OA) was removed by hand using a peeling tool and immediately was frozen at minus 18 °C until being required and before processing of burger the albedo was minced to obtain a pieces with particle size 1–2 mm. Frozen lean beef (6% fat) bought from hypermarket and kidney fat obtained from the local market in Giza, Egypt. Subcutaneous and intramuscular fat, as well as thick and visible connective tissue, were removed from lean beef cuts obtained from boneless rounds.

Preparation of beef burger and final recipe

The control samples were prepared without the (OA). Recipe for "Burger Patties" (control), according to Egyptian standard of frozen beef burger (ES: 1688/2005 ICS: 67.120.10) Egyptian organization for standardization Arab Republic of Egypt (Table 1), and the treatment samples contained 5% of the (OA) used to substitute the same amounts of fat. The selection of the 5% percentage based on previous experiments and this percentage was the best percentage in terms of sensory characteristics. Convenient quantity of each formulation mixed and formed into patties with a weight 100 g and diameter 10 cm. Then wrapped and stored at minus 18 °C until analysis.

Table 1. Beef burger formulation containing orange albedo (OA)

Components %	*Control	Treatment 5% (OA)
Lean meat	65	65
Animal fat	15	10
Orange albedo	—	5
Soy flour	10	10
Onion	3	3
Salt	1.7	1.7
Skimmed milk	1.5	1.5
Bread crust	1.5	1.5
Spices mixture	1.3	1.3
Starch	1	1

* Control prepared according to (ES: 1688/2005 ICS: 67.120.10) Egyptian Standard of frozen beef burger

Method of analysis

Chemical composition

Moisture content was by oven method and ash content of the samples determined by the method described in [23]. In addition, Crude protein by Kjeldahl method and crude fat content using the Soxhlet fat extraction method were determined according to [23]. Crude fiber was determined after boiling 5 g defatted sample in refluxing sulphuric acid and sodium hydroxide. Carbohydrate content was measured by difference according to the following equation:

$$\% \text{ Carbohydrate} = 100 - (\% \text{ protein} + \% \text{ fat} + \\ + \% \text{ moisture} + \% \text{ fiber} + \% \text{ ash}).$$

Caloric values

Based on a 100 g sample, total calories (Kcal) for uncooked patties were determined using the following values for fat (9 Kcal/g), protein (4.02 Kcal/g), and carbohydrates (3.87 Kcal/g) as defined by [24].

The pH value

The pH value of meat model samples determined by mixing 10 gm of the samples with distilled water (100 ml) for 30 s. values of pH measured at 20 °C with a pH meter (Jenway 3510 pH meter) as stated by [25].

Cooking loss%

The meat samples cooked on a preheated (148 °C) electric grill, the samples were cooked for about six minutes, then converted and heated for another four minutes. To determine the percentage of cooking yield samples weighed before and after cooking as described by [26].

The Thiobarbituric acid value (TBA) determined by the distillation method outlined by [27,28].

Water holding capacity (WHC)

Water holding capacity was determined as described by [29] with some modifications. In summary, each sample

(1 g) mixed with 10 ml of distilled water in centrifuge tube, vortexed for 5 minutes and then centrifuged for 30 min at 5000 rpm. The removal of water layer was done then, the weights (wt.) of centrifuge tubes were measured and the following equation was used to calculate WHC:% WHC = [(wt. of tube after decanting (g) — wt. of dry tube (g)) — wt. of total sample (g)] x100/ wt. of total sample (g).

Microbiological studies

Total plate count, Mold and yeast were conducted according to the protocol described by [30]. Coliform group and Pathogenic (*Salmonella)* determined as recommended by [31,32].

Sensorial analysis

Sensorial analysis of cooked samples carried out as stated by [33]. Pieces from samples cooked as described before, prepared and served warm to evaluation, ten qualified and trained panelists were nominated from the staff of Cairo University, faculty of agriculture, department of food science.

Statistical analysis

The statistical analysis of the results was conducted (one-way ANOVA) by XLSTAT software (Addinsoft, New York, USA). Which Duncan test was used to evaluate the differences between all treatments at significance levels ($p \le 0.05$).

Results and discussion

Proximate chemical composition of orange albedo

Data presented in Table 2 shows the gross chemical compositions of orange albedo. The results indicated that orange albedo has a high content of fiber and moisture. These results were in agreement with [34].

Table 2. Proximate chemical composition of orange albedo (g/100 g wet basis)

Contents %	Fresh Orange Albedo
Moisture	65.46 ± 0.21
Total fibers	24.61 ± 0.13
Ash	3.21 ± 0.06
Protein	1.42 ± 0.11
Fat	1.47 ± 0.12
Carbohydrate	3.83

Chemical composition of burgers formulas

The quality characteristics of Burger patties presented in Table 3. Based on the analysis of Table 3, it was found that when the (OA) added to the recipe in an amount of 5%, the moisture content increased by 3.05%, WHC by 8.38%, protein by 0.13%, carbohydrates by 0.25%, dietary fiber by 1.26% and pH by 0.19. Along with this, there is a noticed decrease by 4.97% in fat content, 17.97% in caloric value of the product, and weight loss during heat treatment by 5.62%. The decrease in fat content caused by the fact that beef fat in the recipe replaced with the (OA). The reduction of fat in burger by 4.97% and caloric content by 18,23% suggests the development of a low-calorie product. [35,36,37,38,39,40].

Table 3. Chemical, water-holding capacity, cooking loss% and pH values of burger patties

Studied parameters	Control	Treatment 5% (OA)
Moisture, %	59.73 ± 0.14	62.78 ± 0.09
Ash, %	2.21 ± 0.03	2.49 ± 0.04
Protein, %	16.53 ± 0.08	16.66 ± 0.09
Fat, %	18.26 ± 0.07	13.29 ± 0.04
Carbohydrates, %	1.64	1.89
Dietary fiber, %	1.63 ± 0.05	2.89 ± 0.08
Caloric value, k. cal /100g	237.02	193.81
Water holding capacity (WHC) %	67.45 ± 0.03	75.83 ± 0.04
Cooking loss during heat treatment, %	21.04 ± 0.23	15.42 ± 0.23
pH	5.86 ± 0.10	6.05 ± 0.12

TBA values (mg malonaldehyde/kg of beef burger) for control and treatment as affected by using the (OA) as a partial fat replacer presented in Figure 2. Thiobarbituric values (TBA) during the storage of semi-finished products showed that the treatment samples significantly developed using the (OA) instead of animal fat, have a lower level of lipid oxidation, compared to control samples. According to the obtained data, it could be concluded that the partial substitute of animal fat with the (OA) had a positive effect on the quality characteristics and shelf life of products.

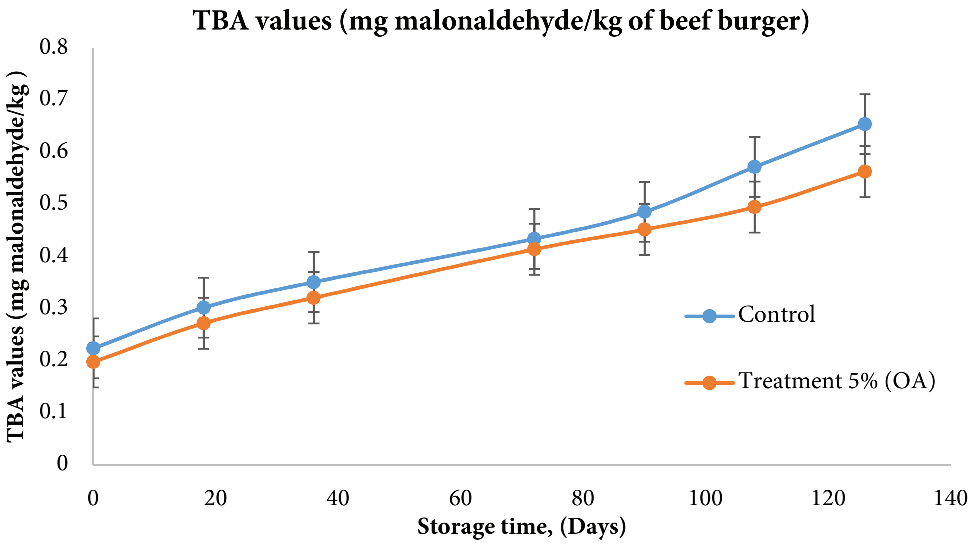

Figure 2. Thiobarbituric values (TBA) during the storage at –18 °C for 126 days (mg malonaldehyde/kg)

Microbiological analysis

Microbiological analysis of samples of burger patties during 126 days of storage at minus18 °C presented in Table 4. Results showed that after 4 months of storage, there is an increase in the total number of microorganisms in both samples. During further storage, there is a slight increase in mesophilic aerobic and facultative anaerobic counts in samples of cutlets for the control Burger. Thus, mesophilic aerobic and facultative anaerobic counts in sample prepared with the (OA) increased to 5,0 * 10^6 CFU/g, during 108 days of storage.

Table 4. Microbiological analysis of frozen minced meat semi-finished products (Burger patties, at minus 18 °C) for 126 days

Microbiological indicators, day	Control	Treatment 5% (OA)
Total plate count CFU/g, no more than 5*10^6		
0	2.2 * 10^{3a}	2.1 * 10^{3b}
18	2.6 * 10^{4a}	2.3 * 10^{4b}
36	4.9 * 10^{5a}	4.5 * 10^{5b}
72	3.7 * 10^{6a}	3.2 * 10^{6b}
90	4.9 * 10^{6a}	4.3 * 10^{6b}
108	5.3 * 10^{6a}	5.0 * 10^{6b}
126	6.1 * 10^{6a}	5.4 * 10^{6b}
Mold and yeast CFU/g, no more than 500		
0	61^a	54^b
18	114^a	86^b
36	156^a	144^b
72	198^a	189^b
90	236^a	213^b
108	262^a	245^b
126	286^a	264^b

* Letters a, b, c indicated to the significant differences among various treatments ($p \leq 0.05$).

In the control sample, the increase to 4.9 * 10^6 cfu/g occurred after only 90 days. Pathogenic organisms, including *Salmonella* bacteria, enteropathogenic Escherichia in the samples of burger and control after storage were not detected. According to the obtained data, it can be concluded that the use of (OA) instead of animal fat increased the shelf life of the frozen product by 18 days, compared to the control sample, which means an increase in the shelf life by 20%. The results of microbiological studies indicated that the control and experimental samples of cutlets were free of pathogens including bacteria of the genus *Salmonella*, enteropathogenic, and acute intestinal infections. Also, *Escherichia coli* and *Staphylococcus* bacteria were not detected during the entire storage period.

Organoleptic analysis

From the point of view of consumers, organoleptic analysis is commonly the ultimate guide to assessment the quality of the products. Therefore, organoleptic analysis conducted in order to assess the color, odor, taste, texture and overall acceptability of cooked samples of control and treatment produced with the (OA). Data presented in Figure 3, reveals that in taste of control samples and treatment there a difference was noticed. The analysis of the obtained data shows that the use of (OA) has a positive effect on all organoleptic indicators. Improvement of organoleptic characteristics such as texture and taste vary in the range of 0.1–0.2 points. The improvement in overall acceptability in relation to the control sample is 0.2 points.

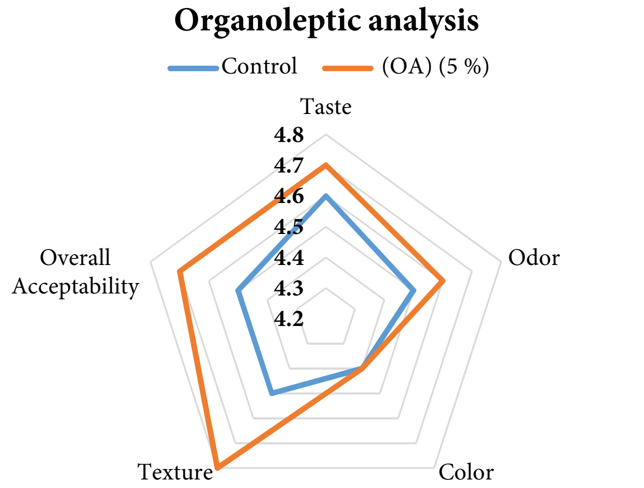

Figure 3. Organoleptic analysis of control and treated beef burger

Conclusion

The use of orange albedo (OA) as a functional additive has a major impact on the quality attributes and storage stability of beef burgers. Furthermore, the use of the OA would increase the fiber content, which is a supplementary nutritional advantage for the customers if an enhancement in dietary fiber is usually recommended. Orange albedo could be beneficial to some of the producers of meat products as a less expensive alternative to conventional meat additives.

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A STUDY ON THE CHEMICAL AND MINERAL COMPOSITION OF THE PROTEIN-MINERAL PASTE FROM POULTRY AND CATTLE BONE RAW MATERIALS

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Abstract
When processing cattle and poultry, a large quantity of secondary slaughter products in the form of bone raw materials are formed in enterprises of the meat and poultry processing industry. Nowadays, its use is not optimal and rational. One of the promising directions of using bone raw materials in enterprises is production of finely ground meat and bone paste. The aim of this research was to study the chemical and mineral compositions, as well as the content of toxic elements in meat and bone paste from poultry and cattle bones obtained after complex grinding on a grinder and ultra-fine grinder. Chicken bone products (chicken necks, drumsticks, wings, breasts) and cattle costal and vertebral bones with residues of muscle tissue were used for investigation. The comparative analysis of the nutritional value of the meat and bone paste showed the following results: protein mass fraction was 18.5% in the meat and bone paste obtained from poultry bones, and 12.1% in the meat and bone paste obtained from cattle bones. The mass fraction of fat was two times higher in the meat and bone paste from poultry bones. As for the mineral composition, it has been established that meat and bone paste is a rich source of calcium: the calcium content was 1,654.02 mg/100g in the poultry meat and bone paste, and 5,318.13 mg/100g in the cattle meat and bone paste. In regard to the toxic element content, the normed values of lead and arsenic, cadmium and mercury were not revealed in the poultry and cattle meat and bone paste. The obtained meat and bone paste can be used for food purposes as a food additive in meat product manufacture, which will allow rational and economic use of bone raw materials in cattle and poultry processing.

Introduction

Nowadays, most meat processing enterprises face an acute problem of the maximum and rational use of secondary products of processing farm animals and poultry. When processing initial raw materials, valuable kinds of secondary raw material resources are obtained, such as blood, bone, by-products of the 2nd category, crude fat, nonedible by-products and others. They can be used for manufacturing additional food, feed and technical products [1,2]. Wider introduction of complex processing of secondary raw materials will enable its rational use as the main components in the meat product technology increasing product output and assortment [3].

A carcass or half-carcass obtained by slaughter of farm animals and poultry is a complex of muscle, fatty, connective and bone tissues. The rational use of bones from slaughter animals and implementation of non-waste and low-waste technological processes into production has assumed great importance allowing exclusion or minimization of losses and assurance of high-quality products. Moreover, bone raw materials is a source of mineral, protein and fat substances [4,5].

In the Republic of Kazakhstan and CIS states, there are few technologies for processing cattle and poultry meat and bone raw materials to the state of finely ground paste. In the foreign countries, meat and bone paste production is common in Japan and China [6,7,8,9].

At present, there are many technological solutions for bone raw material processing that differ from each other by technological parameters, equipment, process duration and so on. Their common feature is striving for maximum extraction of edible components, edible and technical fat, collagen, mineral substances (components) from raw materials by the mechanical, physical, chemical and thermal impact on bones [10].

L. V. Antipova et al. [11] studied the poultry bone residue regarding the nutritional value and proposed a recipe and technology for dry concentrate production based on broth from poultry bone residue. The authors noted that in terms of the chemical composition, the bone residue contained 25% of protein, 18.9% of fat and 11.1% of ash. In regard to the mineral composition, the high content of calcium (3900 mg/kg), phosphorus (2000 mg/kg) and iron (101.8 mg/kg) was observed.

Meat and bone raw materials are used for food purposes as protein hydrolysates and mineral additives, bone broth and fat are produced after corresponding technological processing. For example, an effective and cost efficient technology for processing poultry meat and bone raw materials was created [12]. By hydrolysis of chicken necks in

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the presence of an inorganic catalyzer, a protein product was obtained, which can be used in sausage production, in production of minced semi-finished products, meat fillings and delicacies. Addition of the protein mixture enables increasing the nutritional value and yield of finished delicacy products, improving their sensory properties and rheological characteristics.

Kutsakova et al. [13] developed technologies of three-stage hydrolysis of meat and bone residue of broiler chickens and carcasses of laying hens that allow obtaining food-grade protein hydrolysates that differ by composition and functional-technological properties. Recommendations on the use of hydrolysates as protein additives in meat product manufacture were developed.

The study [14] was devoted to investigation of bone raw material hydrolysis and production of hydrolysates using bone waste from meat, poultry and fish with food additive production. As a result of the hydrolysis of protein mixture from meat and bone raw materials and blood with enzymes of the activated yeast biomass and pancreas, the hydrolysate with the amino acid composition close to optimal was obtained.

Mezenova et al. [15] developed and determined modification parameters for meat and bone raw materials by the hydrolytic method under an action of high temperatures (140 °C) and increased pressure in the aqueous medium (0.62 MPa) to remove valuable protein, fatty and mineral substances. The method of high-temperature modification of meat and bone raw materials allows the complex use of all organic components of raw materials (proteins, minerals, fatty acids) while preserving their native nature and ensuring their sanitary safety. Products of modification of meat and bone raw materials are recommended for the use as food and feed additives, in the composition of biologically active food additives with the osteotropic and gerodietetic direction, microbiological media, feeds for aquaculture, fatty compositions.

Wang et al. [16] used the method of hot-pressure extraction (HPE) for extraction of nutrients (protein, collagen, and minerals) from chicken bone residues. High ratios of protein (83.51%) and collagen (96.81%) were obtained at 135 °C and 120 min. Essential amino acids accounted for 31.03% to 47.73% of total amino acids in the extract of chicken bones. However, the calcium content in the extract of chicken bones (4.2 to 4.8 mg/100 g) was relatively low compared to that in the chicken bone residue (1078 mg/100 g). Extracts of chicken bones were obtained by heating at 130±0.5 °C for 120 min. with following filtration, sedimentation, defatting and concentration. Extracts contain ideal protein and fat, which makes them an excellent substrate for hydrolysis. Extract hydrolysates are potentially suitable food additives in the food industry [17]. It can be concluded that hot-pressure extraction is a method for transformation of chicken bone residues into a nutritional substrate with a flavoring agent; however, it is not an effective way for calcium extraction.

Zhang et al. [18] obtained collagen from chicken bone. Fat, minerals and hybrid protein were removed using ethyl acetate, hydrochloric acid and calcium hydroxide, respectively. The optimal process of collagen extraction envisaged the solid-liquid ratio 1: 8, extraction temperature 100 °C, extraction time 4h and pH 4.0. The content of extracted protein was 88.75%, collagen 86.02%. An ultra-fine bone powder was prepared from bone residues using an ultra-fine pulverizer. Then, the content of nutrients was determined. This method not only solves the environmental problems of livestock and poultry processing industry but also opens new channels and new areas for studying functional foods, and has good social and economic benefits.

Another direction of using bone raw materials is collagen production. Cansu et al. [19] developed a multi-step procedure for collagen isolation from chicken bones that allowed removing 87.5% of minerals and 57.1% of fats with protein losses of about 18.6% and hydroxyproline losses of 14.9%. The collagen yield was about 85% of the initial content, its quality and functional properties were assessed.

Suparno et al. [20] and Kodous et al. [21] described isolation of collagen from chicken feet in the acetic acid solution at 4 **°C** for 24 hours using papain and pepsin with the yield of 18.16% and 22.94%. The obtained collagen contained large amounts of glycine, glutamic acid, proline and hydroxiproline.

These methods of bone raw material processing are based on thermal, acid treatment, which main disadvantage is the loss of nutrients. Moreover, there are significant energy expenditures and labor intensity in several processes.

In this connection, it is necessary to emphasize particularly such direction in bone processing for food purposes as its processing into the finely dispersed mass for using in production of different meat product types. Processing of meat and bone raw materials into finely dispersed paste allows adding this product into sausages, meat semi-finished products such as pelmeni, cutlets, meat balls and so on enriching them with valuable mineral and protein components. The proposed technology for processing meat and bone raw materials allows obtaining meat and bone paste with the high proportion of mineral substances, in particular, calcium, magnesium, iron satisfying the daily requirement up to 50%. The obtained meat and bone paste is recommended to use as a food additive in production of combined meat products.

Meat product manufacture using meat and bone paste ensures an increase in the product yield, broadens an assortment of manufactured products, contributes to an improvement of the ecological state of production and enables the rational use of secondary raw materials, thereby increasing economic efficiency. Moreover, this will give a noticeable economic effect with an increase in profitability of a product in the market [22]. With that, it is necessary to note that the ecological problem is solved simultaneously as all raw materials including those of low value are subjected to processing. Therefore, the proportion of industrial waste generated in case of consumption of poultry meat as carcasses or meat and bone semi-finished products is significantly reduced.

The above mentioned suggests the topicality of the development of the theoretical foundations and practice for deep non-waste processing of meat and bone raw materials and their use in new technologies for functional meat products to enrich them with valuable macro- and microelements and food nutrients.

The aim of the work was to study the chemical and mineral composition of the meat and bone paste from poultry and cattle bone raw materials.

Materials and methods

The objects of the research were samples of frozen meat and bone paste from poultry and cattle bones. Bones with residues of poultry and cattle muscle tissue were used for grinding. Meat and bone raw materials were obtained from meat processing enterprises and large meat trading pavilions in Semey city (Eastern Kazakhstan region, Kazakhstan Republic).

Production of meat and bone paste for investigations

At the first stage of the experiment, the scheme for meat and bone raw material processing was developed, which consisted in successive grinding of meat and bone raw materials preliminarily frozen to a temperature of –18 °C… — 20 °C in freezers. For the experiment on grinding of meat and bone raw materials, chicken bone products (chicken necks, drumsticks, wings, breasts) and cattle costal and vertebral bones with residues of muscle tissue were used for investigation.

Cattle costal and vertebral bones were preliminarily ground to a size of 50–70 mm. Then, meat and bone raw materials were preliminarily frozen in freezers at a temperature of –18 °C…–20 °C for 60 min. After that, frozen raw materials were fed into a bin of a grinder with a diameter of plate holes of 8 mm. After grinding, the obtained meat and bone mass was frozen to a temperature of –18 °C…–20 °C and was ground again in a grinder with a diameter of plate holes of 5 mm. Ice water was added to the meat and bone mass with the raw materials: water ratio of 1:0.5 and was mixed until obtaining a homogeneous mass [23,24].

Then, meat and bone forcemeat was successively ground using a Supermasscolloider ultra-fine grinder with the following clearance between the nonporous grinders: 0.25 mm, 0.1 mm, 0.02 mm. Meat and bone paste with tender spreadable consistency without a sense of toughness on the tongue was obtained at the exit of the grinder. The obtained meat and bone paste was stored at a temperature of 2–4 °C until the further experiments.

Determination of the total chemical composition was carried out by the method of a single weighed portion of the test sample. The method consisted in the successive determination of moisture[1], protein[2] and fat[3] in a single weighed portion.

Minerals were determined according to the normative documents R 4.1.1672–03[4] "Guidelines for quality control and safety of biologically active food additives", GOST 26928–86[5] "Foodstuffs. Method for determination of iron", GOST 33824–2016[6] "Foodstuffs and food ingredients. Stripping voltammetric method for determination of toxic elements (cadmium, lead, copper and zinc)".

Toxic safety was studied by the standard methods according to TR CU021/2011[7] Technical Regulations of the Customs Union "On the safety of food". Heavy metals (Pb, As, Cd, Hg) were determined according to the normative documents: GOST 30178–96[8] "Raw material and foodstuffs. Atomic absorption method for determination of toxic elements", GOST R 51766–2001[9] "Raw material and food-stuffs. Atomic absorption method for determination of arsenic", MUC 4.1.1472–03[10] "Atomic absorption determination of the mass concentration of mercury in biomaterials of animal and plant origin (food, feed, etc.)".

Statistical analysis was carried out using the software package Statistica 6.0 and Excel 2007.

Results and discussion

At the first stage, the chemical composition of the meat and bone paste from poultry and cattle raw materials was studied. The bone tissue consists of the cellular elements and intercellular substance, which includes intermediate substance, formed particles — collagen fibers and inorganic salts [25]. The results of the chemical composition are presented in Table 1. The results of the comparative analysis of the chemical composition show that the poultry and cattle meat and bone paste is characterized by the high protein content (18.3% and 12.1%,

[1] GOST 33319–2015. "Meat and meat products. Method for determination of moisture content". Moscow: Standartinform, 2018. — 14 p. (In Russian)

[2] GOST 25011–2017 "Meat and meat products. Protein determination methods". Moscow: Standartinform, 2018. — 14 p. (In Russian)

[3] GOST 23042–2015 "Meat and meat products. Methods of fat determination". Moscow: Standartinform, 2019. — 8 p. (In Russian)

[4] R 4.1.1672–03 "Guidelines for quality control and safety of biologically active food additives". Retrieved from https://www.rospotrebnadzor.ru/upload/iblock/33e/r-4.1.1678_03.pdf Accessed December 11, 2020. (In Russian)

[5] GOST 26928–86 "Food-stuffs. Method for determination of iron". Moscow: Standartinform, 2010. — 6 p. (In Russian)

[6] GOST 33824–2016 "Foodstuffs and food ingredients. Stripping voltammetric method for determination of toxic elements (cadmium, lead, copper and zink)". Moscow: Standartinform, 2016. — 23 p. (In Russian)

[7] TR CU021/2011 Technical Regulations of the Customs Union "On the safety of food". Retrieved from http://docs.cntd.ru/document/902320560 Accessed December 11, 2020. (In Russian)

[8] GOST 30178–96 "Raw material and food-stuffs. Atomic absorption method for determination of toxic elements". Moscow: Standartinform, 2010. — 20 p. (In Russian)

[9] GOST R 51766–2001 "Raw material and food-stuffs. Atomic absorption method for determination of arsenic". Moscow: Standartinform, 2011. — 12 p. (In Russian)

[10] MUC 4.1.1472–03 "Atomic absorption determination of the mass concentration of mercury in biomaterials of animal and plant origin (food, feed, etc.)". Retrieved from http://docs.cntd.ru/document/1200034851 Accessed December 11, 2020. (In Russian)

respectively). This suggests the expediency of using meat and bone paste in products aimed towards compensation of deficiency in the human protein ration. This allows regarding meat and bone paste as a valuable raw material for food manufacture. The main protein of bone tissue is collagen [26]. Bone tissue collagen is assigned to fibrillar collagens of type I. Collagen is an important component in the composition of food products with a favorable effect on the state of beneficial microflora. A distinctive feature of collagen is the high content of proline and oxyproline. Moreover, collagen facilitates an increase in the emulsifying capacity of the system [27].

Significant differences in the fat content are seen. For example, the poultry meat and bone paste contains 11.8% of fat, which is higher than that in the cattle meat and bone paste (5.3%). Bone fat is concentrated mainly in bone marrow. The peculiar characteristic of bone fat is the high content of lecithin compared to other types of animal fats. The high content of lecithin determines the good emulsifying capacity of this fat type and predetermines its use in production of emulsions [28].

The ash part of the meat and bone paste from poultry was equal to 4.35%, from cattle — 6.80%. Bones have very high stiffness and resilience, which is achieved by a peculiar combination of the organic base with minerals. Bone tissue is a source of mineral salts. It contains 98% of all inorganic substances in the body including 99% Ca, 87% P, 58% Mg, 46% Na [29].

The chemical composition of bones is variable and depends on an animal breed, age and fatness, as well as on a bone type: the fat and mineral content is increased and water content is decreased with an increase in fatness [30].

The results of the mineral composition of poultry and cattle meat and bone paste are shown in Table 2.

Calcium is one of the most important elements for the human body ensuring its normal vital activities. Calcium improves the blood coagulability and nervous system function [35]. The human body cannot produce calcium by itself. Therefore, to maintain the sufficient level of calcium, it is necessary to intake it from different food sources [36]. The best method for meeting the need for this mineral is balanced nutrition with consumption of foods rich in calcium. However, food additives can also be a calcium source.

Analysis of the mineral composition of the meat and bone paste from poultry and cattle bones indicates the considerable calcium content. For example, the calcium content was 1,654.02 mg/100g in the meat and bone paste from poultry bone raw materials and 5,318.13 mg/100g in the meat and bone paste from cattle bones. The significant difference in the calcium content is explained by the structure of cattle and poultry bone tissue. It is known that chicken bones are less hard and thinner than those of mammals; tubular bones do not contain bone marrow and are filled with air [37].

The comparative analysis of the mineral composition of the meat and bone paste and by-products from cattle (liver — 5.00 mg/100g, heart — 8.00 mg/100g, kidney — 13.00 mg/100g, tongue — 6.39 mg/100g, brain — 43.00 mg/100g) and poultry (chicken liver — 15 mg/100g) revealed a significant excess of the calcium content in the meat and bone paste, excluding eggshell.

Another important macro-element for the human body is magnesium. Magnesium normalizes metabolic processes and prevents the development of allergy [38]. The magnesium content was 14.54 mg/100g in the meat and bone paste from poultry bones and 207.62 mg/100g in the meat and bone paste from cattle bones. The increased magne-

Table 1. Chemical composition of meat and bone paste from poultry and cattle bone raw materials

Type of meat and bone paste	Moisture, %	Protein,%	Fat,%	Ash,%
Poultry meat and bone paste	65.55 ± 1.41	18.3 ± 0.36	11.8 ± 0.28	4.35 ± 0.12
Cattle meat and bone paste	75.8 ± 1.91	12.1 ± 0.21	5.3 ± 0.17	6.80 ± 0.19

Table 2. Mineral composition of poultry and cattle meat and bone paste compared to the mineral composition of by-products

	Minerals, mg/100g			
Type of meat and bone paste	Calcium	Magnesium	Iron	Zinc
Poultry meat and bone paste	1,654.02 ± 330.80	14.54 ± 2.91	3.83 ± 0.77	0.070 ± 0.020
Cattle meat and bone paste	5,318.13 ± 1063.63	207.62 ± 41.52	8.35 ± 1.67	Not detected
Cattle by-products (literature data) [31, 32]				
Liver	5.00 ± 0.67	18.00 ± 2.90	4.80 ± 1.35	4.00 ± 2.85
Heart	8.00 ± 1.67	20.00 ± 1.67	4.30 ± 0.08	1.70 ± 0.12
Kidney	13.00 ± 0.22	17.00 ± 0.37	4.60 ± 0.18	1.95 ± 0.10
Tongue	6.39 ± 1.07	16.00 ± 1.33	2.15 ± 0.33	2.32 ± 0.12
Brain	43.00 ± 22.50	13.00 ± 0.00	2.55 ± 0.43	1.02 ± 0.20
Poultry by-products and eggshell (literature data)				
Chicken liver [33]	15	24	17.5	6.6
Eggshell [34]	33400–37300	406–412.9	2.8–41.3	0.40–0.67

sium content in cattle bones is explained by its important role in balance maintenance and retention of calcium and phosphorous in bone tissue. Compared to other by-products, the magnesium content in the meat and bone paste from cattle bones is much higher than that in cattle and poultry by-products. Only eggshell contains more magnesium (406–412.9 mg/100 g).

Besides calcium and phosphorous compounds, bone tissue contains iron, copper and zinc. Iron in bone tissue facilitates calcification of bones of the skeleton. In addition, it is necessary for the cytochrome oxidase activity, which plays an important role in the bone cell function [39]. The high metabolic role of zinc is conditioned by the fact that it is an activator of several enzymes including alkaline phosphatase of bone tissue and others. An excess in calcium and copper inhibits zinc metabolism, processes of growth and differentiation of bone tissue, especially in tubular bones [40].

As for microelements, the iron content was the highest in the cattle meat and bone paste (8.35 mg/100g); it was two times lower in the poultry meat and bone paste (3.83 mg/100 g) upon the reference daily intake of 1–2 mg. Among by-products, chicken liver was characterized by significant amounts of iron (17.5 mg/100 g) compared to cattle liver (4.80 mg/100 g). Significant differences were observed in the zinc content. For example, the highest zinc content was recorded in the chicken liver (6.6 mg/100 g) and cattle liver (4.00 mg/100 g). Lower amounts of zinc (0.070 mg/100g) were observed in the poultry meat and bone paste.

At the next stage, toxic safety of the meat and bone paste was determined (Table 3). The content and level of accumulation of heavy metals in the animal body depend on the kind of consumed feed and type of feeding, zone of raising, climatic conditions and so on. Heavy and toxic metals entering the animal body are accumulated mostly in bones, liver and kidneys.

As lead is widely distributed in nature in relatively high amounts, it is accumulated in the animal body, mainly in bones (90%), comparatively quickly. Lead negatively affects the hematopoietic, nervous, digestive systems and kidneys [41].

Cadmium has high toxicity. Exposure to cadmium causes the oxidative stress in the animal body disturbing the oxidative and antioxidative balance [42]. Excessive accumulation of cadmium in the human body leads to the impaired renal function, dizziness, nausea, skin disorders, reduced appetite, increased arterial pressure, changes and pain in bones and joints.

The presence of arsenic compounds in foods results in serious human diseases later on. Long-term exposure to arsenic can lead to cancer development and skin lesions [43].

The other dangerous element is mercury. Mercury is toxic for the central and peripheral nervous system (mercurial erethism). Chronic poisoning causes predisposition to tuberculosis, atherosclerotic events, liver and gall bladder disorders, hypertension [44].

The critical levels of heavy metals were not revealed in the meat and bone paste: the lead content was 0.071 mg/kg and 0.056 mg/kg, the arsenic content was 0.016 mg/kg and 0.011 mg/kg. It corresponded to the norms of MACs by the content of toxic elements.

Therefore, animal and poultry bones are a rich source of mineral substances. The use of mineral constituents in the food technology upon proper technological and mechanical processing allows enriching products with mineral additives, in particular, calcium, magnesium and other elements.

Conclusion

As a result of the performed experiments, complex processing of meat and bone raw materials was proposed for production of meat and bone paste. The proposed technology for production of meat and bone paste from poultry and cattle bone products differs from existing ones due to the complex scheme of meat and bone processing including stepwise grinding with freezing and following processing to produce finely dispersed meat and bone paste suitable for the use with food purposes as food additives. Analysis of the chemical composition of meat and bone paste shows that meat and bone paste from poultry and cattle bones is a source of protein (18.3% and 12.1%, respectively), fat and minerals, which indicates its nutritional value. Analysis of the mineral composition revealed that the main constituent of the meat and bone paste was calcium, which content was 1,654.02 mg/100 g in the poultry meat and bone paste and 5,318.13 mg/100g in the meat and bone paste from cattle bones. In regard to the toxic element content, critical levels of lead (0.071 mg/kg) and arsenic (0.016 mg/kg) were not revealed in the poultry and cattle meat and bone paste. The content of toxic elements in the meat and bone paste corresponds to food safety standards. The produced meat and bone paste from poultry and cattle meat and bone raw materials is a valuable source of minerals. Production of combined meat products with addition of meat and bone paste into a recipe allows improving the nutritional value of final products.

Table 3. Content of toxic elements in meat and bone paste

Toxic elements mg/kg, not more:	Norms according to normative documentation	Poultry meat and bone paste	Cattle meat and bone paste
Lead	0.5	0.071	0.056
Arsenic	0.1	0.016	0.011
Cadmium	0.05	Not detected	Not detected
Mercury	0.03	Not detected	Not detected

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USE OF IODINE AND SELENIUM ENRICHED FODDER RATIONS FOR PRODUCTION OF FORTIFIED YOUNG LAMB

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Keywords: *young sheep, lamb, feed additives, essential trace elements, iodine deficiency, healthy nutrition*

Abstract
The article is devoted to preventive measures to eliminate the problem of dysmicroelementosis in order to increase the productivity of young sheep. The article presents the results of studies of the influence of feeding rations on the growth and development of young sheep of the Edilbaev breed, on the level of their meat productivity and the qualitative characteristics of biologically fortified lamb. Feed additives based on Yoddar-Zn and DAFS-25 were added to the main ration of lambs at the age of four months, once a day, being added into a fodder mixture with concentrates. For the experiment the herd of lambs was divided to four groups of 25 heads each.The changes in live weight at the age of four, five, six and seven months were analyzed, and it was found that at the age of seven months, the absolute average weight gain in the experimental groups varied from 3.45 kg to 4.49 kg, in the control group it was 3.1 kg, while the largest live weight gain was recorded in group III which received both feed additives based on Yoddar-Zn and DAFS-25.There were no significant differences in the parameters of body measurements, with the exception of group III, where the chest circumference increased by 7.2%, and the height of a lamb at the withers increased in average by 8.1%. It was found that group III had the highest meat density coefficient, equal to 3.9, and the cross-sectional area of m. Longissimus dorsi was equal to 13.61 cm2. It was noted that the amount of free amino acids of the lamb group III is 18.8% higher than the meat of the control group. The lamb obtained from the animals of the experimental groups showed a higher protein content and less fat. The ratio of water to protein in all samples was slightly higher than 3.7, which corresponds to the Federa number for meat raw materials. Enriching the rations of the Edilbaevskoy sheep with feed additives Yoddar-Zn and DAFS-25 promotes the stimulation of growth and development of animals, increases the productivity and nutritional value of lamb.

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Introduction

Among the other tasks defined in the strategy "The Fundamentals of State policy in the sphere of healthy nutrition of the Russian Federation population for the period up to 2025"[1] the most important issues are the support of domestic production of specialized products for healthy nutrition, the development of animals raising technology and the arrangement of specialized zones for organic animal husbandry.

One of the urgent tasks of the agro-industrial complex is the development of progressive methods to obtain the raw meat from organic livestock with certain functional characteristics and composition, which livestock is cultivated through its lifetime to achieve certain quality properties [1].

Breakthrough in technologies of production and processing of agricultural products in Russia has led to production of very numerous and various products of animal origin, often not conforming to criteria of "Healthy food". The reasons for uprising of products like that most often is found in the technologies of raising of the farm animals, which allow to obtain high productivity performance due to the use of antibacterial stimulants [2,3].

Reducing the time of fattening, increasing the productivity rate of animals without introduction of hormones, antibiotics, and anabolic drugs, as well as the production of environmentally safe meat raw materials with high sensory and functional and technological properties, is a main priority now [4,5]. It's not possible to solve these problems without creating a solid feed base through the development of balanced feed rations for complex of basic nutrients and mineral additives to replenish the missing macro-and microelements. Accordingly, the transition of the meat processing industry to domestic raw materials in the near future is impossible without improving the feed base for animals.

High-quality meat raw materials can only be produced with the help of full-fledged feeding of farm animals in compliance with the due conditions of their farm raising, which make it possible to get the necessary properties without adding feed antibiotics, medicinal, chemical and similar growth stimulants [6].

The problem of feed quality is a problem of global importance, and we need to consider this issue in wider way, thus building a chain: feed-meat-man, which will ultimate-

[1] Basic State Policy of the Russian Federation in the sphere of healthy nutrition for the period up to 2020. Retrieved from https://rg.ru/2010/11/03/pravila-dok.html. Accessed January 11, 2021. (In Russian)

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ly help solve the problem of human health and the nation as a whole [1].

In modern animal husbandry the use of feed additives in rations of animals that contribute to increasing the productivity of animals and improving the quality of the obtained products has the particular importance. It is especially important to replenish the deficit of macro-and micronutrients in the body of animals. At the same time it is necessary to take into account the properties of each introduced component and the rates of their introduction in order to obtain edible products with a balanced chemical composition and high biological value [7].

The problem of lack or shortage of essential elements in the ration of the Russian population is one of the most important. The lack of such vital microelements as selenium and iodine in the soil and water, and, consequently, in animal products, is found everywhere in the territory of the Russian Federation [8].

Coupled with the already known successful approaches to enriching pork and poultry meat with organic forms of iodine and selenium, the technology of enriching the ration of ruminants, such as lamb, is a new direction and is of scientific and practical interest.

One of the ways to obtain high-quality lamb, which provides a consumer with the necessary trace elements, is the lifetime optimization of the chemical composition of meat by correcting the animals' rations by enriching them with essential nutrients [9]. The use of a protein-carbohydrate complex with an organic form of iodine and selenium increases the digestibility of feed, stimulates the growth and development of animals, increases non-specific immunity. Those factor all together lead to high productivity and to improved quality of the obtained meat raw materials [10,11].

The main advantage of the lifetime enrichment of lamb with the essential elements is elimination of risk of a negative impact (i. e. overdose), because the introduction of the feed supplement is already "approved" by the animals. This way it is possible to eliminate the obligatory control over the content of trace elements in the finished product, while observing the prescription and technological parameters. Fortification or lifetime formation of the properties of raw materials, and then the finished product, is a significant tool to design functional and specialized nutrition, especially given the current trend towards the personification of nutrition [1,12].

In this regard, the efficient direction of the lifetime formation of the quality and technological properties of raw materials, its biological full-value adequacy by optimizing the technology of animal fattening and comprehensive studies of lamb quality with new predicted consumer and functional properties, for prevention of dysmicroelementosis, are of particular relevance and practical significance now.

In order to determine the most efficient feeding rations on the basis of the Educational and Production Enterprise "Experimental Animal Husbandry" of the Saratov State Agrarian University named after N. I. Vavilov), scientific and economic experiments were conducted, and a comprehensive assessment was given of the lifetime changes in young sheep herd of Edilbaevskaya breed raised on rations enriched with feed additives based on "Yoddar-Zn" and "DAFS-25", vegetable silicon and protein-carbohydrate complex.

Studies to determine the efficiency of enriched rations introduction were carried out on the population of young sheep of a relatively new breed (Edilbaevskaya) for the Russian Federation, which breed features some certain peculiarities in metabolism of these animals.

Edilbaevsky sheep belong to the meat and fat type, these sheep are characterized by incredible meat productivity, capable for big yield of high-quality low-calorie meat used in rationary nutrition, and have a tough coat.

The features of the metabolism of Edilbaevskiy sheep are primarily the following: these sheep are able to digest the feed of the coarsest composition, and conversion of feed is aimed primarily at the formation of muscle mass in animals, which allows them to increase quickly weight gains and accumulate trace elements more efficiently. Iodine, bound by a chemical covalent bond (by amino acid residues — tyrosine), is easily transformed into organically digestible form and absorbed by the internal secretions (thyroid system) of these animals. Unlike the Edilbaevskiy breed, in fine-wooled sheep of the woolly type the feed conversion is primarily aimed at the formation of the wool cover, so these animals are more sensitive to the lack of mineral substances, which makes it difficult to obtain reliable results.

The peculiarities of the Edilbaevskiy sheep metabolism allowed us to reliably identify the form of organic iodine, to control its amount in the form of iodotyrosins, which allows us to deepen and expand the fundamental principles of the lifetime enrichment of raw materials with organic iodine.

The aim of the scientific work was to study the features of impact of rations, enriched with feed additives based on "Yoddar-Zn" and "DAFS-25", for raising of young sheep on stimulation of sheep growth and development, as well as on level of meat yield and quality properties of biologically fortified lamb.

The research objectives were:

— to develop a biologically safe and efficient method of enrichment of lamb *in vivo* with trace elements using feed additives based on "Yoddar-Zn" and "DAFS-25" introduced to sheep rations;
— to conduct a comprehensive assessment of impact of enriching the sheep rations with additives, including organic forms of iodine and selenium, on sheep growth and development, meat productivity: live weight and slaughter weight of animals, slaughter yield of carcasses, physical and chemical, biochemical, functional and technological, sensory properties of lamb.

The priority of production of lamb enriched with essential trace elements will provide producers with non-expensive raw materials for functional products, and, as a result, will contribute to increasing the profitability of the industry, which is a determining factor in solving the problem of food and raw materials safety.

Reducing the time of small cattle fattening, increasing the intensity of weight gain without hormones, antibiotics and anabolic drugs, obtaining lamb with high sensory and functional-technological properties is a priority and an urgent task.

Materials and methods

In order to increase the productivity of young sheep of Edilbaevsky breed and as the preventive measures to eliminate the problem of iodine and selenium deficiency, the scientists of the Volga Research Institute for the Production and Processing of Meat and Dairy Products and the Saratov State Agrarian University named after N. I. Vavilov developed feed additives based on "Yoddar-Zn" and "DAFS-25", which contain essential trace elements, additionally enriched with a protein-carbohydrate complex and mineral supplement "Coretron". Feed additives were added in addition to the main ration once a day according to the instructions for use. All animals received a general farm ration (FR), the animals of the experimental group in addition to the FR received the proposed feed additive in the amount of 1%.

In order to determine the most efficient rations, the authors carried out scientific and economic experiment. The experiment was carried out at the Educational and Production Enterprise "Experimental Animal Husbandry" of the Krasnokut branch of the Saratov State Agrarian University named after N. I. Vavilov. For this purpose, during weaning of young lambs at the age of four months from their mothers, we formed four groups of young rams of Edilbaevsky breed using the method of pairs-analogues, each group of 25 heads. Feed additives were added in addition to the main ration, according to the instructions for use, once a day in a fodder mixture with concentrates. The dosage of "Yoddar-Zn" is 100 g per 1 ton of concentrated feed, 'DAFS-25" — at the rate of 1.6 mg per 1 kg of feed as part of premixes. The control group received the general farm ration and compound feed only as addition, in amount of 250–300 grams per head per day (FR); I experimental group — compound feed + feed additive "Yoddar-Zn"; II experimental group — compound feed + selenium organic additive "DAFS-25"; III experimental group — compound feed + "Yoddar-Zn" + "DAFS-25".

Feed additives based on "Yoddar-Zn" and "DAFS-25" contain in their composition the supplement "Coretron" and a protein-carbohydrate component — cold-pressed pumpkin cake, in the following ratio (kg/100 kg of additives), are sources of trace elements and minerals (iodine, selenium, silicon) in organic form:

"Yoddar-Zn"	0.01
"DAFS-25"	0.0002
"Coretron"	0.5
Cold-pressed pumpkin cake	99.49

"Yoddar-Zn" is a source of the most bioavailable iodine in organic form and zinc. It contains iodized milk proteins in combination with zinc compounds with amino acids.

The biological properties of "Yoddar-Zn" are attributable to the presence of bound iodine in this additive, which is necessary for the biosynthesis of thyroid hormones (thyrotoxin, triiodothyropine), which play an important role in metabolism, promote enzyme formation and support the body's protective reactions. In case of simultaneous intake of iodine and organic zinc, which is a co-factor of superoxide dismutase enzymes, no increase of level of superoxide radicals is observed, which improves the biosynthesis of thyroid hormones.

"DAFS-25" is an organic substance — diacetophenonyl selenide with a mass fraction of selenium of at least 25%. Being a component of the animals and poultry ration, it normalizes the activity of the immune system, provides antioxidant and detoxifying effect on the living body, eliminates the possibility of white muscle disease and fatty liver hepatosis. "DAFS-25" is used to increase the immune resistance of young farm animals and poultry to pathogens of various infectious diseases.

Selenium is used to normalize the metabolism, to increase the overall immune resistance of body and the productivity performance of animals.

The additive has the similar effect on the animal's body like vitamin E. It participates in the processes of tissue respiration and oxidative phosphorylation, acts as a inhibitor of certain enzyme systems, has antitoxic properties, and also prevents the over-oxidation of fatty acids and the accumulation of toxic substances in the body, which normalizes metabolism.

The supplement "Coretron" is a gray powder which consists of amorphous earth silicon of biogenic origin. It is a mineral supplement used in the production of compound feeds and premixes for various types of farm animals and poultry, which acts as an anti-tracking agent, an adsorbent of mycotoxins, is a source of water-soluble silicon (organic form), necessary for the stable functioning of the smooth muscles of animals and poultry gastrointestinal tract, as well as improving the absorption of calcium. In addition, its positive effect on reduction of quantity of helminth infestations due to the saturation of the body with amorphous silicon was observed in case of systematical use.

Cold-pressed pumpkin cake contains (%): crude protein — 22–37, crude fat — 21.15, crude fiber — 11.18, is a source of essential amino acids (including lysine — up to 3.28% of the protein level), macro- and microelements (selenium — up to 3 mg/kg), is rich in carotenoids and vitamin E. The amino acid *cucurbitin*, for example, has a therapeutic and preventive effect in gastrointestinal invasions.

It is also noted that pumpkin cake contributes to the normalization of the gastrointestinal tract functioning, due to which there is an improvement in the feed intake by animals, and a subsequent increase in live weight gain.

In scientific and economic experiments the identical methods of rams raising and fattening were used. At the end of the experiment (105 days) when the animals reached the age of seven months, a control slaughter was carried out in traditional way in a slaughterhouse of the Experimental Animal Husbandry Training and Production Enterprise in accordance with the requirements of the Technical Regulations of the Customs Union[2]. Before the slaughtering all animals were subjected to 24-hour starvation exposure.

The efficiency of enriching rations with organic iodine- and selenium-containing feed additives on rams raising and development, their meat productivity, and the quality properties of meat were studied. Zootechnical properties of rams were evaluated according to the conventionally accepted methods used to control the parameters of animals raising by certain parameters — the age of reaching the due live weight (days), the absolute increase in live weight gain (kg) and the average daily increase in live weight gain (g).

Rams raising and development were evaluated by weighing and taking basic measurements of the animals' body parts at the beginning (at 4 months of age) and at the end of the experiment (at 7 months of age).

To determine the meat yield capacity, the dynamics of the live weight of the rams and the slaughter qualities were taken into account. The quality of the carcasses was assessed by the morphological composition: the yield of muscle tissue, of fat tissue and of bone, as well as the meat grade composition: the yield of meat cuts of I and II grades of quality.

We used standard methods to examine physicochemical parameters of the mass fraction of water, fat[3], protein[4]; the content of minerals (ash) was determined by dry mineralization of the samples in a muffle furnace; the energy value was determined by calculation. The content of the amino acid tryptophan was determined by colorimetric method according to development of a color reaction between the products of tryptophan decomposition formed during its treatment with concentrated hydrochloric acid and n-dimethylaminobenzaldehyde in presence of sodium nitrate (sodium saltpeter)[5], the content of hydroxyproline was determined by method of alkaline hydrolysis from preliminary defatted sample[6]. The mass fraction of hydroxyproline in meat and meat products was determined in accordance with the procedure specified in this document. The parameter of protein quality was determined by calculation method (the ratio of tryptophan content to hydroxyproline content). To determine the mass fraction of water (W,%) MX-50 infrared analyzer (A&D, Japan) was used for thermogravimetric method. Water mass fraction ranged from 0 to 100%, accuracy was equal to 0.02%, standard measurement mode was applied at temperature of 160 °C. Active acidity (pH) was determined by potentiometric method using an HI 213 microprocessor pH meter (Hanna Instruments, Germany). The pH measurement ranged from 0 to 14, the accuracy was 0.02 pH units [13]. The water activity of raw meat was determined by the cryoscopic method on AVK-10 device. The water activity measurement ranged from 0.75 to 1.00, accuracy was ± 0.0010, resolution: 0.0001, measurement exposure duration varied from 4 to 5 minutes while aw > 0.95) [14]. Water binding capacity (WBC),% of total water, was determined by pressing the sample on filter paper.

To carry out multisensor studies on a "VOCmeter" device, three samples were taken from each analyzed lamb sample. For this, the sample under study was crushed and placed into special glass containers (vials). The vials were tightly closed, sealed and thermostated. At the end of the incubation time, a needle was inserted into the vials for automatic sampling of gas subject to analysis, which gas was transferred to the VOCmeter. The analysis of the received responses of metal oxide sensors (MOS1–4) of an "electronic nose" of the device was run with the help of special software "Argus". Sensors specification:

- aldehydes (M1),
- ketones (M4),
- free amino acids (M3),
- low molecular weight nitrogen-containing compounds (M2)

According to the results of calculating the arithmetic mean and standard deviation for this sample, the standard error of the arithmetic mean and the boundaries of its confidence interval was determined taking into account the Student's coefficient t (n, p) at a significance level of 95% (p = 0.05) and the number of measurements. Significance of differences between the mean values in the empirical and control experiments was assessed by the p-value in case of two-sample unpaired t-test with unequal dispersions. Differences were considered significant and trusted when the inequality $P \geq 0.05$ was achieved. In addition in these cases the adherence of inequality t, t (n, p) was checked up, at

$$n = (df + 1),$$

where

df is the number of degrees of freedom),

$p = 0.05$,

where

$$t = \frac{|x_1 - x_2|}{(s_1^2 + s_2^2)^{1/2}},$$

where

x_1 and x_2 — arithmetic mean values,

s_1 and s_2 are their standard errors for two samples of experimental data [15].

[2] TR CU034/2013 Technical Regulations of the Customs Union "On the safety of meat and meat products" Retrieved from http://docs.cntd.ru/document/499050564. Accessed January 15, 2021. (In Russian)

[3] GOST 23042–2015 "Meat and meat products. Methods of fat determination". Moscow: Standartinform, 2019. — 8 p.

[4] GOST 25011–2017 "Meat and meat products. Protein determination methods". Moscow: Standartinform, 2018. — 14 p.

[5] GOST 34132–2017 "Meat and meat products. Determination of amino acids composition of animal protein". Moscow: Standartinform, 2017. — 16 p.

[6] GOST 23041–2015 "Meat and meat products. Method for determination of oxyproline". Moscow: Standartinform, 2019. — 8 p.

Results and discussion

Studies of the effect of feed additives based on Yoddar-Zn and DAFS-25, as well as their combined use on development and meat yield performance of Edilbaevskiy sheep breed showed that the animals of the experimental groups significantly exceeded the values of the control group.

So, when setting up for an experiment at the age of 4 months, the live weight of rams in the control and experimental groups was approximately the same and amounted to 31.16 ± 0.22 kg in the control group and 31.27 ± 0.19 kg (group I), 31.46 ± 0.17 kg (group II) and 31.68 ± 0.21 kg (group III), respectively. At the end of the experiment at the age of 7 months the animals had the following average weight: in the control group — 40.47 ± 0.31 kg, in the Ist experimental group — 41.63 ± 0.35 kg, the 2nd one — 41.63 ± 0, 35 kg, the IIIrd — 45.21 ± 0.37 kg. During the experiment there was no mortality of the experimental animals caused by diseases. The data on the dynamics of the live weight gain of the experimental young sheep are shown below in Table 1.

At the end of the experiment, the dynamics of the increase in live weight was observed in the rams of the IIIrd experimental group. Those rams received both feed additives (Yoddar-Zn + DAFS-25) in addition to the main farm ration.

The rams of the Ist group, which received an iodine-containing supplement, exceeded the average live weight over the control group by 2.79% ($P > 0.95$), or 1.2 kg.

Animals of the IInd group, which received a selenium-containing additive, had a greater advantage over the control group, respectively by 7.01% ($P > 0.95$), or 3.1 kg. The greatest effect was observed in animals of the IIIrd group, which rations were enriched with both additives. Accordingly, the live weight gain advantage over the control group was 10.48% ($P > 0.95$) or 4.27 kg.

According to the results of calculating the average daily gain in live weight, the rams of the experimental groups surpass their peers in the control group in the age period 120–210 days of age: in the 1st group by 11.70 g, or 10.17% ($P \geq 0.999$); in the second — by 30.4 g, or 22.74% ($P \geq 0.999$); in the third — by 46.40 g, or 31.0% ($P \geq 0.999$), respectively.

The calculation of the absolute weight gains in the live weight of the rams showed that the animals of the experimental groups showed higher body weight gain in comparison with the animals of the control group within the age period 120–210 days: in I group by 0.35 kg, or 10.14% ($P \geq 0.999$); in II group — by 0.91 kg, or 22.69% ($P \geq 0.999$); in III group — by 1.39 kg, or 30.96% ($P \geq 0.999$), respectively (Table 1).

In terms of live weight dynamics, the animals of the experimental groups surpassed the rams of control group, which, in our opinion, was caused by the enrichment of the rations with additives including organic forms of selenium and iodine.

The parameters of the main measurements of the ram body points are presented below in Table 2. According to them, no significant differences were found between the animals of the experimental groups, with the exception of the animals of the third group, which parameters exceeded the control group: in chest circumference — by 6.9 cm ($P > 0.95$), which amounted to 7.2%, in height at withers — by 4.3 cm ($P > 0.99$), that was respectively 8.1%.

Table 1. Dynamics of live weight gain of Edilbaevskiy rams

Parameter	Groups			
	Control group	I	II	II
At birth				
Live weight, kg	3,84 ± 0,03	3,89 ± 0.02	3,93 ± 0,02	3,98 ± 0,03
4 months				
Live weight, kg	31,16 ± 0,22	31,27 ± 0,19	31,46 ± 0,17	31,68 ± 0,21
Absolute gain, kg	27,32	27,38	25,53	27,70
Average daily gain, g	227,7	228,2	229,4	230,8
5 months				
Live weight, kg	34,26 ± 0.18	34,72 ± 0,21	35,48 ± 0,32	36,19 ± 0,21
Absolute gain, kg	3,1	3,45	4,02	4,51
Average daily gain, g	103,3	115,0	134,0	150,3
6 months				
Live weight, kg	37,37 ± 0,21	38,18 ± 0,22	39,51 ± 0,27	40,72 ± 0,25
Absolute gain, kg	3,11	3,46	4,03	4,53
Average daily gain, g	103,7	115,3	134,3	151,0
7 months				
Live weight, kg	40,47 ± 0,31	41,63 ± 0,35	43,52 ± 0,29	45,21 ± 0,37
Absolute gain, kg	3,1	3,45	4,01	4,49
Average daily gain, g	103,3	115,0	133,7	149,7

Table 3 shows the parameters of meat yield performance of rams, which parameters confirm the trend for achieving the body superiority of animals in the experimental groups over the animals of the control group. The superiority was observed: in terms of pre-slaughter weight, i. e. the Ist group exceeded the control group by 1.2 kg, $P > 0.95$ (2.9%), the II[nd] group — by 3.1 kg, $P > 0.95$ (7.01%), the III[rd] group — by 4.7 kg, $P > 0.95$ (10.48%); slaughter yield — by 2.8%; 3.3%; 5.22% respectively.

Parameters of the slaughter yield and meat qualities, obtained as a result of slaughtering animals, also indicate that introduction of new feed additives to the ration contributed to the body superiority of rams at the age of 7 months in comparison with the experimental group over their counterparts from the control group (Table 3).

For the most comprehensive assessment of the meat yield productivity of rams the morphological composition of the carcasses was also studied. Based on the following parameters such as the mass of boneless meat, fat, bones and tendons, the percentage ratios of these parts to the weight of the carcass were determined.

The morphological composition of carcasses was determined by boning of the individual meat cuts (Table 4).

Table 2. Measurements of body points of Edilbaevskiy breed rams, cm

Dimensions	Groups			
	Control group	I	II	III
4 months				
Height at withers	53,21 ± 0,54	53,28 ± 0,14	53,31 ± 0,17	53,37 ± 0,10
Oblique length of torso	54,22 ± 0,18	54,31 ± 0,17	54,39 ± 0,11	54,47 ± 0,14
Chest girth	73,12 ± 0,16	73,22 ± 0,18	73,23 ± 0,16	73,42 ± 0,21
Chest width	18,23 ± 0,21	18,34 ± 0,23	18,37 ± 0,25	18,41 ± 0,23
Chest depth	29,31 ± 0,17	29,33 ± 0,15	29,35 ± 0,18	29,39 ± 0,12
Rump height	59,64 ± 0,12	59,50 ± 0,14	59,76 ± 0,13	59,87 ± 0,18
Pastern girth	9,31 ± 0,18	9,38 ± 0,18	9,42 ± 0,19	9,44 ± 0,16
Width in hips femurs	12,26 ± 0.14	12,28 ± 0,16	12,31 ± 0,17	12,32 ± 0,22
7 months				
Height at withers	62,22 ± 0,22	63,74 ± 0,22	64,26 ± 0,24	66,47 ± 0,22
Oblique torso length	61,75 ± 0,14	62,24 ± 0,13	63,38 ± 0,16	64,01 ± 0,14
Chest girth	88,67 ± 0,21	91,36 ± 0,17	94,29 ± 0,18	95,65 ± 0,21
Chest width	23,54 ± 0,22	24,75 ± 0,22	25,17 ± 0,20	26,22 ± 0,16
Chest depth	32,33 ± 0,17	33,19 ± 0,18	33,77 ± 0,11	34,28 ± 0,14
Rump height	64,89 ± 0,13	65,38 ± 0,12	67,13 ± 0,18	68,05 ± 0,16
Pastern girth	9,74 ± 0,18	9,77 ± 0,17	10,02 ± 0,13	10,14 ± 0,15
Width in hips femurs	14,18 ± 0,12	14,35 ± 0,11	14,57 ± 0,15	14,72 ± 0,14

Table 3. Slaughter parameters of Edilbaevskiy breed rams (n = 5)

Parameter	Groups			
	Control group	I	II	III
4 months				
Pre-slaughter weight, kg	31,16 ± 0,22	31,27 ± 0,19	31,46 ± 0,17	31,68 ± 0,21
Carcass weight, kg	11,94 ± 0,17	11,96 ± 0,21	12,15 ± 0,18	12,24 ± 0,15
Internal fat mass, kg	0,61 ± 0,01	0,63 ± 0,22	0,64 ± 0,01	0,67 ± 0,02
Fat tail weight, kg	1,73 ± 0,18	1,76 ± 0,14	1,77 ± 0,12	1,79 ± 0,15
Slaughter weight, kg	14,28 ± 0,21	14,35 ± 0,14	14,56 ± 0,21	14,70 ± 0,19
Slaughter yield,%	45,83	45,89	46,28	46,40
7 months				
Pre-slaughter weight, kg	40,47 ± 0,31	41,63 ± 0,35	43,52 ± 0,29	45,21 ± 0,37
Carcass weight, kg	16,21 ± 0,21	17,15 ± 0,17	18,05 ± 0,26	19,24 ± 0,21
Internal fat mass, kg	0,78 ± 0,05	0,82 ± 0,04	0,85 ± 0,03	0,97 ± 0,04
Fat tail weight, kg	2,31 ± 0,23	3,05 ± 0,27	3,31 ± 0,18	3,71 ± 0,15
Slaughter weight, kg	19,30 ± 0,21	21,02 ± 0,24	22,21 ± 0,30	23,92 ± 0,28
Slaughter yield,%	47,69	50,49	51,03	52,91

Table 4. Morphological composition of ram carcasses (n = 5)

Parameter	Groups			
	Control group	I	II	III
4 months				
Carcass weight, kg	11,94 ± 0,17	11,96 ± 0,21	12,15 ± 0,18	12,24 ± 0,15
Content in the carcass: of boneless meat, kg	9,26 ± 0,16	9,29 ± 0,18	9,44 ± 0,12	9,53 ± 0,16
%	77,53	77,64	77,71	77,82
of bones, kg	2,68 ± 0,11	2,67 ± 0,14	2,71 ± 0,13	2,71 ± 0,11
%	22,47	22,36	22,29	22,18
Meat factor	3,46	3,48	3,48	3,52
“Muscle eye” area, cm^2	8,86	8,88	9,14	9,27
7 months				
Carcass weight, kg	16,21 ± 0,21	17,15 ± 0,17	18,05 ± 0,26	19,24 ± 0,21
Content in the carcass: of boneless meat, kg	12,69 ± 0,17	13,46 ± 0,23	14,28 ± 0,24	15,31 ± 0,26
%	78,31	78,46	79,11	79,56
of bones, kg	3,52 ± 0,11	3,69 ± 0,15	3,77 ± 0,10	3,93 ± 0,12
%	21,69	21,54	20,89	20,44
Meat factor	3,61	3,65	3,79	3,90
“Muscle eye” area, cm^2	12,54	12,83	13,21	13,61

Cutting carcasses in order to determine the morphological composition showed superiority of experimental rams over the control group animals at 7 months of age. The content of boneless meat in the carcasses of the III[rd] group was higher than in the control group by 2.62 kg (17.0%). Also, the III[rd] group had the highest meat factor — 3.90.

The cross-sectional area of the *m. longissimus dorsi*– the “muscular eye” — was determined on the anterior section of the *m. longissimus dorsi*, along the border between the last thoracic and the first lumbar vertebrae (Table 4). The best parameters of the “muscular eye” also were recorded in carcasses of the III-rd experimental group — 13.61 cm^2.

Table 5 below shows the data of physical and chemical parameters of *m. Longissimus dorsi*. This muscle was chosen as parametrical point because of the high variability of the composition and properties of raw meat.

Lamb from animals of the experimental groups slightly differed for the better, this lamb showed a higher protein content and less fat. The water-to-protein ratio in all samples was slightly higher than 3.7, which corresponds to the Federzahl number for raw meat [16].

The pH parameter in the control sample is slightly higher than in the experimental ones, and is directly proportional to the parameter of water activity: along with decrease in pH the activity of water also decreases. The water binding capacity of the samples also decreases with decreasing of pH. The pH level in all samples was typical for the normal course of the autolysis process.

Table 6 below presents the results of a study of the qualitative protein quotient of lamb muscle tissue based on various feeding rations.

In terms of tryptophan content the meat obtained from animals of the experimental and control groups had no

Table 5. Physical and chemical parameters *m. Longissimus dorsi* **obtained from rams of various feeding rations**

Parameters	Groups under study			
	Control group	I	II	III
Water content,%	75,96 ± 0,62	76,99 ± 0,64	77,13 ± 0,58	76,56 ± 0,96
Mass fraction of fat,%	2,62 ± 0,34	1,62 ± 0,28	1,27 ± 0,19	1,98 ± 0,29
Protein mass fraction,%	16,43 ± 2,01	16,93 ± 2,40	17,04 ± 2,35	16,98 ± 0,41
Mass fraction of ash,%	1,01 ± 0,15	1,05 ± 0,06	1,08 ± 0,08	1,06 ± 0,08
pH	5,716 ± 0,013	5,635 ± 0,080	5,617 ± 0,016	5,597 ± 0,007
Water activity	0,9872 ± 0,0011	0,9859 ± 0,0009	0,9850 ± 0,0010	0,9841 ± 0,0015
WBC,%	82,4 ± 0,5	77,8 ± 0,3	77,1 ± 0,4	76,3 ± 0,3
Dry matter,%	20,06	19,60	19,39	20,02

“M ± m” — mean ± standard error of the mean

Table 6. Qualitative protein quotient of lamb muscle tissue based on various feeding rations

Parameter	Control group	I group (Yoddar-Zn)	II group (DAFS-25)	III group (Yoddar-Zn+DAFS-25)
Tryptophan	0,310 ± 0,023	0,292 ± 0,031	0,661 ± 0,048	0,320 ± 0,030
Oxyproline,%	0,108 ± 0,013	0,063 ± 0,010	0,138 ± 0,016	0,056 ± 0,007
Qualitative protein quotient (QPQ)	2,87	4,64	4,79	5,69

Table 7. Amino acid composition of ram meat

Amino acids	Content of free amino acids (g/100 g of product)			
	Lamb of control group	Lamb from the I group (Yoddar-Zn)	Lamb from the II group (DAFS-25)	Lamb from the III group (Yoddar-Zn+DAFS-25)
Aspartic acid	1,19 ± 0,36	1,33 ± 0,20	1,12 ± 0,17	1,31 ± 0,21
Glutamic acid	2,42 ± 0,09	3,51 ± 0,57	2,52 ± 0,42	3,71 ± 0,60
Serine	0,58 ± 0,08	0,62 ± 0,09	0,50 ± 0,07	0,65 ± 0,10
Histidine	0,51 ± 0,10	0,80 ± 0,12	0,42 ± 0,06	0,84 ± 0,13
Glycine	0,64 ± 0,08	0,58 ± 0,09	0,76 ± 0,11	0,61 ± 0,09
Threonine	0,54 ± 0,06	1,12 ± 0,17	0,49 ± 0,06	1,18 ± 0,18
Arginine	0,39 ± 0,11	0,68 ± 0,10	0,58 ± 0,09	0,72 ± 0,11
Alanine	0,73 ± 0,07	0,71 ± 0,11	0,76 ± 0,11	0,75 ± 0,11
Tyrosine	0,47 ± 0,03	0,69 ± 0,10	0,56 ± 0,08	0,71 ± 0,11
Cystine	0,18 ± 0,11	0,22 ± 0,03	0,16 ± 0,02	0,25 ± 0,03
Valin	0,73 ± 0,07	0,74 ± 0,11	0,75 ± 0,11	0,78 ± 0,12
Methionine	0,42 ± 0,09	0,40 ± 0,06	0,47 ± 0,04	0,48 ± 0,06
Phenylalanine	0,69 ± 0,15	0,67 ± 0,06	0,79 ± 0,12	0,79 ± 0,06
Isoleucine	0,99 ± 0,18	1,03 ± 0,15	0,86 ± 0,13	1,08 ± 0,16
Leucine	1,18 ± 0,15	0,98 ± 0,15	0,95 ± 0,14	1,03 ± 0,15
Lysine	0,99 ± 0,07	0,96 ± 0,14	1,02 ± 0,15	1,01 ± 0,15
Proline	0,59 ± 0,36	0,50 ± 0,08	0,66 ± 0,10	0,53 ± 0,08
Total	13,30 ± 2,00	15,54 ± 2,33	13,37 ± 2,01	16,37 ± 2,46

significant difference. The content of oxyproline was the smallest in lamb of the III[rd] group, which contributed to an increase in the qualitative protein quotient from 2.87 in the control group to 5.69, i. e. by 49.56%.

The results of study of amino acid composition of lamb (Table 7) obtained from the rams of various feeding rations, showed the increase of total amount of free amino acids in lamb of experimental groups.

The lamb from the III[rd] experimental group has a higher nutritional value. It showed its superiority in amount of free amino acids by 18.8% in comparison with meat obtained from the control group.

The content of nonessential amino acids in the protein also was increased, in particular, the greatest increase in content of cystine, arginine, aspartic and glutamic acids was observed [12].

Taking into consideration the fact that the lamb of the III experimental group had a higher nutritional value, the multisensory studies of lamb obtained from animals of this group were carried out in comparison with the control group.

Figure 1 below shows the profile diagrams of the "visual fingerprint" of the samples smell being under study.

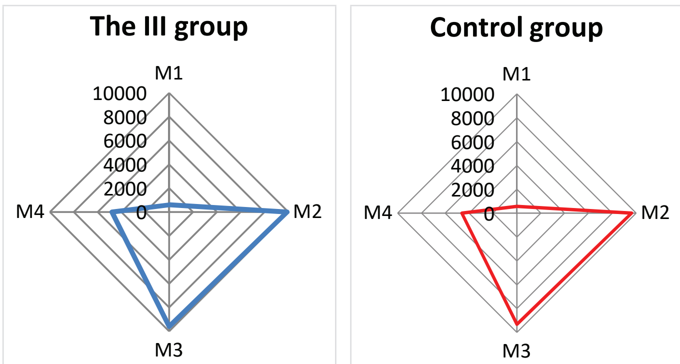

Figure 1. Profile diagrams of the "visual print" of the smell

The Table 8 below shows the "visual prints" of the lamb samples smell.

Table 8. Areas of the "visual prints" of smell

Sample	Area of the "visual prints" of smell, cond. units × 10^7
Control group	7,04
III group	7,49

According to the results of the study, it was found that the intensity of the smell of lamb sample from the III group is 0.58% higher than the lamb sample of the control group, as evidenced by the area of "visual prints" of smell (Table 8). The profiles of "visual prints" of lamb samples from the III and control groups differ in reading of M3 sensor, which showed a slightly higher content of free amino acids in the gas phase of the lamb sample obtained from the IIIrd group.

The higher sensory readings of lamb obtained from the IIIrd experimental group are explained by the fact that the sum of aroma-forming amino acids (valine, serine, methionine, glutamine, histidine, alanine) in meat of these animals was 25.2% higher than in the control group (Table 7). Lamb obtained from the IIIrd experimental group features a higher content of nitrogenous extractives (methionine) involved in formation of a specific flavor and taste and stimulating the secretory function of the digestive tract, which improves the digestibility of the lamb [17].

Conclusion

Enriching the diets of Edilbaevskiy breed sheep with feed additives based on "Yoddar-Zn" and "DAFS-25" in combination with a protein-carbohydrate complex stimulates the growth and development of animals, increases the meat yield performance and nutritional value of lamb:

— the dynamics of increase in live weight gain in rams of the experimental groups exceeded the analogous parameters of control group;

— the animals of the IIIrd group showed better body parameters in comparison with the control group: in chest circumference — by 6.9 cm ($P > 0.95$), which was 7.2%, in height at withers — by 4.3 cm ($P > 0.99$), respectively 8.1%.;

— in terms of meat yield performance of rams the experiment confirmed the tendency of the advantage of animals from the experimental groups over the control group, the superiority in terms of pre-slaughter weight was from 1.2 kg to 4.7 kg;

— the content of boneless meat in the carcasses of the IIIrd group exceeded the same parameters of the control group by 2.62 kg (17.0%);

In terms of content and qualitative quotient of protein and fat, the lamb of the experimental batches outdid the parameters of the control group, which confirms the efficiency of introduction of the rations enriched with essential microelements, the lamb obtained from the animals of the experimental groups also has a higher nutritional value.

The obtained results allow stating that the development and implementation of technologies for formation of meat yield performance of small ruminants, the production of lamb of the predictable composition and quality due to lifetime enrichment of the animal body with essential microelements, is a priority sphere of activity, which solution has scientific and practical importance for implementation of the Food Safety program of the Russian Federation.

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RISK IDENTIFICATION AND ASSESSMENT IN PRODUCTION OF MEAT PRODUCT PACKAGING

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Keywords: *meat packaging, polypropylene packaging, packaging materials, risk assessment, safety system*

Abstract
Production of convenient and inexpensive packaging materials is increasing due to the constant growth of consumer demand for safer food products. The paper examines the questions dedicated to the development and introduction of the safety and quality management system in enterprises producing packaging for the meat industry. The authors analyze the elements of safety and quality management for polypropylene packaging in a form of a tray, which is a final element of the united chain in production of whole-piece meat semi-finished products. The investigations were carried out in the operating enterprise in the Moscow region. Hazardous factors in production of polypropylene packaging were identified, risk analysis with assessment of the probability of emergence and realization of hazardous factors was carried out, a Pareto chart was built, unacceptable risks were determined, critical control points (CCP) were revealed, preventive and corrective measures were developed with account for the established critical limits and requirements for CCP monitoring were formulated. All stages of production process were subjected to risk analysis; the severity of consequences from hazardous factor realization and the probability of such realization were assessed by experts for each of these stages. It was established that "injection molding and chilling in a press-mold" is a CCT as there is a risk of increasing the maximum allowable concentrations of chemical substances and compounds (formaldehyde, ethyl acetate, alcohols and others) as a chemical factor with the severity of consequences of 3 and the probability of realization of 3. Using the Pareto chart, causes that had the highest effects on safety and quality of polypropylene packaging were grouped. It was established that nonobservance of preventive maintenance schedule for equipment and, as a consequence, possible equipment failure (80%) influenced to the higher degree the realization of chemical hazardous factor.

Introduction

In the modern world, high requirements are imposed to quality and safety of food products. Food enterprises that strive to be competitive and want to keep their place in the market should take into account risks associated with product safety assurance. This explains the active development and worldwide introduction of such management systems that would guarantee stable quality and safety of manufactured products for consumers [1,2].

Nowadays, enterprises use many certification standards and schemes according to the requirements of food safety and quality management systems; however, a basic model is the HACCP system (Hazard Analysis and Critical Control Points) [2,3,4].

HACCP is an instrument used to assess hazards and risks, and establish specific control measures that emphasize prevention, rather than final product testing. An increase in public awareness about food safety has led to the fact that manufacturers began to demand higher standards from their suppliers. Suppliers of raw materials, ingredients and food packaging should bring their hygiene standards in correspondence with expectations of the meat industry. Food manufacturers should be guaranteed that packaging has no negative effects on their products. HACCP is a method that can be used for safety assurance [5].

Based on the research carried out in 100 companies in the packaging sector in Poland [6], it was confirmed that hazard analysis and critical control points (HACCP) are the most effective management instrument for ensuring safety of manufactured products (50%). Moreover, enterprises develop, introduce, maintain and actualize programs of prerequisite measures based on Good Manufacturing Practice (GMP-74%) and Good Hygiene Practice (GHP). They include sanitation, zonation of territories according to a type of production process, storage, distribution and transportation, personal hygiene of employees, deratization and disinsection, water supply, illumination and ventilation, proper wastewater disposal, proper work of equipment; that is, they ensure the main conditions and measures necessary to maintain the basic level of sanitary and hygiene in an enterprise. Only 8% of the surveyed manufacturers introduced BRC. The fact that 10% of manufactures and even 45% distributers have not introduced any system (Figure 1) is also of concern [6].

Introduction of safety and quality management systems give enterprises several advantages:

— system approach to safety assurance for manufactured products;
— increase in consumer confidence in manufactured products;
— possibility to enter new markets;

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— savings due to an increase in efficiency and improvement in labor performance;
— additional advantages when taking part in important tenders;
— optimization of management processes;
— distribution of authority, responsibility and communication of personnel;
— use of preventive measures rather than late actions on correction of defects and product recall;
— identification of food risks and introduction of the necessary work order;
— reduction of the number of claims due to assurance of stable product quality;
— increase in competitiveness of an enterprise;
— creation of the reputation as a manufacturer of safe and quality products;
— significant decrease in the level of non-compliant products due to the use of preventive and corrective measures;
— documentation of safety of manufactured products [7,8,9].

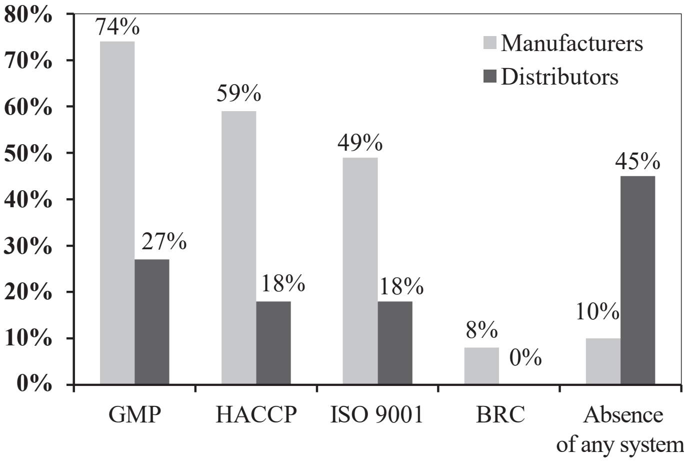


Figure 1. Introduction of safety and quality management systems in the packaging industry in Poland [6]

The role of packaging in the meat industry is quite high. First of all, it is food product preservation, their protection from chemical and physical damage, minimization of losses and reduction of preserving agents used in products. Packaging also helps to protect a shape and texture of meat products, prevent the loss of aroma and odor and extend shelf life due to the long-term preservation of their freshness [10].

Therefore, formation of meat product safety directly depends on packaging material quality. Food contact packaging should not be a source of biological (or microbiological), chemical or physical hazards [11,12,13].

At present, the production and use of polypropylene packaging is growing with increasing rates, which can be explained by low specific mass at relatively high density, chemical inertness, low brittleness, easiness in staining, high processability and replaceability. However, several disadvantages of this packaging type can be highlighted, such as:

— off-odor from packaging that is not peculiar to a product;
— aging caused by the action of air oxygen, aggressive media and sun sight (in other words, photoaging);
— possible migration of organic/chemical substances into a packed product.

According to a functional purpose, polypropylene packaging is classified into consumer, industrial and transport. Consumer packaging from polypropylene became most common in agriculture, and in particular, in the meat industry. Its main task is to protect meat products from deformations, damage, spilling, drying and other types of losses. A shape, design and capacity of such packaging are determined according to properties and configuration of packed products and a method for its production. Packaging can have a capacity from several kilograms to several tens of kilograms. Consumer polypropylene packaging can be tough and soft. The main methods of its production are injection molding, thermal and vacuum forming and pressing [14].

Polypropylene is a product of propylene polymerization; i. e., it is a synthetic polymer. Over the last years, polypropylene has been more often used in manufacturing packaging for meat products. It is driving back polystyrene due to its larger strength and chemical stability, as well as many kinds of polyethylene due to its toughness and glossiness.

Chemical stability of polypropylene resides in the fact that only highly concentrated strong oxidizers can exert a notable effect on it, namely, chlorosulfonic acid, fuming nitric acid, sulfuric acid and halogens. Polypropylene has high chemical stability to contacts with alkali, salt solutions, mineral and vegetable oils, as well as alcohol containing products.

Polypropylene is quite sensitive to action of oxygen, especially at high temperatures, due to the presence of tertiary carbon atoms. This explains it proneness to aging, which can occur very quickly compared to other polymers and can be accompanied by a sharp decrease in its mechanical properties. Therefore, low doses of low molecular weight additives (stabilizers) that protect it from destruction both in the process of processing and during its use are added to avoid aging.

To protect polypropylene from light aging, light stabilizers are used. Their action resides in filtration of ultraviolet irradiation and its transformation into heat energy. Antioxidants help to avoid thermal oxidative aging of polypropylene.

Polypropylene is also quite water-stable material. After long contact with water during six month, water absorption is less than 0.5% (at a room temperature).

Plastic masses serving as raw materials for production of a polypropylene tray practically never consist of a single polymer material. To impart different performance characteristics, many various additives are introduced such as plasticizing agents, filling agents, stabilizing agents, crosslinking agents, colorants, foaming agents, lubricating agents and so on. Compositions of plastic masses with equal distribution of raw materials are produced by mixing. To impart specific shape and size to polypropylene particles, granulation is carried out followed by the plasticization process — heating and homogenization of produced granules. To facilitate the process of the following formation when making a tray, polypropylene is solved in different types of liquids [15].

A wide geometric variety of packaging for meat products is presented in the modern market. There are polypropylene films, bags, containers and trays with different shapes and designs. All packaging types take into consideration product peculiarities and properties in one way or another. As meat products are perishable, most manufacturers prefer to pack a finished product immediately after production before chilling. Moreover, packaging of whole-piece meat semi-finished products should attract attention of buyers. These goals can be achieved by using different technologies, materials and substances [15,16].

One of the most important packaging types for meat semi-finished products is a polypropylene tray that performs a range of the above mentioned functions, which in combination with special methods for designing and processing often allows obtaining economically beneficial solutions that justify its leading position in the market of packaging materials. In this regard, many Russian and foreign manufacturers prefer to use this type of packaging. With that, trays can be hermetically sealed, filled with a special gas or vacuumized, which allows the significant extension of product shelf life due to a decrease in the microbial ability to multiply. As a gas environment, inert gases mixed in certain proportions are used, which composition always depends on a particular packed product.

Packaging materials in normal or predicted conditions of the use should not lead to a risk of meat product contamination with chemical components in amounts that can present a risk for consumer health; changes in the finished product composition and deterioration of their sensory characteristics are also unacceptable. This requirement is applicable to all materials that come into contact with a product through direct contact or as a result of air exchange between them.

Packaging materials mainly present hazard of meat product contamination due to migration of packaging chemical substances (Figure 2). It is a multi-factor process that depends on the nature of a product and contacting material, as well as storage conditions (contact duration, temperature, humidity, light access and so on). Undoubtedly, emergence of modern inexpensive materials with antiseptic properties in the market and their introduction into production of packaging enable extending product shelf life. However, in many cases, their components are a threat to consumer health per se. A strict control of incoming raw materials and conditions of technological operations is necessary to avoid contamination of packaging materials and, as a consequence, meat products with chemical substances and compounds [16].

A serious threat for safety of polypropylene packaging can present such contaminants as molds, residues of cleaning agents, disinfectants, lubricating agents, foreign substances that enter the packaging environment due to non-observance of personal hygiene by employees or violation of sanitary rules in production as well as chemical risks. An unintentional introduction of foreign substances into the composition of raw plastic masses or directly to finished packages is also possible [16].

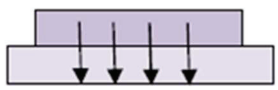
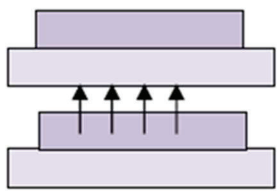
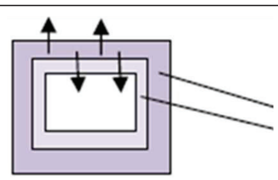
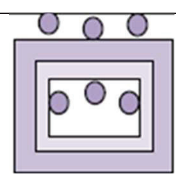
1.	**Penetration** through the substrate to the back side (thin film)		ink substrate
2.	**Contact (set off)** Transfer of print to the back side of the print in a stack or roll during drying		ink substrate ink substrate
3.	**Evaporation** Migration of components when cooking products in packaging		ink substrate
4.	**Extraction upon condensation** Migration of hazardous components during cooking/sterilization		ink substrate

Figure 2. Migration of packaging chemical substances

It is necessary to note that typical hazardous factors, measures for their prevention and correction and monitoring procedures regarding production of packaging materials for meat products are not established in Russia up to date. Therefore, based on the above stated, studies aimed towards developing elements of safety and quality management in their production are timely and topical.

Materials and methods

Taking into consideration the necessity of the effective risk analysis, in 2020, the Gorbatov Research Center for Food Systems carried out the research aimed towards developing elements of safety and quality management of packaging in a form of a tray, which is a final element of the united chain in production of whole-piece meat semi-finished products. The object of the research was packaging in a form of a tray from polypropylene as well as the system of management of hazardous factors in its production.

Within the framework of the studies carried out at the first stage of the work, the provisions of the HACCP system were realized, including:

— hazardous factors typical for production of polypropylene packaging were identified and described successively for each stage of the technological process;
— risk analysis was carried out — the probability of occurrence and realization of hazardous factors in the production process, as well as severity of consequences of their realization for an ultimate consumer were assessed;
— critical control points (CCPs) were revealed; for each CCP, the critical control limits were established;
— a Pareto chart was built to reveal the main causes influencing CCP realization;
— unacceptable risks were determined as well as operations and processes in production of polypropylene packaging where their appearance that leads to a negative effect on safety and quality of whole-piece meat semi-finished products is possible;
— preventive and corrective measures were developed with consideration for the established critical limits;
— requirements for CCP monitoring were established.

It is necessary to note that:

1. All studies of the production process were analyzed. First of all, the revealed risk assigned to the category of unacceptable risks (the zone of the high and medium risk) were taken into account.
2. If the same unacceptable risk could occur at several successive stages of the production process, the probability of its realization at later stages with regard to preventive actions on the preceding stages of the production process was analyzed (for example, regarding the microbial growth);
3. If the same unacceptable risk could occur at several successive stages of the production process and its realization at later stages in no way depended on control and preventive actions at the preceding stages of the production process, then the control was carried out at all indicated stages (for example, introduction of foreign substances).

To reveal critical control points, the method was used that envisaged the expert comparative assessment of the severity of consequences from realization of this factor and the probability of occurrence of this risk for each determined hazardous factor in production of a polypropylene tray for whole-piece meat semi-finished products using designations [17,18].

Probability of realization	Score	Severity of consequences
Unlikely (for example, once in several years)	1	Insignificant consequences (for example, minor ailment that did not lead to serious disorders in the human body);
Very seldom (for example, once a year)	2	Consequences of medium severity (for example, infliction of harm that disappears over time without hospitalization);
Seldom (for example, once a month)	3	Severe consequences (for example, serious disorders in the human body leading to hospitalization);
Quite often (for example, every week, every day)	4	Critical consequences (for example, serious deterioration of human health that caused long-term disability or death).

To detect CCT, it is necessary to calculate the aggregate coefficient, which was determined by the following way:

aggregate coefficient = product of scores / sum of scores

After all calculations, a scale for CCP determination from the min to max values (in this case from 0 to 1.5) was constructed (Figure 3).

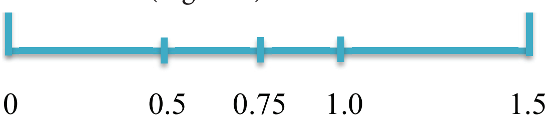


Figure 3. The scale for CCP determination
from 0 to 0.75 — are not CCPs;
from 0.76 to 1.1 — points that envisage the use of control to prevent a hazard but are not critical;
from 1.2 and higher are CCPs.

Results and discussion

The first stage of activities towards developing elements of safety and quality management of polypropylene packaging for whole-piece meat semi-finished products was a construction of a production flow-chart. In this experimental work, packaging in a form of a tray was investigated. One of the widespread technological methods used in production of this type of packaging from polypropylene is injection molding.

In this method, initial polypropylene material in a form of granules or powder is loaded into a bin of a compression machine, where it is taken by the screw and is transported along the axis of the heated cylinder into its nozzle end being transformed from the solid to molten state. As the necessary volume of polymer melt is accumulated, it is injected due to the translational motion of the screw through the special nozzle to the closed injection mold, where chilling is performed. After the cavity of the form is filled, the polymer melt stays in it for some time under pressure and then is chilled. Then the injection mold is opened and a final product is removed from its cavity. After that, an excess of polypropylene is removed and the product edges are cut with special mechanisms.

The flow chart for production of polypropylene packaging used in the present study is presented in Figure 4.

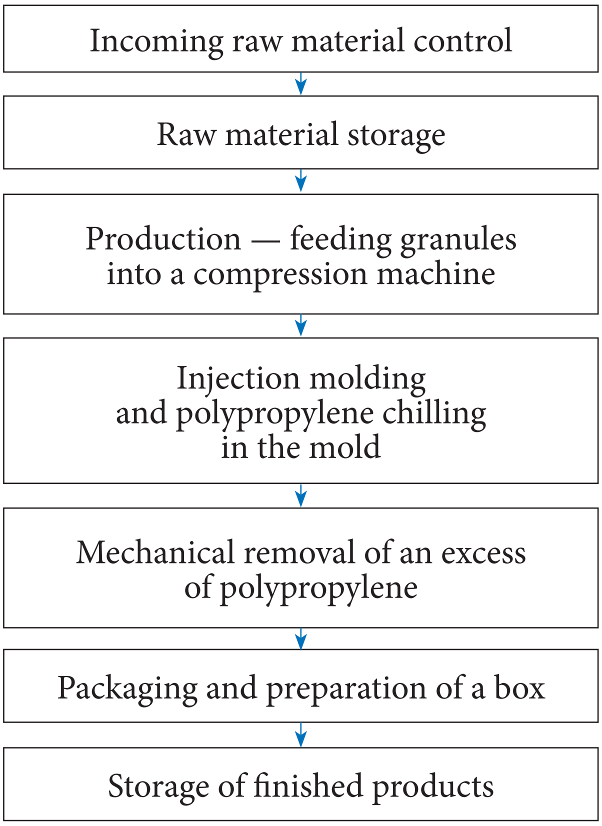


Figure 4. Flow chart for production of polypropylene packaging for whole- piece meat semi-finished products

The method of injection molding has several advantages compared to other methods of forming products from polypropylene. Among them are the high level of productivity, mechanization and automation of the realized process, a small amount of production waste and a possibility of setting practically any distribution of the product wall thickness.

After making a flow chart of production of packaging from polypropylene, potential hazardous factors (biological, chemical, physical), which can be realized at any technological stage, were identified. The result of determination that include their name, brief characteristics and assessment of the degree of their hazard for human life and health is presented in detail in Table 1.

It can be seen from this table that the serious threat for finished product safety can be posed by contaminants such as opportunistic microorganisms, molds, residues of cleaning agents and disinfectants, lubricating agents entering the packaging environment due to non-compliance of personal hygiene by employees or violation of sanitary rules in production. An unintentional introduction of foreign objects into the composition of plastic masses or directly final packaging is also possible. With that, it is worth noting that polypropylene packaging being an object of the investigation in the present study poses a hazard directly by migration of plasticizing agents, heat stabilizers (epoxidized vegetable oils), lubricating agents, light stabilizers, antioxidants, solvents (carbohydrates, alcohols, glycol ethers, ketones and esters), as well as other chemical compounds [16].

When analyzing chemical hazardous factors, it is also necessary to consider procedures of internal control as emerging risks can be minimized due to the use of permitted and nontoxic cleaning agents for disinfection with corresponding supporting documentation (certificates of correspondence, declarations, instructions). It is also important to note appropriate training of cleaning personnel, control of disinfection procedures, effectiveness of cleaning of equipment, utensils and containers [13].

After identification of hazardous factors, the experts performed the risk analysis, which results are presented in Table 2.

As a result of analysis of risks influencing safety and quality of finished products, it was found that the main cause of the possible realization of a biological hazardous factor is non-observance of the established temperature and humidity conditions in warehouses for storage of raw materials and finished products. Absence or insufficient use of bactericidal lamps in the production zone, which work duration should be strictly regulated in the recording documents, also leads to an increase in undesirable microorganisms.

Personnel of an enterprise, in turn, also influence an appearance of the biological risk due to non-observance of rules of personal and production hygiene. Therefore, when developing and introducing preventive measures, manufacturers of packaging should plan timely training of each employee taking part in a technological process, including by the development of work instructions on the rules of personal hygiene, cleaning and disinfection of equipment, containers and utensils, as well as requirements for visitors of an enterprise and corresponding briefing.

During analysis of appearance of the physical risk, it was established that the most significant causes are personnel (the presence of foreign objects, non-observance of deratization and disinsection measures) and equipment failure (penetration of details and pieces).

Based on the revealed values, the expert group detected CCPs by using the scale presented in Figure 2.

Therefore, the stage “injection molding and chilling of polypropylene in a mold” was classified as a critical control point. As was described above, the process of polypropylene packaging production for meat products envisages introduction of acetaldehyde, formaldehyde, ethyl acetate,

Table 1. Identification of hazardous factors for production of polypropylene tray

Biological hazardous factors		
Name	Brief characteristics	Degree of hazard. Severity of consequences
QMAFAnM, CFU/g	QMAFAnM includes different taxonomic groups of microorganisms — bacteria, yeasts, molds. Exceeding indicates non-observance of the sanitary-hygienic regimes.	Foodborne Toxicoinfections. Severity of consequences — low/medium. Inflammatory process in the gastrointestinal tract after eating food products containing living microorganisms in large quantity (not less than 107–108 in 1 g).
Coliforms, *E. coli*	Indicator microorganisms Assigned to opportunistic microorganisms Their presence indicates non-observance of the sanitary-hygienic regimes. Their presence in the finished products indicate the initial high contamination of raw materials.	Foodborne toxicoinfections. Severity of consequences — low/medium. Inflammatory process in the gastrointestinal tract after ingestion of enteropathogenic strains.
Molds	Molds secrete more than 400 different mycotoxins, all of which are toxic for any individual.	Severity of consequences — low/medium. Mold colonies produce aflatoxins, which can accumulate in the body and cause liver oncological diseases over time. Moreover, molds can be a cause of diseases of the gastrointestinal tract of different types and severity.
Giardia cysts	Giardia exists in two forms: motile (vegetative) and nonmotile (cyst form). Cysts are a nonmotile form of parasite life, which ensures survival of Giardia during long periods of time. At high humidity, cysts survive for over 2 months.The spore form of the parasite is tolerant to chlorine containing disinfectants. The motile form of Giardia has four pairs of flagella and the adhesive disk, by which it attaches to small intestinal mucosa. Infection occurs after ingestion of food and water contaminated by Giardia cysts, as well as through hands and surfaces contaminated by cysts.	Giardiasis Giardiasis is one of the most common parasitic diseases. Giardiasis is dangerous both for adults and children. In terms of human infection, a dose of about 10–100 is considered dangerous. Therefore, for disease onset and development, even minimal contamination of the adult or child environment is sufficient. If from one to 10 cysts enter the body, the risk of infection is about 10–30%. Severity of consequences: high
Chemical hazardous factors		
Formaldehyde	Formaldehyde has properties of an excellent antiseptic. Therefore, it is actively used in packaging production.	Severity of consequences — medium/ severe. Formaldehyde can cause oncological diseases.
Acetaldehyde	Acetaldehyde is formed upon polypropylene thermal processing.	Severity of consequences — medium/ severe. Depending on the concentration, symptoms range from slight irritation of the conjunctiva and upper respiratory tract to asphyxia, severe cough and stupor.
Ethyl acetate	Ethyl acetate forms during production of the plastic mass upon reaction of acetic acid and ethanol.	Severity of consequences —мmedium/ severe. Skin irritation; long inhalation can cause kidney and liver damage; it is toxic for the nervous system.
Hexane, hexene, heptane, heptene	These organic compounds are used for polypropylene production and preliminary processing.	Severity of consequences — medium/ severe Exert irritating action on skin; when ingested — lung damage; when inhaled, causes dizziness.
Acetone	Organic substance used as a solvent in polypropylene production.	Severity of consequences — medium/severe Symptoms, such as abdominal pain, cyanosis, vomiting, nausea, can be observed
Alcohols (methyl, propyl, isopropyl, butyl, isobutyl)	Alcohols are used as solvents in polypropylene production.	Severity of consequences — severe/critical Appearance of severe headache, nausea, vomiting, disorder of the digestive system, intoxication of the whole body.
Residues of cleaning agents and disinfectants, lubricating agents	Elements of cleaning agents and disinfectants: sodium hypochlorite, chloramine, sodium carbonate, liquid hand soap. Abundant lubrication of the working surface of the equipment with oils can lead to contamination of raw materials and finished products.	Severity of consequences — low/medium. Depending on the chemical composition of a disinfectant: from mild intoxication to foodborne poisoning.
Physical hazardous factors		
Foreign objects from equipment	Small parts of the equipment (nuts, wood screws, bolts, screws, pieces of electrical wires, pieces of the transporter belt).	Severity of consequences — low/medium. When ingesting small objects (less than 0.5–1 mm) that are not sharp — unpleasant sensations. When ingesting large objects or objects with sharp edges, it is possible to injure teeth, oral cavity, esophagus and stomach up to bleeding.
Foreign objects from personnel	Personal effects: buttons, earrings, jewelry, small objects, gloves. Waste products from personnel — hair, fingernails.	Severity of consequences — low/medium. They can possibly cause physical traumas, as well as aesthetic aversion.
Polypropylene residues	Introduction of polypropylene particles into final packaging for whole-piece meat semi-prepared products, which lead to changes in their taste and deterioration of consumer properties.	Severity of consequences — low/medium.
Pests (rodents, insects)	Introduction of waste products from different pests into finished products due to the insufficient level of hygiene in production facilities	Severity of consequences — low/medium. They can cause aesthetic aversion, as well as infectious diseases transmitted by pests.
Dust	It is a mechanical contaminant and a carrier of saprophytic microflora causative agent of spoilage, mold spores)	Severity of consequences -low

Table 2. Analysis of risks in production of polypropylene packaging

A stage of the production process	Description of a hazardous factor	Type of a hazardous factor	Preventive actions	Controlled traits	Responsible person	Severity of consequences	Probability of realization	Aggregate coefficient = product of scores/ sum of scores
1	2	3	4	5	6	7	8	9
Incoming raw material control	Presence of pathogenic microorganisms in raw materials and other materials	B	— control of the hygienic state of supplied raw materials; — work with approved suppliers; — control of supporting documentation.	- correspondence of the values of microbiological indicators specified in supporting documentation to the requirements of the regulatory documentation; — correspondence of the values of the physicochemical indicators specified in supporting documentation to the requirements of the regulatory documentation; — shelf life; — presence of the corresponding package of documents.	warehouse superintendent	2	2	4/4=1
	Increased or decreased content of chemical substances	Ch	— work with approved suppliers; — control of supporting documentation.	-presence of the corresponding package of documents (safety data sheet, certificate of quality).	warehouse superintendent	2	2	4/4 = 1
	Presence of foreign objects in raw materials, contamination	Ph	— briefing of personnel (visual control of raw materials on the presence of foreign objects in it); — work with approved suppliers.	-absence of foreign objects in raw materials; — absence of defects, including defects of packaging units.	warehouse superintendent	1	2	2/3=0.6
Storage of raw materials and auxiliary materials	Contamination of raw materials from personnel, containers, utensils during storage.	B	— assurance of keeping warehouse facilities in conditions corresponding to the sanitary-hygienic norms; — briefing of personnel regarding the requirements of personal hygiene; — control of temperature-humidity conditions of the warehouse.	— observance of the sanitary-hygienic requirements of warehouse facilities and utensils according to the established norms.	shop foreman	2	2	4/4=1
	Contamination with residues of cleaning agents and disinfectants from containers and utensils	Ch	— control of container and utensil cleanliness; — briefing of personnel; — visual control.	— observance of the sanitary-hygienic condition of containers and utensils; — control of cleaning effectiveness.	shop foreman	2	2	4/4=1
	Introduction of foreign objects from personnel, contamination due to damage of packaging integrity	Ph	— briefing of personnel regarding the requirements of personal hygiene; — visual control.	— absence of foreign objects in raw materials; — absence of contamination.	shop foreman	2	2	4/4=1

A stage of the production process	Description of a hazardous factor	Type of a hazardous factor	Preventive actions	Controlled traits	Responsible person	Severity of consequences	Probability of realization	Aggregate coefficient = product of scores/ sum of scores
1	2	3	4	5	6	7	8	9
Production — granule feeding into the compression machine	Absent	B	—	—	—	—	—	—
	Contamination with residues of cleaning agents and disinfectants from equipment	Ch	— control of equipment cleanliness; — briefing of personnel; — visual control.	— observance of the sanitary-hygienic condition of equipment; — control of cleaning effectiveness.	shop foreman	2	2	4/4=1
	Contamination of raw materials with lubricating agents and from equipment	Ch	— control of equipment condition, its cleaning from lubricating oils; — visual control.	— use of lubricating agents permitted for food enterprises	chief engineer	2	2	4/4=1
	Introduction of foreign objects into raw materials from equipment.	Ph	— briefing of personnel; — visual control. — observance of preventive maintenance schedule for equipment;	— integrity of technological equipment.	shop foreman	2	1	2/3=0.6
Injection molding and chilling in a mold	absent	B	—	—	—	—	—	—
	Exceeded MACs of chemical substances and compounds (formaldehyde, ethyl acetate, alcohols and others)	Ch	- control over the course of production operations; — briefing of personnel.	— control over adherence to the rules and conditions of performing technological operations. — briefing of personnel, observance of the work instruction	shop foreman	3	3	9/6=1.5
	Absent	Ph	—	—	—	—	—	—
Mechanical removal of an excess of polypropylene	Absent	B	—	—	—	—	—	—
	Absent	Ch	—	—	—	—	—	—
	Introduction of residues of packaging materials into finished products	Ph	— visual control of products after removing the flash; — briefing of personnel.	— absence of foreign inclusions in finished products	shop foreman	2	2	4/4=1
Packaging and preparation of a box	Microbial contamination from personnel	B	— briefing of personnel; — observance of the instruction on the personal hygiene of employees	— sanitary-hygienic requirements imposed on personnel.	shop foreman	2	2	4/4=1
	Absent	Ch	—	—	—	—	—	—
	Absent	Ph	—	—	—	—	—	—
Storage of finished products	Spreading of molds over the surface of packaging materials	B	— strict adherence to the rules of raw material storage; — assurance of hermeticity upon finished product storage.	— correspondence of storage conditions (temperature regime, ventilation and others) to established norm.	warehouse superintendent	2	2	4/4=1
	Absent	Ch	—	—	—	—	—	
	Introduction of foreign objects (insects, rodents, dust, personal effects of personnel).	Ph	— control over deratization and disinsection in an enterprise; — briefing of personnel; — visual control.	— sanitary requirements to deratization and disinsection in the food enterprise — adherence to the instruction on personal hygiene of employees	shop foreman	1	2	2/3=0.6

lubricating agents, light stabilizers, antioxidants, solvents (carbohydrates, alcohols, glycol ethers, ketones and esters), as well as other chemical compounds that can lead to the risk of chemical migration into food products [16]. In this connection, the excess of the level of hazardous chemical compounds and substances in a polypropylene tray is possible at this stage of its production, which later on can lead to appearance of the threat to safety and quality of packed whole-piece meat semi-finished products.

In the process of the detailed analysis, it is necessary to determine interrelationships of safety and quality indicators of both raw materials and finished products with all possible causes and to reveal an effect of causes at all stages of the technological process. Based on the analysis of risks and data obtained during the work, a Pareto chart was built (Figure 5), which allowed visual determination and assessment of the main causes influencing the CCP realization.

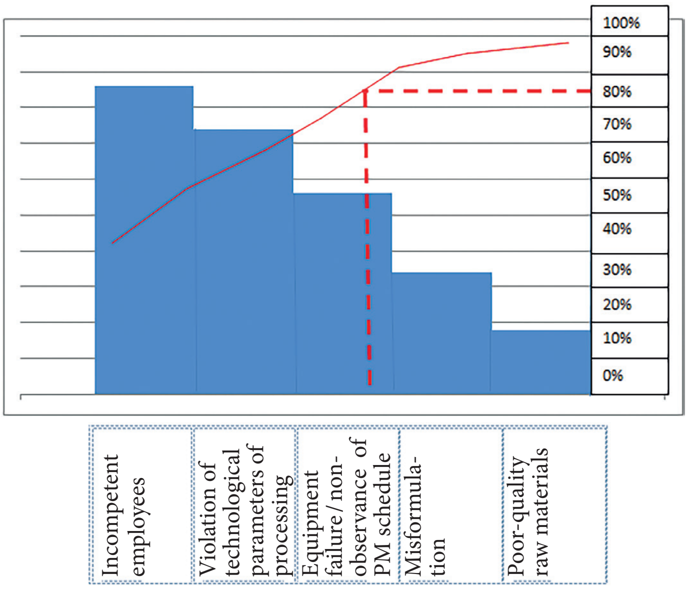


Figure 5. The Pareto chart for analysis of hazardous factors

The Pareto chart presents graphical interpretation of the so-called 80/20 rule. These are causes sorted by a degree of importance, frequency of occurrence, costs, a level of indicators and so on. When ranking causes on the Pareto chart, the most important of them are arranged on the left side of the chart so that this vital minority can be easily identified. To increase informativeness of the Pareto chart, a cumulative frequency curve is usually plotted on it [17].

During the study of the Pareto chart, it was established that the realization of the chemical hazardous factor for the identified CCP was influenced to a large extent by non-observance of the preventive maintenance schedule for equipment (hereinafter, the PM schedule) and, as a consequence, possible equipment failure (80%).

For minimization or prevention of this threat in production, therefore, it is necessary to introduce an effective monitoring system, which may include strict control of the PM schedule, maintenance support of equipment by competent personnel, briefing of personnel directly working at this technological stage as well as adherence to a given recipe and filling in established reporting documentation.

It is also worth noting that in case of using different dyes for printing on packaging, the content of hazardous substances in them that can migrate into meat products with favorable moist environment should be excluded.

At the last stage of the performed risk analysis, the monitoring procedure and corrective actions were determined. The developed measures on management of a critical control point are presented in Table 3.

Therefore, by developing measures on prevention and correction of a hazardous factor in case of its realization and, subsequently, also a prerequisite program and introducing these measures into the production process of polypropylene film manufacture, it is possible, in principle, to exclude or significantly reduce the risk realization, which later on will ensure safety and quality of whole-piece meat products packed in this film.

Conclusion

Safety of packaging for meat products is achieved by interrelation of different indicators of materials used in its production, adherence to the sanitary-hygienic norms and absence of chemical contamination. With that, these requirements are used to all packaging types irrespective of the used material (natural, metal, polymer, carton, glass or combined materials).

The use of the results of the investigation allows organizing safety and quality control of manufactured produce (packaging), increasing its competitiveness, ensuring openness in relations with consumers and regulatory authorities. The hazard factor management, strict mechanism of preventive and corrective measures in production of polypropylene packaging give a manufacture confidence that safety and quality of manufactured products are maintained and controlled, which finally will lead to a decrease in the percent of nonconforming product outputs and, consequently, cost of quality.

Table 3. Critical limits, monitoring procedure and corrective actions

CCP	CCP No.	Hazardous factor	Controlled parameter and its limits	Monitoring procedure	Corrective actions
Injection molding and chilling in a press-mold	**1**	**Exceeded MACs of chemical substances and compounds due to equipment failure**	**Acetaldehyde — 0.200 mg/l. Presence of formaldehyde, ethyl acetate, acetone, hexane, hexene, heptane, heptene and alcohols is not allowed**	**Daily control of the PM schedule. Monitoring of the record book for equipment maintenance on a shift basis. Visual control of equipment integrity**	**Remove produced plastic masses from production with entering a corresponding record into the check-list of the nonconforming products. Call technical service to check equipment integrity**

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Review article

SCIENTIFIC RATIONALE OF INGREDIENTS CHOICE FOR FUNCTIONAL FISH PASTES

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Keywords: *multicomponent product, formulation, functional food, ingredient, technological properties*

Abstract
In recent years, the global demand for finished fish products is increased. Aquaculture raw materials are a rich source of nutrients for health. At the same time a large amount of fished fish raw materials are rejected and representing a losses. High-quality raw materials with technological defects are not in demand by the food production of fish products. Perishable substandard fish raw materials can be used in the production of combined fish products. The analysis of the fish catches global scale in the seas, oceans, farmed marine and freshwater aquaculture is carried out. An assessment of the market trends for the production and fish products consumption is made. This article provides an overview of targeted ingredient selection for balanced fish formulations. The characteristics of the most popular plant origin ingredients and their derivatives in the fish pates production are given. A comprehensive assessment of the biological, energy and nutritional value of plant products is given. Described technological approaches and production methods, the safety of vegetable ingredients. The results of research, technological solutions, substantiation of the choice of ingredients adopted by the developers for the formulations of multicomponent pate are summarized. The use of unclaimed raw materials, leftover fish meat with plant ingredients represents additional profit. Fish pate formulations are the basis for the production of complete, ready-to-eat products. Tests of multicomponent fish and vegetable products confirm their high performance. Pates, balanced in nutritional value, minerals, are a finished product that does not require additional preparation. The review article will generate awareness among the researchers, food technologists for the production of fish products and the general public.

Introduction

Providing the population with adequate nutrition is included in the list of global problems of the world. Many countries are interested in resource efficiency in food production. Scientific achievements in the food industry aimed at improving the food quality are relevant for all countries of the world. The food industry should make the most of the available resources for the raw materials processing. Scientists are considering the possibilities of elaborating product formulations to quality improve and profitability of production.

It is advisable to make the most of available and traditional sources of raw materials in food production technology. Food products from raw materials with specified characteristics will provide high quality products. Thus, the production of multicomponent products will provide an opportunity to expand the range of nutritionally balanced food sources.

Comprehensive approaches to formulation development promote complementarity of ingredients in the finished product.

The selection of ingredients for simulating new formulations must be scientifically evidenced. The components of this products should complement each other, be synergistic. The product with the specified technological characteristics will positively affect the consumer preferences of the finished product.

Planning, quality management and scientific rationale for the selection of ingredients are important steps in the development of new product formulations. Scientific approaches, research work related to the improvement of food formulations are relevant in modern conditions.

The development of new food products consists of theoretical and specific methods for implementing the balance principles. When modeling the formulation, the compatibility of the ingredients and the energy value of the product are assessed. Comparison of the content of essential nutrients in raw materials and finished products is crucial for formulation development. The quality characteristics of raw materials are the evidence to obtaining a finished product with the specified parameters. The composition of the products should include ballast substances, they have the ability to swell, retain moisture. Dietary fibers stabilize aqueous suspensions, improve the structural and mechanical properties of finished products [1,2,3]. Analysis of proteins, fats, carbohydrates, minerals, is the main assessment when modeling a product [4].

Balancing nutrition is one of the constituent factors affecting human health. The consumption of animal and plant products contributes to the correct metabolism. Proteins and fats of vegetable and animal origin are the source for the vital activity of the organism. The enzymes activity in metabolic processes depends on the presence of vitamins, macro- and microelements in the diet. The nutritional and biological value of the finished product characterizes its composition. The composition of products must be balanced, easily digestible, to ensure the normal functioning of the human organism.

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For many consumers, the sensory attributes of the product and the emotional component when choosing it are important. Products must be attractive to the human senses and satisfy their physiological needs. The acceptability of the product must be balanced against the expectations of the purchaser in order to generate consumer interest in proper nutrition.

Consumer behavior when choosing a product is a complex process. The most unifying characteristics of buyers are numerous personal, social and psychological factors. By offering new gastronomic combinations that can diversify the traditional menu, it is necessary not to lose consumer confidence. The growing interest of the population in a healthy lifestyle forms the basis for proper nutrition [5].

The nutrient requirements of the human organism vary and depend on age. Other factors and living conditions require adjustments in food intake. The compliance of food with the biological needs of a person is an important issue for a healthy diet. The solution to this problem is to expand the assortment variety of products, including fish products. The balanced combination of fish with vegetable, grain ingredients help to increase the nutritional value of the product. Research related to the improvement of food formulations is receiving a lot of attention.

The purpose of the work is to scientifically grounded the choice of ingredients for obtaining a multicomponent product of a functional tendency.

It is generally accepted that functional food products, when consumed, reduce the risk of developing diseases associated with metabolic processes in the organism. Proposals for the production of formulations for functional fish products are currently timely. The tasks set are aimed at solving the maximum use of high-quality fish raw materials. Scientific technologies of processing and quality management will make it possible to effectively use fish raw materials by increasing the range of finished products.

The purpose selection of research articles will provide information on the existing areas of production of fish products. The plan is to determine the functional ingredients promise when changing product formulations to meet the changing needs of the food market.

Materials and methods

The article presents research papers and overview scientific information in the field of the use of fish meat in the industry.

We carried out a targeted review of literary references. Selected research articles from domestic, foreign magazines, published in recent years. The criteria for considering the articles were the rationale and significance of studies of the muscle tissue of fish for the development of fish pates. Used scientific data from research in the food industry in different countries. Analysis of literature data is aimed at the prospects of scientific technologies in fish production. When selecting components for formulations, the functional orientation of the final product is desirable.

Results and discussion

Fish is a highly valuable food source. In a number of countries, fish products traditionally form the basis of the diet. Fish is considered one of the demanded products included in the people menu of the world. With its high nutritional characteristics, the fish is not inferior to other animal products. In terms of biological value, the muscle tissue of fish is better digested and is more quickly involved in metabolic processes [6]. Fish has high digestibility and nutritional value compared to meat from farm animals. Reduces the possibility of wider use of fish in the daily diet, its rather high price.

The fish presence in the diet provides the organism with biologically complete protein. The protein content in fish of different species ranges from 12 to 25% with a set of essential amino acids. Protein consists of the complex protein ichtulin, albumin and phosphorus-containing nucleoproteins. Albumin, water-soluble proteins globulin, myoalbumin, myogen, myoglobulin represent the structural part of the sarcolemma constituent. Salt-soluble proteins, globulins, actin, actomyosin, myosin, tropomyosin form myofibrils in fish muscle cells. The connective tissue of fish protein contains only collagen, elastin is absent. The connective tissue is evenly distributed in the muscles and accounts for 10 to 12%. Low connective tissue content, high water-holding capacity ensure quick readiness of fish products. It takes much less time to cook fish than to cook meat from animals. During heat treatment, fish collagen is converted to highly hydrophilic glutin. This transition of fish collagen gives tenderness and juiciness to the finished product. The amino acids glycine and L-alanine, when exposed to heat, give the fish a sweetish taste. Fish protein differs from the productive animals' protein by the absence of hydroxyproline, high content of lysine, methionine, cysteine, tryptophan.

Fish is a source of complete fats. Fish oil has better digestibility by the human organism compared to animal fats. The fat of marine fish contains eicosapentaenoic and docosahexaenoic acids, which have a known biological activity as ω-3.

Marine freshwater fish are rich in a significant amount of the group of water-soluble vitamins. Fat-soluble vitamins A, D, E are concentrated in the tissues of medium-fat and fat fish.

In the muscle tissue of fish, water is contained in a free and associate state. During heat treatment, water loss is about 20%. The moisture loss in meat of productive animals after heat treatment is up to 40% of water, which reduces digestibility.

Fish contains 2 times less extractive substances than meat of productive animals. During heat treatment, most of the extractives are released with fish juice and pass into broth. Fish products in an easily digestible form contain essential fatty acids, vitamins, iodine, selenium. The minerals of fish meat are represented by phosphorus, potassium, sodium, calcium, sulfur, chlorine, and manganese.

Fish raw materials and finished products are characterized by a large amount of microelements.

The chemical composition of commercial fish is unstable and depends on many factors: fish species, age, habitats. The proteins amount in the muscle tissue of fish averages up to 22%, fat from 0,1% to 55%. The concentration of free and bound water in fish can range from 48% to 93%. The fish minerals composition is stable within 2–3%. The fish meat calorie content is determined by the chemical composition of 100 g of the product. Average energy values of fish vary greatly from 100 kcal to 900 kcal. The energy consumption of sea fish oily species can be up to 1500 kcal. According to the degree of fatness, the following are subdivided: not fatty 3%; medium fat content up to 8%; fatty more than 10%. Fish products are classified by grades 1 and 2, by weight and length of the specimen.

Numerous scientific and practical studies confirm the advisability of using fish as a valuable food product. The exclusivity of the muscle tissue of fish lies in the ability to quickly digest and join the metabolic processes of the organism. Fish is rich in amino acids and is an important source of omega-3 fatty acids necessary for the human organism. The biological and nutritional value of fish is defined as a source of nutrients for a full human life. Fish and fish semi-finished products should be present in the human diet for a healthy lifestyle [7].

In recent years, in the food market, the demand for commercial fish and aquaculture products has increased. In a number of countries, fish farming is developing rapidly (Figure 1).

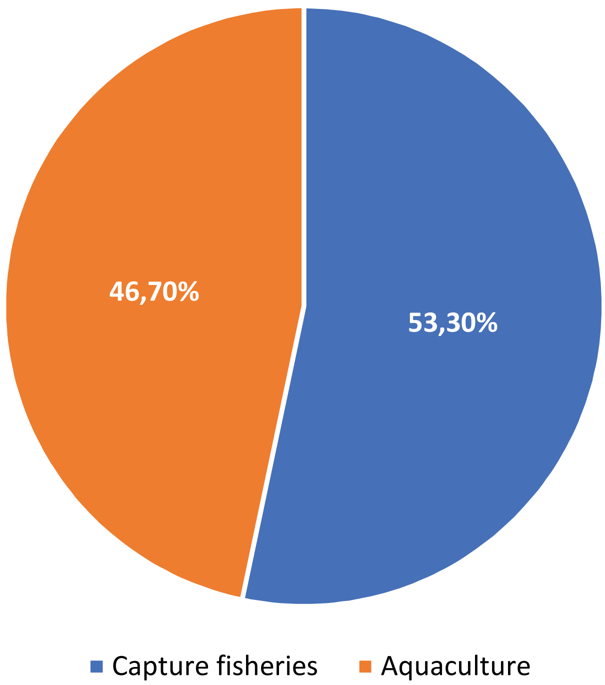

Figure 1. Aquaculture chart of world fish production

The global increase in the catch of farmed aquaculture is more than 8% annually. It is predicted that by 2030 aquaculture will account for more than 60% of the total fish production.

There are some peculiarities associated with the cost-effectiveness of artificial fish reproduction. The instability of the weight gain of reared individuals leads to the appearance of small specimens. At the same time, the cost of raising fish involves obtaining individuals with forecasted parameters [8].

By-catches occur when catching commercial fish species. By-catch usually has no commercial value and is therefore not used for product manufacturing. In order to save resources, it is necessary to make the most of the caught fish. By-catch should be involved in food production, where textural characteristics are determined by processing methods [9].

Food products with a modified texture of fish meat are minced fish, pates, riets and pastas. It is rational to use high-quality fish that is not standard in terms of size characteristics in the technology for the production of the above-mentioned products [10,11].

Fish raw materials must be safe in terms of chemical and microbiological parameters. Aquatic biological resources (WBR) caught in natural water bodies are characterized by instability. The quantitative and qualitative composition, size characteristics of wild fish have a fairly wide range of values.

Aquaculture produces fish with more stable technological characteristics. In the Russian Federation, the main objects of freshwater aquaculture are trout, carp, whitefish, salmon and catfish. The most promising species of the catfish order is the North African catfish (*Clarias gariepinus*). Its natural habitat is freshwater reservoirs in Africa, in the Jordan River basin, in South and Southeast Asia. The fishery development of clary catfish in Russia began in 1996. The production technology of this type of fish is characterized by the rapidity of growing to marketable weight [12].

In the Russian Federation, the fishing industry is being modernized in the areas of waste-free processing of raw materials and expanding the range of products. The geographic location of the country allows the use of rich natural waters for fishing. There is a trend towards a reduction in the share of imported fish products in the country's food market.

For 2020, science knows and describes 35 768 species of existing fish. Approximately 1500 fish species of commercial interest. In accordance with habitats and migration, fish are conventionally divided into marine, freshwater, anadromous and semi-anadromous. The water area of the Russian Federation is home to about 3 000 species of fish. In fresh water of rivers, ponds and reservoirs, there are up to 300 species of fish.

In addition to fresh fish, there are fish raw materials that can be chilled, slightly frozen, frozen, glazed.

The compatibility of products of animal and fish origin with plant representatives is now widely advertised. The technologies for the production of canned food have been studied quite comprehensively. Canned fish is produced from pieces of carcasses, liver, caviar and mict of commercial fish. In the production of canned fish and vegetable products, vegetable components are added to fish raw materials. Depending on the plant component, canned fish and vegetables or fish cereal canned foods are produced. Combined canned food is produced for the purpose of combining nutritional factors in the form of meatballs, fricassee, cabbage rolls, cutlets.

Plant products represent a large group with many subgroups. As a result of cereals technological processing, grains are the raw material. In the production of combined products, cereals or flour are more often used. The most popular are rice, buckwheat, corn, barley.

Grain crops and their cereals, flour is characterized by a stable content of protein, fat and carbohydrates. The average nutritional composition of cereals is 10–12% protein, 4% fat, and up to 70% carbohydrates. At the same time, the grain part of the human diet can account for up to 40% of the daily protein requirement. From the carbohydrates of cereals supplied with food, the organism is provided with energy for 90%. Cereal fats have a high nutritional value due to polyunsaturated fatty acids. The germ of cereals has a high biological activity, which is widely used in the food and other industries. Cereal processing products are a source of water-soluble vitamins and minerals.

The legume is widely used in the production of combined food products. Most often, peas, beans, soybeans and various types of beans are used for the production of fish products. Legumes are characterized by a high protein concentration of up to 24%, and in soybeans up to 35%. The biological value of legumes is inferior to animal meat in terms of digestibility and balance of protein components. Peas and beans are used as independent dishes after prolonged thermal and culinary preparation. Technological processing and fermentation of these crops allows you to reduce the cost of manufacturing raw materials, finished, canned products.

The most popular crop is soybeans, with a high level of vegetable fat 15–17%, low starch content. Soy is popular as a protein product after industrial processing. As a result of high-tech impact, soy flour, isolate, soy protein hydrolyzate are obtained. Textured forms of soy are used in formulations for meat, fish semi-finished products, sausages, and canned food. Soybean biologically active substances, trypsin inhibitors and oligosaccharides, should be limited. Their uncontrolled presence in products can have an undesirable effect on human health.

The most numerous groups of plant products is represented by vegetables, fruits, berries and garnish. This group is represented by a significant range of traditional products that complement cereals and legumes. Representatives of this group are sources of ascorbic acid, β-carotene, bioflavonoids. Plant foods, especially their fruits, contain a lot of carbohydrates, potassium, magnesium, iron. The vegetable and fruit group is a source of biologically active compounds. Organic acids, essential oils promote enzymatic activity and regulation of gastrointestinal motility.

The chemical composition of vegetables indicates a high water content of up to 92%, carbohydrates 8%. Proteins and minerals account for approximately 1% in fresh vegetables. The roots of turnips, beets, carrots, pumpkin have a high content of easily digestible fiber. Dietary fiber includes parts of hemicelluloses and encrusting substances of cutin, lignin, suberin. Root hemicellulose is represented by hexose and pentose sugars of plant cells and is a source of nutrients. Some varieties of sugar beets contain 17 and more than 20% sugars. High carbohydrate energy content is not always in demand in the production of finished products. The biological value of plant materials with high fiber values in the formulation of combined products will be appropriate. The components introduced into the formulation change the chemical composition and quality of the finished products. Plant pigments during heat treatment will give a pleasant color to the finished product. Carrots, beets, pumpkins, turnips with many color shades will neutralize the taste of the fish component in the product. The sugar contained in root vegetables will pass into the product after mechanical and thermal processing with water. In addition, they will enrich the products with pleasant sweetish taste properties. The main indicators that determine the attractiveness of a product are color, aroma and taste [13,14].

The use of carrots and beets allows you to significantly expand the existing range of ready-made food products. The nutritional value of beets lies in the anticarcinogenic and antiradiation properties of betanin [15].

Turnip refers to low-calorie foods, when consumed has the ability to give a feeling of fullness. Saturation of the organism occurs thanks to sugars, easily settled polysaccharides, satisfying the feeling of hunger. The chemical composition of turnips is represented by proteins 1%, fats 0,1%, carbohydrates 7%, minerals 1%, fiber up to 2%. High content of vitamin C, water-soluble vitamins, provitamin A. A wide range of minerals is present in turnip fruits. Macro- and microelements of turnip are in the optimal compounds for assimilation by the organism. Magnesium, which is found in large quantities in turnips in humans and animals, helps to absorb calcium. Turnip has a number of therapeutic and wellness properties. In the modern rhythm of life, turnip is becoming a demanded raw material and product in the food industry. With the development of interest in functional products using dietary fiber, a turnip drying technology has been developed. Experimentally obtained samples of powdered turnip contain 7,2% protein, 3% ash, 2,8% starch, and 33,6% cellulose [16].

Pumpkin is a carotene source, the content of which is higher than in some varieties of carrots. Most of the pumpkin mineral's iron, copper, cobalt, zinc are involved in the hematopoietic metabolism. The concentrated trace elements complex and vitamins in pumpkin is recommended as a prophylactic product for cardiovascular diseases. The statistical chemical composition of pumpkin is represented by 1% protein, 0,1% fat, microminerals up to 3%. The product contains up to 90,5% of bound water. Due to its high fiber content, dietary fiber plays a preventive role in the work of gastrointestinal motility. Possessing many positive properties and rich chemical composition, pumpkin is widely used in the multicomponent products technology. Currently, pumpkin powder is actively used in the food industry.

There are about 30 genuses and about 650 species of onions in the world. Onions are one of the high nutritional value vegetables with antibacterial properties. Onion protein is 50% nitrogen-containing substances. Carbohydrates contain up to 9%, which are represented by a wide range of sugars. Onions contain a lot of essential oils, vitamin C, water-soluble vitamins, citric and malic acid. The phytoncides contained in onions have a disinfecting effect. Onions are widely used in medicine as a stimulant, a sedative, for vitamin deficiency, a source of vitamin C. For prophylactic purposes, green onions and bitter varieties are consumed. Medicines are prepared from onion juice.

Onions are considered the most common. By chemical composition, onions are conventionally divided into varieties of spicy, semi-sharp and sweet. Sharp has a high solids content of 15%, with a essential oils high concentration 155 mg / 100 g, and sugars from 12% to 15%. Onion glycosides impart a bitter taste to the product and reduce the sweetness sensation. When fried in animal or vegetable fats, the onion takes on a yellow to golden color. Heat treatment destroys glycosides, essential oils give the onion a specific smell and a sweet taste appears. Onions are widely known in the cooking of many cuisines of the world, fresh, frozen and dry. The food industry uses onions in the production of many meat and fish products.

White cabbage is used in cooking in many countries around the world. Its advantages are represented by dietary properties and use in medical nutrition. Fresh cabbage is an average water content of 90% to 95%. The main energy part of cabbage is sugar content up to 5.4%, 2.2% protein. The fiber content in cabbage does not exceed 1.1%. White cabbage is especially rich in vitamin C up to 70.0 mg%, mineral salts and water-soluble vitamins. Many different first, second and independent dishes are prepared from cabbage. Fresh cabbage, fermented, boiled, stewed cabbage is used for making salads and fillings. In the technology of cooking vegetable, meat, fish semi-finished products, combined canned food, cabbage is included in the products formulation.

After drying vegetables, fruits, berries and garnish, the quality characteristics of the raw materials practically do not change. The preservation of the obtained product is influenced by the preparation carried out and the technological regime of drying and packaging. Dried carrots, beets, onions, cabbage are included in the formulations for meat and fish products. For a short and long shelf life of dried vegetables, a dry place and a moisture content of 14% are required. The introduction of dried vegetables, fruits, berries and garnish into formulations enriches products with biological nutrients. They increase the amount of minerals, give the finished product new aroma and taste properties.

In the 21st century, a difficult situation has developed for agriculture and producers of fat and oil products. The human society does not have comprehensive information from competent government organizations about the benefits and dangers of fats. Lack of information from the population led to a decrease in interest in fat and oil products: margarines, mayonnaise, combined fats. The economic situation on the market of food raw materials and fat and oil products poses complex and responsible tasks. Currently, more than 50 types of oilseeds are cultivated.

Agricultural specialists are developing improved technologies for growing oilseeds. Manufacturers are called upon to find new efficient production of healthy food products based on fat and oil products. Oilseed scientists have focused their efforts on improving quality and yield. The sphere of technological solutions is aimed at the development of raw materials processing processes and the functional orientation of product formulations. Finished products should be focused on improving quality and consumer properties.

After a long stable decline in demand for vegetable oils, their consumption began to increase since 2018. According to nutritional standards, the physiological human need for fats should be 65–120 g, but not less than 25–35 g per day. Vegetable fats should make up 30% of your daily fat intake.

In England, the annual consumption of vegetable fats is 18 kg per capita per year, in the USA and the Netherlands about 25 kg. To a large extent, this amount of vegetable oil is explained by preferences for vegetables in the diet of the population. The society of these countries is offered products to reduce the consumption of animal fats and oils. The health food policy in the Netherlands has resulted in the consumption of animal oil up to 4 kg per year. The consumption of fat stimulates the appetite, improves the taste of food and makes you feel full for a long time. A complete edible fat in the diet of a healthy organism should contain about 10% PFA, 60% MUFA (oleic) and 30% saturated. The recommended ratio of polyunsaturated to saturated fatty acids in the human diet is 2: 1. It was found that an increase in the amount of PUFA in the human diet reduces the risk of cardiovascular diseases. The essential fatty acids of fish have an antibacterial effect, inhibiting the growth of bacteria.

In recent years, the animal fat consumption is decreased in a number of countries. Interest in healthy eating, low price influenced the higher consumption of vegetable oil. With the change in lifestyle, human nutrition, the norms of vegetable oil consumption per capita have been changed.

Lipids are esters of glycerol and fatty acids of different carbon chain lengths and degrees of saturation. The main function of lipids in the human organism is to provide energy. Fat-soluble vitamins A, D, K, E are necessary for the regulation of metabolism in the organism. Fats are part of the cells, are involved in the organism thermoregulation.

Vegetable oil is obtained from seeds or fruit pulp. Separately, there is a group of processed modified fats, in particular margarine, culinary, combined fats.

Hard fats containing low molecular weight fatty acids include coconut oil, mutton fat. The greatest amount of lauric, myristic, palmitic, palmitoleic low molecular weight acids is concentrated in milk fat. It is believed that there are no low molecular weight fatty acids in cocoa butter, pork, bone, and beef fats. Most animal fats are hard at room temperature.

Vegetable oils have a liquid consistency at 20 °C, with the exception of cocoa butter. Depending on the ability to polymerize, vegetable fats are divided into drying, semi-drying, non-drying. The drying group includes linseed and hemp oils containing acid esters with 2–3 double bonds. Semi-drying oils, sunflower, corn, soybean, cottonseed, sesame, poppy, contain acid residues with 1–2 double bonds. Castor, palm oil contains mainly esters of glycerin and saturated acids, a small amount of monounsaturated oleic acid.

When heated, liquid fats of marine animals and fish thicken and form soft, fragile polymer films. Fish oil is used as a dietary supplement due to its beneficial properties for the human organism.

Depending on the raw materials used, 16 types of vegetable oils are distinguished. The most popular are sunflower, olive, cottonseed, soybean, peanut, mustard, sesame, corn, linseed and hemp oils. Rapeseed, coconut, cocoa, palm, palm kernel, tung, poppy, and almond oils are considered specific or technical types.

Vegetable oil is obtained by pressing, pressing under high pressure or extraction with a chemical solvent.

Oils that have undergone only mechanical treatment by filtration, centrifugation or settling are unrefined. From the seeds from which the oil is made has a specific color, pronounced smell and taste. Hydrated oil is made from unrefined oil. Hydrated oil has a less intense color than unrefined oil.

Refined oils are used in food production technology. The oil is purified from mechanical impurities, treated with alkali, so fatty acids and phospholipids are removed from it. Thus, the purified oil rises up, and the sediment remains at the bottom, then the vegetable oil is bleached. Refined sunflower oil is characterized by weak sensory attribute. Its biological value is lower, since it contains less tocopherols and does not contain phosphatides.

Deodorized sunflower oil is obtained from refined in the process of exposure to water vapor under vacuum. The purpose of this process is to eliminate all aromatic substances that can lead to premature oil deterioration. Deodorized D-grade sunflower oil is used in the production of baby and dietary food. The acid number of grade "D" has no more than 0,4 mgKOH / g, and grade "P" — 0,6 mgKOH / g.

Vegetable fats enter the organism through the use of nuts, with the fruits of oilseeds. Vegetable oils are consumed with salads or as part of sauces, seasonings.

Animal fats in large quantities are contained in pork fat up to 92%, butter 82,5%. In animal fats, there is a lot of cholesterol, which plays an important role in the life of the organism, its daily rate is 300 mg. Plant products contain p-sitosterol, which forms insoluble complexes with cholesterol. The combination of vegetable and animal fats in the diet improves the technological, biochemical and sensory attributes of the product.

The population is saving time by consuming semi-finished products and high-calorie foods. Often, the diet of the working-age population consists of a large amount of fat, table salt and sugar or substitutes. Lack of fat-soluble and most B vitamins is detrimental to health.

It is important to develop formulations for products that do not require additional culinary processing. Balancing fatty acid, amino acid and vitamin compositions will allow us to offer the buyer products for a healthy diet. The design of functional multicomponent products is aimed at balancing the composition of vegetable ingredients. Pates based on fish raw materials are promising in terms of nutritional and biological values.

The tendency in the global food market since 2016 is assessed as positive. According to experts, by 2025, the demand for fish products should increase by 1,3% annually [17]. The growth rate in the food market is associated with changes in consumer preferences for food. With the development of the tourism industry and the adoption of a Western diet, the range of world cuisine is expanding. This path is popular due to its convenience, it fits into the tempo of modern human life. Today's food culture is based on saving time by snacking on fish products (Table 1).

Table 1. The tendency of the market for the consumption of fish products

Usage data	Average annual volume (million tons)					
	1990	2001	2015	2016	2017	2018
Food use	70,8	97,8	128,3	148,7	151,8	155,2
Non-food use	30,9	28,7	21,1	18,7	19,3	23,5
Population (billion)	5,4	6,2	7,0	7,5	7,5	7,6
Per capita consumption (kg)	13,1	15,8	18,3	19,8	20,2	20,4

The global aquaculture market is represented by fish suppliers, product manufacturers and country merchants. The main representatives determining the fish market policy are the countries of Europe and the USA. Agreements are accepted for the export of fresh, chilled, frozen, canned, pickled aquaculture products. The variety of fish and aquaculture species has a significant impact on the catch, production of finished products, semi-finished products. The abundance of aquatic organisms in the world's oceans has led to the formation of traditional fish products and a culture of consumption (Table 2).

Table 2. The tendency fish raw material production market

Production	Average annual volume (million tons)					
	1990	2001	2015	2016	2017	2018
In inland waters	6,3	8,4	10,1	11,5	11,7	11,9
In the seas and oceans	80,6	83,1	79,4	78,2	81,5	84,0
Industrialised fishing	86,9	95,5	89,5	89,7	94,2	95,9
Aquiculture in inland waters	8,5	19,7	36,7	48,1	49,5	51,2
Aquiculture in the seas and oceans	6,3	14,3	22,6	28,4	30,3	30,7
Aquiculture, total amount	14,8	35,0	59,3	76,5	79,8	81,9
World fisheries and aquaculture, total amount	101,7	125,5	148,8	166,2	174,0	177,8

The fish food processing industry additionally produces fish products with plant components. The fillers of such products are vegetable oils, sauces with a wide range of spices. The formulation for fish pates consists of fillets, spices, seasonings, vegetable fillers. All ingredients of the pate during the production process are brought to a homogeneous pasty consistence. In addition to fresh fish fillets, frozen, salted, pickled, smoked fish are used. Pates can be made from many species of fish. Fillets of tuna, mackerel, trout, salmon are often used in the production of pâtés. The popularity of fish pates is growing thanks to the possibility of a quick snack. Packaged in jars, fish pastes are convenient for making sandwiches, as an addition to garnish [18].

Europe is the largest regional market with very high consumption. Fast food is especially developed in France, where 68% of people use this service. Initially, dietary food advertisements focused on chicken liver products. Many nutritional theories that exist today focus on the consumption of beef, lean pork. Fish contains about 20% more easily digestible protein compared to the muscle tissue of animals. Fish products can be useful in improving and enriching the human diet. Consuming even small amounts of fish can have a positive nutritional effect on metabolism. Modern food production facilities are equipped with technologies that can avoid strong fishy smell and taste. Among the consumers of fish products, there are many supporters of a healthy diet.

The psychological processes study that the authors describe is one of the consciousness determining factors. Memory potential that lasts throughout life and accumulates all previously received information. The changes in the effect of random learning on memory related to the texture and taste of foods are very important. Experiments show that nutritional characteristics are memorized at a certain level of memory.

The research authors note that the combination of sensory and rheological characteristics supports the objective determination of food texture. t is important for experts in the classification of the texture of the food profile for all segments of the population. Food producers ultimately rely on the preferences of the consumer for whom their regional market is created.

In the European part, depending on the region, there is a common and specific formulation for a fish product. Preferences are determined by the species composition of the fish. Formulations for cooking fish products are based on preferences for water, type of fish, vegetable fats, salt, spices. The competition for smell and taste is the desire and speed of adaptation of consumers to new fish products. The specific production technology of various fish species helps to adapt to new products. The fate of the product depends on the amount of fish and other ingredients, the method of production, nutritional value, and the effectiveness of the ways of implementation. Product distribution, already at the formulation stage, is linked to the quality of the finished product. Appearance, smell, texture, taste is compared with similar products competing in the food market. For a permanent category of consumers of fish, fish products, there is an attribute standard of sensory characteristics [19].

Among the fast food products derived from fish are pates. They represent a pasty product of different consistency, depending on the ingredients. The formulation basis for minced fish is fish, fish liver, fat, chopped vegetable ingredients, garnish, spices. In industrial production, it is possible to add chemical preservatives to increase the shelf life of fish pates.

Currently, there is a tendency in consumer demand for the supply of products with no chemical preparations. Official publishing sources report the demand for natural preservatives. Over the past 10 years, there has been an increasing interest in biological and natural preservation in food production. The answer to the demand for preservatives is scientific publications on preventing bacterial spoilage of products. Competitive bacteria are used for natural preservation. The result of such work was fish sausages, shrimps, squid, mussels, scallops, raw and cooked trepangs [20, 21].

Having received the status of biological preservatives, not all bacteria meet the safety requirements for the food category [22]. Research work on the study of the bacteriological product of nisin bacteriocin has shown that the strain can be free from virulence factors, antibiotic-resistant models and biogenic amines. During the experiments, high resistance to heat, pH environment and chemicals was noted. The bacterial product was able to inhibit the growth of microflora. In fish paste, the microbiological load during storage decreased, without affecting the properties of the finished product.

There are known studies of the moisture-retaining capacity in fish pates made from barracuda meat with fat replacement. Water, inulin and monioca starch are added to the fish. The inclusion of ingredients in the fish paste formulation significantly influenced the texture and consistence of the product. To obtain a fish paste with the desired properties, a comprehensive knowledge of the raw materials characteristics study used is required. The inclusion of cassava starch and inulin in the formulation reduced the water content with a high water-holding capacity of the product. To obtain a product of low hardness, it is suggested to use low concentrations of cassava starch. High values of the bound water content in the fish product improve its plasticity. The researchers recommend revising the quality characteristics for the standardization of fish pates. This need is associated with the tendency of quality to fish and fish raw materials in demand on the food market [23].

Studies on the physical and chemical properties of pate when replacing animal fat with vegetable oils are relevant. The texture characteristics of the new pate have less hardness and viscidity. Fat replacement studies with olive oil do not affect the protein, fat, moisture content of the finished product. Olive oil has changed the fatty acid composition by increasing monounsaturated and lowering saturated and polyunsaturated fatty acids. With the addition of olive oil to the pate formulation, the cholesterol amount is significantly reduced. Olive oil enriches its composition and, being a natural antioxidant, gives new properties to the pate. The authors insist on the need for a comprehensive study. It is required to study the influence of the fat substitution and oils on the sensory abilities and characteristics of pates [24, 25, 26].

When developing functional foods with enhanced nutritional properties, interest in natural antioxidants is emerging. Taking care of your health requires a balanced diet. The fruits antioxidant activity, berries and extracts are important for the beneficial physiological activity of metabolism at the cellular level. The potential of some fruits and berries used in nutrition has not been fully studied for many years. Preliminary tests to study the antioxidant activity of the strawberry tree confirm the oxidative stability of meat and fish products. Strawberry tree extract 3% and 6% does not affect the energy value of fish pates. The crushed fraction of the fruit significantly increases the fiber content in finished fish products. The vegetable fiber source changes texture when mixed to give the product a uniform consistency. A comprehensive study showed high values when included in the product 6% active extract of strawberry tree. Natural antioxidant activity was shown within 90 days. Oxidative stability, improvement in uniformity and nutritional value allowed to obtain results of confirmation of a quality product [27,28,29].

To maintain a continuous demand for fish, fish products, it is necessary to improve the management of non-standard aquaculture samples. Non-standard, high-quality fish raw materials are used for the production of non-food products or disposed. Due to non-standard quality raw materials, it is intended for non-food products. Through the efforts of the scientific community, research and technologies for the processing of fishery losses are encouraged. The strategy of maximizing the fish use for food consumption is becoming an important incentive for production. Technological alternative solutions must be urgently found and offered to production in the form of additional profit. The solution to waste-free use of aquaculture is the production of multicomponent nutritionally balanced fish and fish pates.

The authors of the articles assessed the replacement of commercial fish with fish grown under artificially created conditions. With a maximum feeding base, stable climatic conditions, up to 10% of fish specimens differ from standard specimens. The methods of catching, processing, storing, processing are of great importance for the final result of fish products. As a result, it was concluded that the protein source has a significant potential to meet human needs [30,31,32].

The modern consumer is selective, picky about food products, excluding any health risks. The development of innovative, functional organic food products is a hot topic. A productive dialogue of scientific potential with industrialists with the interest of governments and state institutions is the way to provide the world's population with high-quality products.

The main part of salt, up to 80%, comes with products produced by the food industry. In addition, salt is consumed by a person with cooked food or in the process of eating [33]. An excess of salt raises blood pressure and provokes the development of cardiovascular diseases. This situation leads to financial costs for society, costs associated with treatment [34,35].

Salt performs many important functions, reduces water activity, growth and development of microorganisms. The shelf life of finished products and semi-finished products depends on the salt concentration. Salt affects the sensory attributes of the products consumed. Food manufacturers do not have the technological ability to abandon salt as an ingredient in flavor and preservative. Changes in the products formulation with a lower salt concentration may adversely affect competitiveness in the food market [36].

There is a strong demand on the part of manufacturers for the production of low salt products. Satisfying the requirements of consumers, the food industry is interested in maintaining the sensory properties of products, shelf life, purchasing power [37, 38, 39].

All seafood contains a small concentration of table salt 20–150 mg per 100 g of fresh raw materials. During the processing of fish and seafood, the concentration of sodium salts differs significantly. Significant differences in products are associated with food preferences and habits of the population. Large differences are observed in salty foods from 1000 to 6000 mg per 100 g. A high concentration is typical for canned foods. Salted and smoked fish products usually do not exceed the salt content of more than 1100 mg per 100 g.

A number of strategic decisions help to reduce sodium content in ready-made seafood, while maintaining quality and shelf life. The application of a strategic decisions number can reduce the sodium content in the finished seafood, while maintaining the quality and shelf life. Compliance with regulations and consumer interests is of paramount importance to food production. The offered flavor enhancers, herbs, spices are used in combination with mineral salts. Other solutions are not capable of producing the desired characteristics, achieving acceptable shelf life. Only mineral salts can provide with their functional properties the processes associated with preservation [40].

Fish pates prepared in the Swedish city of Gothenburg contained eggs, cream, sour cream, dill, salt, water. The basis of the pate is freshly caught salmon, the fillet of which is processed into minced meat. The amount of salt in the standard recipe is 1%. To reduce sodium chloride, a mixture of sodium and potassium salts is proposed, with a content of 35% less sodium. During testing of prototypes, a decrease in sodium by 22% is found, compared to the standard formulation. Microbiological, taste properties are stable during the entire experimental period. Organoleptic differences in texture, taste, salinity of fish pates are not significant. The proposed salt mixture can be used in the recipe for fish pastes based on farmed salmon. The work noted the specificity of replacing sodium chloride with potassium in high concentrations. Products from other fish species with potassium chloride are often bitter and leave a metallic aftertaste [41]. In recent years, commercial proposals have been received for a mixture of salts with a sodium chloride content of less than 35% [42].

Freshwater fish species in many countries, due to the seasonality of the catch, are not used effectively. The main reasons are bony flesh, textural properties, specific taste, odor, uneven weight and size. For the industrial production of products from freshwater fish, it is necessary to assess the nutritional properties and food processing. A commercial approach to the use of local fish raw materials is of economic importance for expanding the products range [43].

In Italy, studies have been carried out on the possibility of using freshwater fish tenchu from Lake Trasimeno in pate. The caught fish specimens were salted in 22% brine, followed by drying. The dried fish were smoked by convection for 6 hours. Prepared smoked minced meat from fish muscle tissue. In 1 experimental group, minced meat was mixed with olive oil 41.5%, parsley 1,65%, lemon juice 1.65%. Group 2 added 42,55% mascarpone cheese, 12% cream, 1,28% parsley, 0,85% lemon juice. The difference between group 3 and group 2 was 40% cream. All samples were sterilized according to heat treatment standards for canned fish. Analysis of pates showed high nutritional and energy values. The images with olive oil received the optimal evaluation characteristics. Thanks to the selected ingredients, 2 experimental groups showed specific sensory sensory attributes, compared to group 3. The sample with olive oil received a higher rating from consumers; the formulation is recommended for smoked fish pate [44].

The most economically inexpensive freshwater fish are bream and perch. These types of fish are not demanding on feeding and living conditions. The nutritional value of these fish is maximized in the fall. Among the large number of freshwater fish, bream has a high nutritional value and low cost with large catches. A sample of an average bream contains 75% water, 18% protein, 1% minerals, and up to 6% fat. When making fish pate, minced fish was prepared from bream meat. The vegetable product pumpkin, seaweed, vegetable oil, fish broth, and dill were added to the minced meat. A homogeneous pasty mixture was prepared. After heat treatment and obtaining a canned fish paste, samples were taken for analysis. As a result of comparing the energy value of fish products, an increase in protein fractions and a low content of fat were noted. The protein content ranged from 17 to 18%, with a high content of essential amino acids. The mineral composition was significantly higher than the control sample. Dietary fiber, a rich set of mineral complexes in fish paste are a complete product [45,46,47]. Expanding the range of products, offering formulations for fish pates from freshwater fish meat is relevant for the food industry. Multicomponent fish pate has a high biological value with functional properties of a food product [48].

The staff of the Brazilian University is developed a method for preparing fish paste with fiber elements. The basis of the pate is a mustache-striped catfish of the genus pseudoplastistoma, which lives in the fresh waters of South America. Muscle tissue is valued for its juiciness and is in demand among consumers. This type of catfish grows well and reproduces in artificially created conditions. For the preparation of fish pate, fragments of meat left after the separation of the fish fillet were used. Frozen losses after technological processing are used as plant material. The composition of the fish pate formulation is represented by catfish meat 60%, sunflower oil 20%, water 16,7%, spices, additives 3,3%. The ingredients were crushed and homogenized. Heat treatment of the pate was carried out in a steam autoclave in accordance with the developed technology. Jars with fish paste were stored for 180 days at 25 °C. As a result of experimental studies, the positive effect of the thermal preservation process on the increase in the shelf life of fish paste. The nutritional value of the finished product was 15 g of protein and 7,92 g of fat per 100 g, respectively. The average moisture content was 75,5% and 1% minerals. A comprehensive analysis of the fish product is confirmed the safety for humans throughout the entire experience. The transformation of quality food losses can compete with seafood and aquaculture products [49].

Studies of the fish resources of the Baltic Sea indicate the concentration of small herring for the sprat production. As a result of the analytical work of the processing enterprises, a high catch culling rate of 25% was established. Cull fish represents quality food loss. After processing the caught fish, up to 80% of the raw material is discharged into solid loss. Developing new approaches to fish processing requires expanding the range of ready-to-eat foods. An increase in the range of culinary and canned products based on fish raw materials will be similar to existing delicacies [50].

From losses of by-products, smoked fish, a fish pate has been developed with variations of flavor, color and flavor additives. To remove the smell of smoke, smoky taste, strong spices are included in the formulations. Vegetable ingredients of garlic, pepper, fried onions, carrots help to improve the smell and taste in the finished product. Fatty dairy products, starch, fried flour, fragrant herbal fiber reduce the concentration of smoke odor in the product. From the ethical point of view, the consideration of smoked heads as a raw material in the production of products for humans causes protest. Such pates have a non-uniform structure with fragments of the raw product, strong smoke odor and satisfactory taste. The formulations made it possible to produce fish pates with acceptable sensory attribute. A pate with a neutral aroma can be used to produce a product with a wide range of smells and tastes [51].

The ratio of components in fish pates is considered optimal from the point of view of the balance of fats, proteins, plant components. The functional purpose in comparison with the proposed classic formulations expands the range of fish pates. The consumption of functional pates will have a beneficial effect on digestion and absorption of nutrients by the human organism.

Conclusions

Based on the results of research on the selection of ingredients, it should be noted its versatility. Biological compatibility, nutritional balance, individual approach, taking into account the preferences of consumers, determines the recipe for the finished product. The process constituting the selection of components consists of scientific research, technological solutions to problems in the fishing industry. Targeted management of technological processes for the use of related raw materials stimulates the production of full-fledged functional products. Research has allowed us to determine the preferences of fish products, sustainability, the value of the main attributes of consumption. By objectively combining ingredients from the food industry, multicomponent, balanced food products can be developed.

Additional research on the compatibility of ingredients for fish pates is needed to expand the range of finished products. Extending shelf life requires formulations to incorporate functional ingredients.

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ASSESSMENT OF QUALITY AND SAFETY OF PORK TREATED WITH LOW-TEMPERATURE ATMOSPHERIC-PRESSURE PLASMA

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Abstract
It is known that processing methods ensuring partial or full microbial inactivation are quite limited. Therefore, it is of great interest to develop technique and technologies allowing the effective action on microorganisms without a significant influence on product properties. The use of cold plasma can be one of the promising methods of meat product treatment by cold sterilization. The present work examines a possibility of chilled meat treatment with low-temperature atmospheric-pressure plasma to increase its stability to microbial spoilage and extend shelf life. To obtain low temperature plasma, the equipment developed by the designing department "Plasmamed" was used. Chilled meat was treated with low-temperature atmospheric-pressure argon plasma for 5, 10, 20 and 30 min. Samples were stored at a temperature of 2–4 °C for 10 days. Organoleptic indices, moisture weight fraction, changes in pH and water activity were analyzed before treatment and during storage. Sanitary microbiological analyses were carried out by the following indicators: quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), the presence and quantity of coliforms, Salmonella, Escherichia coli, Listeria monocytogenes, Proteus. It was shown that meat cold treatment with argon plasma inhibited the development of mesophilic microorganisms. The colony forming units detected in the samples after ten days of storage were determined by the duration of exposure to plasma. It was proved that meat treatment for 15 and 30 min had the bactericidal effect and facilitated an improvement in meat color during storage. The organoleptic indices of the samples treated with plasma corresponded to the requirements of standards and approved consumer characteristics.

Introduction

Modern food market conditions dictate a necessity to search for new approaches to their processing that would ensure high stability at storage and sales. A problem of preservation of non-treated food products (raw meat, chilled by-products, semi-finished products in pieces), in which possibilities of shelf life duration are limited, as a rule, by the use of packaging materials and gas atmosphere, has acquired special topicality. Modern packaging technologies are based on domination of the anabiosis principles — inhibition of microbial vital activities. Shelf life of products obtained using such methods is limited by the effectiveness of the microbial growth retardation and increase in duration of the lag-phase, during which microorganisms are not capable of significant damage to product safety and consumer characteristics due to their low concentration.

Immediately after production, meat raw materials with the intact structure are exposed only to surface contamination remaining practically sterile inside. Therefore, it can be expected that treatment of raw material surface based on abiosis principles will be significantly more effective regarding product safety.

From the practical point of view, realization of methods ensuring partial or almost full termination of vital activities in microbial cells is quite limited. Therefore, the studies aimed towards the development of equipment and technologies effectively influencing the condition of microflora without significant changes in the initial product properties are of particular interest. For these reasons, the electrophysical methods found application in the food industry.

Treatment with ultraviolet radiation (UV-radiation), ozone, gas atmosphere modified in the electrical field and some other bactericidal treatments are considered promising in regard to a decrease in product contamination and an increase in their storability [1,2,3,4].

For example, Graça et al. [4] studied an effect of UV radiation on the yeast growth on fresh Royal gala apples stored at a temperature of 4 °C. It was proved that treatment of apple samples contaminated with yeasts

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using ultra-violet radiation (2.5–10 kJ/m^2) reduced yeast growth rate by four times. UV radiation at doses of 7.5 and 10 kJ/m^2 showed the high bactericidal effect, which allowed preventing cross-contamination of food products with yeasts.

When studying UV treatment of chilled beef with and without ozone, the authors [5] found an effective reduction of meat microbial contamination during storage. In the experiments, meat was inoculated with cocktails of Shiga toxin-producing *Escherichia coli* (STEC) strains (serotypes O26, O45, O103, O111, O121, O145, and O157: H7), *Salmonella,* and *Listeria monocytogenes*. Then, inoculated beef was treated with UV radiation or UV radiation and ozone. The treatment reduced populations of *E. coli* O157: H7, *Salmonella, L. monocytogenes,* and aerobic bacteria from 1.49 to 0.86, 1.33 to 0.76, 1.14 to 0.5, and 1.23 to 0.64 log CFU, respectively. Gaseous ozone alone reduced populations of *E. coli* O157: H7, *Salmonella,* and *L. monocytogenes* by 0.65, 0.70, and 0.33 log CFU, respectively. It was also found that exposure to UVC and ozone did not lead to noticeable changes in fresh meat color and did not accelerate the formation of products of oxidative and hydrolytic spoilage of the fat fraction of products, which indicates an expediency of using UV treatment including with the use of active gas atmosphere to enhance meat microbiological stability without deterioration of consumer characteristics.

Magnetic induction heating (MIH) is considered promising in regard to increasing microbiological safety of pasteurized products. In the study that compared the convective heating protocol and MIH, death of Salmonella was achieved at temperatures of 60 °C and 68 °C. Exposure to the alternating magnetic field (AMF) led to heat generation by microbeads with a size of about 50 µm via the magnetic hysteresis effect, which later on ensured homogeneous product heating due to the high specific rate of heat distribution by homogeneous dispersed particles in high-viscosity food products. Reduction of treatment duration allows preventing coagulation of egg proteins and ensures high preservation of the initial state of food nutrients [6,7].

It was proved in the study [8] of high pressure processing (HPP) of chicken meat in combination with active packaging based on an essential oil that the synergetic effect of applied impacts facilitated a reduction in the quantity of *Listeria monocytogenes* lower than the detection limit during 60-day storage at low positive temperatures (0 °C to 4 °C). However, the growth of *L. monocytogenes* began again when the temperature was increased up to 8 °C. The authors suggest that high pressure processing (HPP) of poultry meat can be beneficial for reducing the risk of raw material contamination with *L. monocytogenes* without deterioration of its quality.

The effectiveness of high pressure processing regarding the reduction of total aerobic and lactic acid bacteria counts in frozen beef was reported by Fernández et al. [9]. According to the authors' data, moisture losses upon thawing and meat color were within limits comparable with those after thawing non-treated beef.

Undoubtedly, food sterilization with ionizing radiation is considered the most effective. Nowadays, however, this method is not accepted as safe. It was found that an ionizing radiation dose of 3 kGy prevents the development of food microbiological spoilage during storage [5,10].

Over the last years, new methods of thermal and non-thermal food processing have been designed, for example, ohmic heating, microwave treatment, high pressure processing, cold plasma, which turned to be less detrimental to processed food quality than traditional heating methods [8,11].

The use of low-temperature atmospheric-pressure plasma (LTAP) is an innovative technology that envisages food processing with the ionized gas — cold plasma [12,13]. Ionization occurs due to an effect of an electric discharge emerged in the regulated conditions.

Multiple studies point to the high effectiveness of LTAP in regard to decontamination of different objects. The method of cold sterilization is widely used in processing medical instruments and materials [14,15]. The use of cold plasma in the food industry is introduced to a lower degree; however, the effectiveness of the bactericidal effect of LTAP on a wide range of causative agents of food spoilage has been studied and presented in literature quite extensive [16,17,18].

The plasma composition and functionality significantly depend on ionization parameters — the voltage and frequency of an electric field, composition of a gas atmosphere and design of a generator. For example, cold plasma formed due to ionization of the atmospheric air is saturated with the reactive oxygen species (ROS) and reactive nitrogen species (RNS) that ensure the antimicrobial effect of treatment. Due to high temperatures, this technological gas can be used for simultaneous drying and microbial inactivation in free-flowing materials. Helium and argon plasmas have the effective antimicrobial activity. The peculiarity of inert gas ionization is their easy flammability [18].

An effect of a gas atmosphere on the reactivity of cold plasma is explained by non-uniformity of energy distribution between its particles due to differences in thermodynamic conditions of electrons and larger particles — ions and neutral molecules. Contrary to high-temperature plasma, the thermodynamic temperature of free electrons in cold plasma reaches tens of thousands of degrees Kelvin, while the temperature of molecules and ions is close to an ambient temperature. When colliding, free electrons transfer energy that leads to excitation of larger particles; however, a degree of reactivity remains non-uniform [19].

With respect to the use of cold plasma in the meat product technology, one of the most promising properties is its high electrical conductivity. The ability of the plasma medium to transfer charged particles under an effect of external electric fields, while at the same time retaining the ionized condition of the gas can be used for the development of methods for thermal food processing; however, it is not useful for preservation due to quick heating of a processed object.

An influence of design peculiarities of plasma generators (plasmatrons) is expressed in the possibility to realize different types of charges inducing plasma flows with different characteristics [20].

One of the main methods for generation of cold atmospheric-pressure plasma is a dielectric barrier discharge (DBD) emerging due to electrical breakdown between two electrodes under an impact of high pressure. At the moment of the discharge, high current is induced due to high breakdown conductivity; however, short exposure duration (8–10 s) does not allow inducing high amounts of energy (Figure 1A). As a rule, dielectric discharges emerge at alternating voltage in a frequency range of 50 to several thousands of Hz. Voltage varies from 1 to 100 kV and more [20].

A dielectric barrier discharge has a low threshold of cold plasma ignition, and is characterized by a large area of impact, which makes it the most acceptable for product decontamination. The effectiveness of the DBD plasma treatment method was established with regard to eggs [21], packaging materials and packed foods including sausage products, packed fruit and vegetables [22,23]. Treatment of a product with a dielectric barrier discharge for 15 s fully inactivated microorganisms in a quantity of 10^7 CFU/g, while 2-min treatment practically killed most pathogenic microorganisms [24].

Modification of the method for obtaining cold atmospheric-pressure plasma is generation of a discharge in a frequency range from several kHz to tens of MHz and power from several W to kW in the dielectric tube (Figure 1B) [25]. The generated electric discharge runs out of the tube with the flow of ionized gas forming plasma jets. Characteristics of plasma jets depend on the type of electrode location and parameters of power supply (Figure 2).

This method is the prevalent source of low-temperature plasma. However, to ensure high performance of the generator that is sufficient for treatment of large areas, the simultaneous impact of several jets is necessary. It should be noted that the high plasma concentration in the jet conditions ensures effectiveness of antimicrobial treatment [26].

There is a method of microwave plasma striking. Microwave discharges at atmospheric pressure usually are plasma torches that work with large gas flows similar to plasma jets (Figure 1C). Flares induce plasma at a frequen-

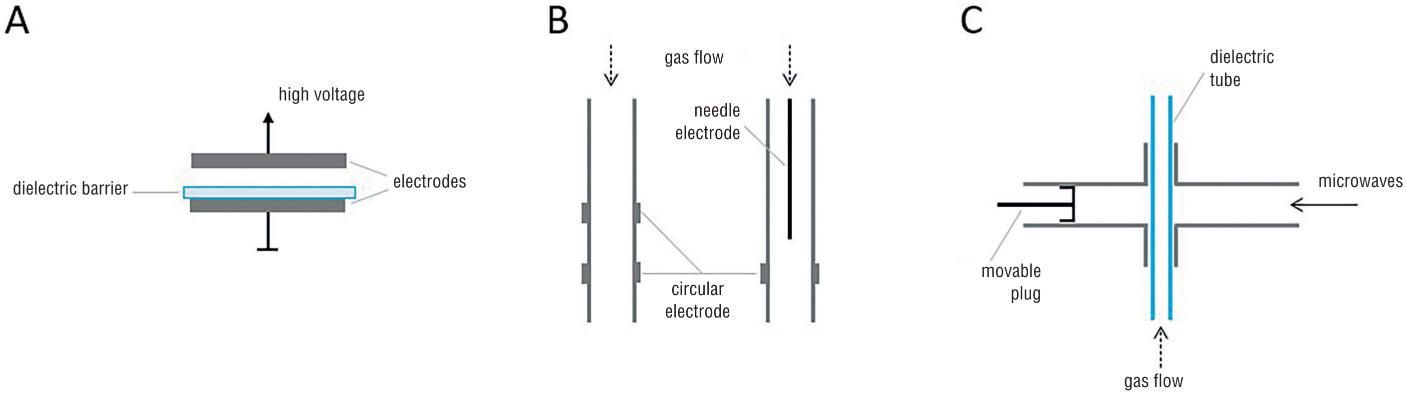

Figure 1. Schematic illustration of (A) the dielectric barrier discharge, (B) different units for plasma jets and (C) simplified microwave plasma torch

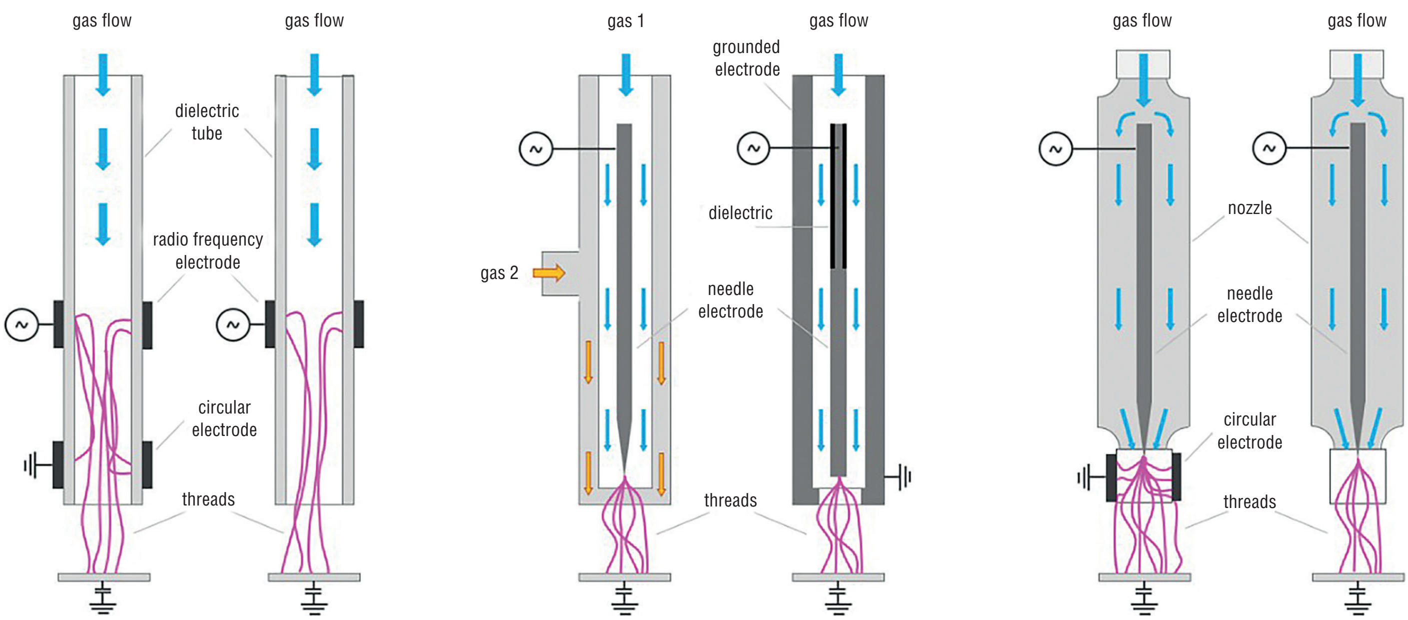

Figure 2. Configuration of electrodes

cy from several GHz in a power range from 10 W to about 6 kW. However, the plasma gas is heated in these conditions, which is a significant limitation for using this method for food processing.

Summarizing an experience in sterilization of different objects, the authors [17] propose to use several plasma sources (microplasma matrices, surface and coplanar dielectric barrier discharge) as a promising technology.

To use plasma in the food industry, its temperature should not exceed 20 °C to avoid denaturation changes in protein and induction of oxidative processes. When processing thermosensitive food products, the necessary plasma temperature is achieved mainly by regulation of the ionizing gas composition. Differences in the thermodynamic condition of electrons, ions and molecules, non-uniformity of energy distribution upon their collision and, as a consequence, changes in a plasma temperature can be managed by the composition of the initial gas atmosphere [27,28].

Wang et al. [29] stated that cold plasma processing of food products is regarded as promising sterilization technology. At the same time, studies on inactivation of microbial cells in solutions are practically absent. It was proved in the experiments with a computer simulation model of a plasma reactor that high background concentration of yeasts negatively affected their inactivation.

Sahebkar et al. [30] presented the study on an effect of cold plasma treatment of marinades, which were injected into chicken fillets infected with *Escherichia coli* and *Staphylococcus aureus*, on microbial contamination of the product. The study proved a decrease in the growth of the above mentioned microorganisms.

Another important aspect of food treatment with ionized low-temperature plasma is a method of its contact with the object surface. Contact of the plasma gas and a product can be considered most effective; as a result, many reactive compounds directly affecting microorganisms are concentrated on the treated surface [31]. The mechanism of the bactericidal effect of low-temperature plasma is similar to ultraviolet radiation, which has the high penetrating ability and causes the death of a microbial cell linked with destruction of the DNA structure [32]. UV radiation weakens DNA replication process and, thus, hinders microbial multiplication [33]. Effectiveness of UV radiation range (200–220 nm) is linked with the fact that maximum inactivation of the cell genetic composition is achieved under the action of short-wave radiation. It is necessary to note that only 7% of low-temperature plasma action occur in the above mentioned wavelength range. With that, however, plasma has the high bactericidal effect, which to a large extent is conditioned by the intensity within this particular wavelength range, where the maximum inactivation of microbial DNA takes place. Moreover, the bactericidal action of plasma is enhanced due to food treatment throughout the whole volume and the development of pulse-periodic electric discharges within food systems [34,35].

The aim of this research was to study a possibility of using the equipment for low-temperature plasma generation in the conditions of atmospheric pressure developed by the designing department "Plasmamed" [36] (hereafter, plasmatron) for treatment of chilled meat to increase its storability at low positive temperatures.

Materials and methods

M. longissimus dorsi from chilled pork produced by Rodnikovsky pig-breeding farm (Chelyabinsk Oblast, Miasskoye settlement) was chosen as an object of research. During the experiments, the following samples were made: 1 — control; 2 ÷5 — experimental samples treated for 5, 10, 20 and 30 minutes on the plasmatron under the same conditions of plasma generation (Figure 3). The weight of samples was 1.5–1.6 kg; sizes were 16 cm × 22 cm × 10 cm; a temperature of the samples before the experiment was 4 °C.

Figure 3. Chilled meat samples for analyses

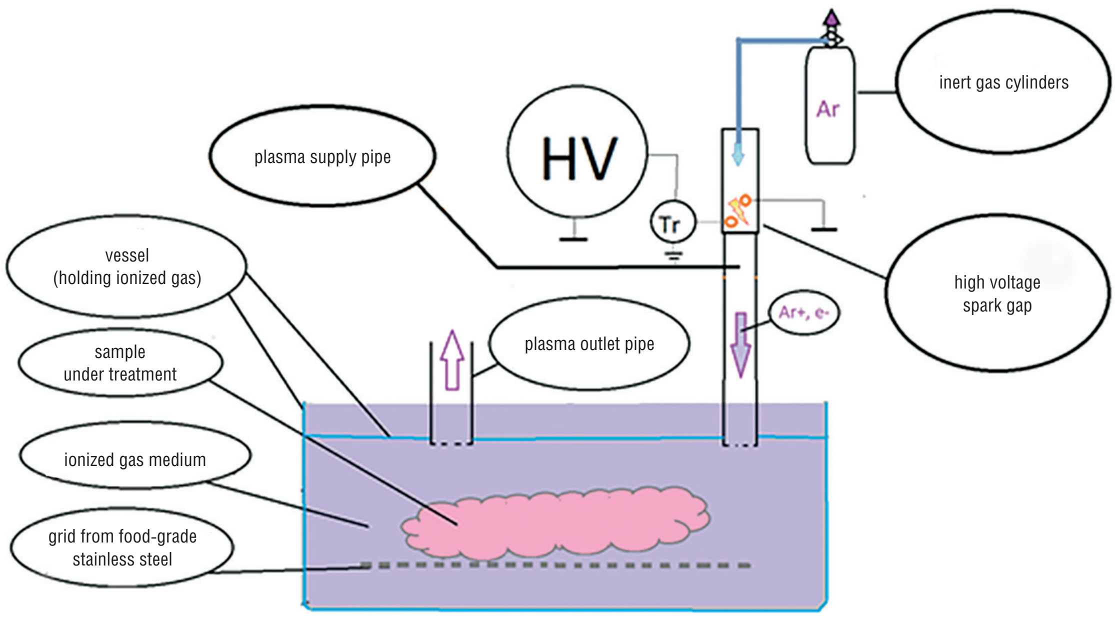

Figure 4. Scheme of meat treatment with low-temperature atmospheric-pressure argon plasma

To create a modified atmosphere, inert gas argon[1] was used, which ionization took place in the working chamber of the plasmatron using the DBD method under the impact of discharges with frequencies of 450 to 550 kHz induced by the current supplied to the electrodes. The voltage between electrodes was 12–14 kV, the power of the generator was 25–30 W.

The principal scheme of the developed equipment for chilled meat treatment with low-temperature atmospheric-pressure argon plasma is presented in Figure 4.

In the experiment, meat was placed unpacked into 3 L plastic containers with lids fitted with valves for supplying and removing modified atmosphere (Figure 3). To control a level of air replacement in a container with the ionized gas, the outlet pipe was fitted with a bag, which volume corresponded to the volume of the container. When plasma was supplied, air was displaced from the container into the bag. After filling the container, the bag was detached, the exhaust valve was closed and the ionized plasma gas continued to be supplied to the container. Treatment time was recorded for each sample using a timer with the accuracy of up to 5 s. After treatment, containers with the samples were placed into a refrigerator chamber for storage at a temperature of 2 °C ...4 °C.

An effect of treatment on meat preservation in the chilled condition was assessed according to the recommendation[2] by the scheme for products with expected shelf-life up to 10 days in the test-laboratory SBI of the Sverdlovsk Region "Sverdlovsk Regional Veterinary Laboratory". Sanitary microbiological analyses were carried out by the following indicators: quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM)[3], the presence and quantity of coliforms[4], *Salmonella*[5], *Escherichia coli*[6], *L. monocytogenes*[7], *Proteus*[8]. Organoleptic indices[9] and moisture weight fraction[10] were determined in the plasma treated and control samples. The pH indicator was determined by the potentiometric method using a digital pH-meter (model 2696) with a measurement range of 0–14 pH units and the measurement accuracy of 0.05 pH units. Water activity (aw) in the initial samples and after storage was recorded using a water activity analyzer AWC-4 with a measurement range of 1.00 to 0.75 with the measurement accuracy of 0.0001 units with the computer communication interface.

Results and discussion

Organoleptic indices in the samples were analyzed before their treatment and controlled every day of the following storage. At the initial stage, all muscles had similar condition and were scored identically. After a day of storage at low positive temperatures, the samples treated with plasma differed from the control by more intensive surface color; with that, the color intensity in the samples increased with

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Table 1. Physico-chemical indicators of the control and experimental samples of chilled pork before and after storage

Indicator		Control samples[3)]		Samples treated with argon LTAP (duration in min.) before and after storage							
				5		10		20		30	
		before	after	before	after	before	after	before	after	before	after
pH		5.72	5.86	5.67	5.74	5.85	5.91	5.76	5.82	5.77	5.84
W[1)], %	S[2)]	—	—	53.67	51.43	54.16	52.98	55.15	53.02	55.19	53.54
	C[2)]	54.33	54.21	53.95	53.42	53.89	53.27	54.68	53.88	54.62	54.07
a_w	S[2)]	—	—	0.9860	0.9839	0.9882	0.9827	0.9864	0.9811	0.9825	0.9816
	C[2)]	—	—	0.9887	0.9874	0.9864	0.9849	0.9844	0.9844	0.9854	0.9841

Notes:
1) W — moisture weight fraction;
2) S — for samples taken from the surface layer with a thickness of not more than 3 mm;
C — for samples taken from the central part of meat;
3) After 5 days of storage.

increasing treatment duration. The revealed differences in the color intensity remained up to the sixth day of storage. During following storage, differences in the color intensity in the treated samples were not significant; the samples had the dark pink color.

When storing non-treated pork, the surface color intensity insignificantly grew; however, the color remained to be paler compared to plasma treated samples. After six days of storage, organoleptic traits of spoilage began to appear in the control sample as moisturization and weak discoloration of the surface.

An effect of storage on the color intensity in the LTAP treated samples can be explained by a change in the condition of the meat surface layer due to partial drying, and, probably, due to more intensive and directed transformation of heme pigments with prevalence of oxyforms.

Organoleptic analyses of the experimental samples during and after storage did not reveal significant differences in appearance, consistency and odor. Signs of spoilage were not found.

The values of pH, moisture weight fraction (W) and water activity (a_w) determined in the surface layer with a depth of not more than 3 mm (S) and in the samples from the meat center (C) before treatment and after 10 days of storage are presented in Table 1.

It was established that meat storage in containers without additional packaging led to some weight losses due to moisture removal mainly from the surface layer. By the end of storage, the value of moisture weight fraction in the surface layer of the experimental samples decreased on average by 3.30 ±0.87% relative to the value in the surface layer of the samples sent to storage. Moisture of the surface samples and the samples taken from the central layers of meat before storage did not have significant differences.

It is impossible to explain directly the observed reduction of moisture in the surface layer of the samples treated with LTAP by an effect of low-temperature plasma. However, it can be assumed that the sterilization effect of treatment significantly inhibits the microbial development on the meat surface. As a result of treatment, the concentration of the products of microbial vital activities also decreased. These products facilitate moisturization and following slime formation on the surface, which was observed in the control sample after six days of storage.

The pH values in fresh and stored chilled meat were different by not more than 0.6 units, which is a consequence of the biochemical changes in chilled muscle tissue during storage and indirectly confirm the absence of intense microbial growth.

The water activity values correlate with the data on the moisture content.

The results of the microbial analyses are of critical importance for assessment of the LTAP effect on meat storability. The control and plasma treated samples were analyzed after five and ten days of storage, respectively.

According to the results of the investigation, *L. monocytogenes*, *Salmonella*, *Proteus*, *E.coli* were not detected. Changes were noted in QMAFAnM values and the presence of coliforms. The results of the experiments are given in Table 2.

Table 2. QMAFAnM values and presence of coliforms in the samples after storage

Samples	Value of the indicator	
	QMAFAnM, not more than, CFU/g	Coliforms
Control	$7.4 \cdot 10^5$	Detected in 1 g
Обработанные НПАД, мин		
5	$6.0 \cdot 10^5$	Not detected
10	$5.1 \cdot 10^3$	Not detected
20	$3.1 \cdot 10^3$	Not detected
30	$2.8 \cdot 10^3$	Not detected

According to the obtained data, treatment of chilled meat with argon plasma inhibits the development of mesophilic microorganisms. It was established that the colony forming units detected in the samples after ten days of storage depended on the duration of exposure to plasma. Comparison of the obtained values with the data from literature sources [37] confirmed the high potential of meat treatment in the flow of argon plasma to preserve chilled products.

The obtained results on the reduction of bacterial contamination of chilled meat after treatment with cold atmospheric-pressure plasma correspond to the study [38], which linked the plasma effect with an impact on cellular DNA. One of the mechanisms of microbial cell destruction is breakage and modification of cellular DNA. Another one is dimerization of thymine and oxidation of bases in the DNA composition with formation of 8-OHdG in prokaryotic cells under the impact of cold plasma. Reparation of DNA damages or cell death was initiated depending on a damage degree.

There are also other theories that explain mechanisms of the bactericidal effect of cold plasma on a microbial cell.

According to study [39], nitrogen reactive species can accumulate on the surface of microbial cells and easily diffuse through cell membranes causing a decrease in the intracellular pH. This indicator plays an important part in cell functioning. It affects the enzyme activity, rate of reaction, protein stability and nucleic acid structure. A decrease in the intracellular pH causes destruction of the microbial cells. It was established in our investigations that an increase in the time of meat treatment with cold plasma insignificantly influenced microbial contamination, which corresponds to data [40]. The authors also did not reveal a significant effect of the treatment duration on microbial deactivation. At the same time, repetition of ten-minute treatment of meat products with cold plasma increases the intensity of microbial inactivation, which depends not only on conditions of plasma treatment but also on a food product type and, in particular, on characteristics of their surfaces. It is always necessary to take into account this factor when choosing parameters for effective microbial inactivation.

In our investigations, meat samples were treated throughout the whole area, which allowed inactivation of most microorganisms and corresponds to the results of study [41]. The authors state that two-sided treatment of chicken breasts during 2.5 min facilitated deeper microbial inactivation than one-sided treatment for 5 min. Similar conclusions were also obtained for pork loins. According to investigations, food product treatment with oxygen atmospheric-pressure plasma enhances the intensity of UV radiation. UV photons and particles reactivated in plasma enhance its bactericidal action. Based on the obtained results, the authors emphasize the necessity of the careful approach when applying longer product treatment with plasma as their organoleptic indicators can change. Taking into account our own results and opinions of foreign researchers, we recommend chilled meat treatment with LTAP for 20 min.

When studying an effect of helium and argon plasma on microbiota of the pork and beef surface, the authors [42] established that 10-min treatment with helium plasma led to reduction of the total microbial count, the number of yeasts and psychrotrophic microorganisms by 1.14–1.48 log cycles for pork and 0.98–2.09 log cycles for beef. More significant reduction of microbial counts by 2.00 log cycles was observed for *Bacillus subtilis* and *Yersinia enterocolitica* after 2 min of helium plasma treatment. Similar results were established for *Staphylococcus aureus*, *E. coli* and *Pseudomonas fluorescens* after 5 min and 10 min of exposure. Disruption and lysis of *E. coli* cells treated with helium plasma for 10 min were revealed. The presented data about the effectiveness of contaminated meat treatment with low-temperature plasma correspond to our results.

Ulbin-Figlewicz et al. [43] studied an effect of cold plasma treatment on microbial inactivation of meat surface, meat color and pH value. Nitrogen, argon and helium plasmas generated by high voltage discharge in a vacuum chamber were used for the experiment. The final plasma pressure was 0.8 MPa; the exposure time of samples in the active medium was 5 and 10 min. The authors proved that the number of psychrotrophic bacteria and total microbial counts on the surface of the samples treated with helium and argon plasmas for 10 min reduced by about 3 log CFU/cm^2 and 2 log CFU/cm^2, respectively. In addition, more intense reduction of yeasts and molds was revealed, which was about 3 CFU/cm^2 and 2.6 CFU/cm^2 for helium and argon plasma, respectively. Significant differences in the pH values before and after cold plasma treatment were not observed, which confirm the results of our investigations.

Our results obtained regarding an effect of treatment with low-temperature plasma on the moisture content in pork samples are in agreement with data of other researchers [44,45]. Assessment of cold atmospheric-pressure plasma effectiveness for inactivation of two bacterial pathogens and its effect on changes in the moisture content showed that treatment inactivated *Staphylococcus aureus* and *L.monocytogenes*, reduced yeast–mold counts, and led to reduction of the moisture content in a food product by 7.34% due to water evaporation from the sample surface.

The presented analysis of the data about an effect of plasma on microorganisms is confirmed by our data about changes in the water activity in plasma treated and non-treated pork samples, which also indirectly confirm an increase in meat microbiological stability and revealed a decrease in the moisture weight fraction in the surface layers of the studied objects.

Conclusion

The results of the microbiological investigations unambiguously suggest the inhibitory action of ionized argon produced in the unit for generation of low-temperature plasma of the designing department "Plasmamed" in regard to mesophilic microorganisms developing on the surface of chilled meat during storage. Meat holding in the ionized atmosphere under the accepted conditions significantly reduced QMAFAnM and had a detrimental effect on coliforms, which was confirmed by the water activity value before and after storage. The organoleptic indices of the plasma treated samples corresponded to the requirements of standards and conventional consumer characteristics.

The effect of argon plasma on color stability revealed during investigations should be further studied.

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WAYS OF IMPROVEMENT OF TECHNOLOGICAL EQUIPMENT PERFORMANCE

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Abstract
Increasing of production performance of production systems and equipment in the meat-processing industry is integrally linked to maintenance activities. The article analyzes the influence of technological effectiveness, level of hygiene, reliability and simplicity of equipment design on the main parameters of meat products quality. Here it is shown that the strategy of Total Productive Maintenance (TRM) and Lean production is important tool for assessment of parameter of the Overall Equipment Effectiveness (OEE), which is used to assess the main types of losses that reduce the equipment productivity. The link of influence between the structural and mechanical properties of food masses, the moisture content of the initial food raw material, the temperature and viscosity, the processing pressure, and the density for the optimal operating terms of the equipment are analyzed in this research. With the help of OEE concept, the time losses related to the features of the equipment functioning are determined. The types of losses are classified as follows: downtime and readjustment (availability or readiness for operation) of equipment, short-term shutdown of equipment and a decrease in processing speed (productivity or performance rate of the equipment), product rejects and product losses (quality losses) during startup of equipment. While analyzing of the operation of vacuum filler for sausages production, the values of parameters of equipment availability, it productivity and product quality are calculated. The availability index is 0.79, the productivity index is 0.76, and the product quality index is 0.95. Taking into account the obtained data on equipment availability, productivity and product quality, it is determined that the overall equipment effectiveness (OEE) accounts for 57%. The application of this method for studying the overall equipment effectiveness operation can be applied to any technological equipment used in the meat-processing industry and allows eliminating many problems that arise during the operation of technological equipment, improving its technical and economic parameters, and developing a system of measures for improvement of its maintenance and repair.

Introduction

Due to the intensive increase in food consumption and change in structure of consumed food towards the higher quality and more diverse range of food products, the development of food industry currently has dramatic importance. Its efficient functioning is of critical importance for ensuring the food and economic security of a country and for improvement of the living standards of the population1.

Despite the fact that the current state of the industries engaged in processing of agricultural products is characterized by relative stability and significant potential to grow, the further development of the country's economy requires increasing the efficiency of domestic production, increasing its volumes, taking into account the competitiveness of the produced food products, in order to meet the needs of industry in raw materials in full range and to provide the population with food. Based on the basic socio-economic requirements for the development of the food industry, in the future, along with an increase in food production, it is necessary to raise the technical and technological level of production, create new types of products, and improve the consumer quality of food products. The solution of these main tasks is possible on the basis of the introduction of scientific and technologic achievements in the most important areas of scientific and technological progress regarding to production, storage and processing of food products.

Among many branches of food industry in the Republic of Kazakhstan, the production of meat and meat products is traditionally considered one of the main and prior spheres of agriculture. However, the technical condition of the most of meat processing enterprises requires a thorough renovation and reconstruction. Taking into account the features of an enterprise, its production goals and objectives, the market for production equipment to produce semi-finished meat products can offer domestic or imported technological lines, as well as some separate units of equipment.

Despite this, the management of each enterprise encounters questions related in some way to improvement of equipment effectiveness, its maintenance and its repair. To solve these issues during the operation of equipment it is necessary to apply approaches and methods which ensure its high productivity, produce high-quality product, and reduce idle time by reducing of unplanned downtime.

[1] Ponomareva, N.I., Nikitenko. V.S. (2005). The current state of the food industry in Kazakhstan: Analytical review. Almaty: KazGNINTI. 2005. — 69. (In Russian)

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In this case, the introduction of lean production methods makes it possible to form a more responsible arrangement of production processes, to find reserves for increasing the production rate due to more efficient use of existing technological capacities, and also to reduce the cost of production [1]. Currently, the term "lean production" is being implemented at enterprises in many countries and in various industries [2,3].

Within the "lean production" system a range of methods can be offered, which use will increase production effectiveness, for instant the principle of "kaizen" (continuous improvement of production processes), "5S System" (due arrangement of the workspace and optimization of labor), "TPM" (Total Productive Maintenance — a system of total productive equipment maintenance), etc.

In particular the target of the TPM program is to eliminate losses related with equipment maintenance. The "TPM" is based on eight principles aimed at preventive methods of maintaining the reliability of equipment [4,5]:

1 **Autonomous maintenance** (making operators responsible for routine maintenance);
2 **Scheduled maintenance** (development of procedure and schedule of maintenance);
3 **High-quality service** (finding and prevention of errors);
4 **Continuous improvement** (based on the concept of "kaizen");
5 **Early management of the equipment** (creation of a management system for development and introduction of new equipment);
6 **Training of employees** (improving the awareness of staff involved in the service);
7 **Safety, health, environment** (creation of a safe and healthy working environment in the workplace);
8 **Administrative and office TPM** (elimination of losses in the administrative functions).

Due to preventive repair and maintenance of equipment in operable condition, its maximally productive, economical use is achieved [6].

The obligatory condition for production of high-quality products is the perfect operable state of machinery (equipment, tooling), materials and methods (service system, methods of operation and measuring methods). The efficiency of the major production becomes increasingly dependent on the services and servicemen assigned to ensure the maintaining the working equipment and tools in operable condition with minimal costs of repair. For the main production, it is necessary to supply raw materials, other materials, semi-finished products, power of various types, tools, transport [7].

The implementation of all these diverse functions is the task of various auxiliary divisions of an enterprise — department of repair, tools, power supply, transport, warehouse, etc. Improving the efficiency of technical maintenance of production in whole to the greatest extent depends on correct organization of those departments and further improvement of their work.

Like any other process the TPM function must be continuously improved to minimize maintenance and repair costs and maximize equipment availability and performance. While that an important tool for the strategy of Total Productive Maintenance (TPM) and of Lean production is the parameter of the overall equipment effectiveness (OEE), which is used to estimate the main types of losses which cause reduction of equipment operation effectiveness [6,7,8,9]. KPIs are three metrics as follows, namely: equipment availability, productivity, quality. The first two parameters (availability and productivity) take into account the loss of time from equipment downtime, and the quality metric takes into account the loss of quality during production of the finished goods.

Improving the equipment availability and productivity usually requires reengineering, which does not always require replacement of existing equipment with the new ones. The desired result can be achieved due to the high availability of equipment, caused by reliability and maintainability of the equipment. Therefore, the maintenance function is vital for sustainability of any manufacturing facility, and according to Pareto rule of 80–20, which implies that most part of efforts is inefficient and must be reduced, it follows that 80% of maintenance costs and repair costs are spent on maintenance of 20% of equipment [10,11]. This very problematic equipment must be revealed and special attention must be focused on it.

Materials and methods

Depending on the processing and cooking methods, the composition of raw materials of the minced meat is quite diverse. It is explained by the varying degree of mechanical processing, moisture level, fat content and other factors. Therefore, the production compositions of minced meat used in sausages production were selected as the objects of research.

The important element in determining the efficiency of equipment operation is the arrangement and monitoring of work to collect information on losses that cause the greatest problems in the process of operation of technological equipment. It is recommended to use Pareto analysis to process the obtained data. Pareto chart is a way of graphing data that allows identifying a few, but important factors that make the greatest impact on losses, in order to focus the primary efforts on eliminating or reducing the influence of these factors. Continuous monitoring of the overall efficiency of the equipment will reveal not only downtime due to breakdowns, but also losses due to ineffective adjustment or tuning of equipment, decrease in its performance or idle time due to waiting for the materials [12,13].

Results and discussion

Technological processes in the food industry are numerous and various, however they have in common that the original organic raw materials are processed into finished food products. This circumstance determines the specific feature of food production, and that is the absolute cleanliness for acceptance of raw materials, storage and processing of food products, and protection of food products from pollution and spoilage, as well as from accidental admixture of foreign substances and objects.

Taking into account the specifics of food production, i. e. the manufacture of semi-finished or finished food products, it is necessary to comply with a number of requirements for food equipment [14]:

- technological effectiveness of equipment — implementation of established technological processes at each stage of processing of food raw materials (maximum use of the raw materials and preservation of its nutritional value);
- high level of hygiene of the equipment, which defines the quality of the produced food (high level of hygiene of working parts and their surfaces in contact with the product must be made of corrosion-resistant construction materials);
- equipment reliability — the maximum probable duration of operation (failures in operation lead to a decrease in rate of productivity, to disruption of the technological process and loss of product);
- simplicity of equipment design (ease of disassembly and assembly of the working mechanisms, availability of free access for repairs, sanitization and maintenance, simplicity of units, working and most wearing parts must be easily replaceable).

The design of the working parts of a machine must exclude unnecessary grinding of the processed raw materials, chance of getting the lubricating oils, rust or metal dust caused by wear of parts into the processed food mass.

Currently, food industry enterprises use high-performance equipment, create production lines for the manufacture of various semi-finished products and finished products; introduce new continuous technological processes into production based on cut-the-edge technologies. Meanwhile any measures, applied to ensure the growth of production, must provide for a more complete and efficient use of machinery and equipment, elimination of downtime, reduction of the time required to master the new equipment, and for further intensification of production. In addition, it is necessary to keep in mind that equipment is one of the factors which influence the quality of products. The properties of equipment can be determined through the structural and mechanical characteristics (SMC) of the used raw materials and the influence of various technological factors on SMC [15]. The necessity to comply with technological modes and conditions of production to reduce rejects and improve quality during production of cooked sausages was stated by L. S. Kudryashov in his research [16].

The influence of various technological factors on structural and mechanical properties of food masses.

The quality of finished food products depends on quality of the raw materials. The quality of raw materials is greatly influenced by processing and compliance with the technological recipe.

The wide variety of raw materials and products make it inevitable to use many fundamentally different technologies, types of machines and devices. Compliance with the optimal technological and mechanical parameters in the production of semi-finished food products at certain stages of their processing, using rheological methods, allows stabilization of yield and quality of the finished products. A set of functional, technological, structural and mechanical parameters is used to predict the behavior of raw meat during technological processing. The set of parameters objectively reflect its quality (pH value, water-binding and fat-holding capacity, water activity, stickiness, viscosity, plasticity, etc.) [17].

The process of food media forming is one of the most complex processes in the food industry because of the specificity and variety of types of the processed raw materials. The entire range of physical and chemical properties of the formed material is observed in this process in all its wide diversity, as well as changes in structural and mechanical properties during processing and in dependence with various technological factors. Therefore, the design schemes of the forming machines are completely determined by the technological properties of the food media accordingly.

Accurate observance of the minced meat recipe in terms of the quantitative and qualitative composition of raw materials and additives, ensuring optimal conditions for its preparation and optimizing the processes of extrusion and forming the sausages guarantee the obtaining high-quality products. Meanwhile it is necessary to note that the processes of extrusion, dispensing and forming (molding) are not yet mechanized enough and still very manual labor consuming. When forming sausages, the structural and mechanical properties of minced meat and their changes during processing, the working pressure when stuffing (filling) the minced meat into a sausage casing or mold, temperature changes in the casing during heat treatment of sausage loaves are not taken into account. Many of these disadvantages occur in dosing and forming / molding machines used in both large and small meat processing plants. One of the important directions in optimization of stuffing process is the correct selection of the stuffer and determination of the range of its practical use [18].

In order to regulate the technological processes, determine the optimal operating modes of the sausage forming equipment, it is necessary to monitor constantly the quality properties of the processed product based on its structural and mechanical properties, which are most sensitive to changes in various technological and mechanical factors.

Duration of ripening. To determine the influence of various factors on the structural and mechanical properties of minced meat, it is important to know the duration of period during which its properties (Θ_0, η, B, m) after their forming remain practically constant.

Studying the effect of ripening on shear properties, A. V. Gorbatov noted that in the first two hours of ripening of minced meat its properties practically do not change [19].

In case of sausages ripening for 4–10 h (second period), all parameters (Θ_0, η, B, m) increase to a maximum (critical ripening time). While that period the process of spontaneous hardening finishes, moisture completely saturates all existing bonds. This is important to know when preparing sausages for heat treatment.

Further exposure (third period) causes a decrease in values of all properties due to weakening of the sausage structure under the influence of microbiological and biochemical processes.

Moisture. Moisture is one of the main technological parameters for many food products. The water content and moisture content determine the quality of the food. They have a significant impact on the flavor, appearance, texture and shelf life of foods.

For sausage minced meat, along with an increase in humidity the liquid layers of dispersion medium between the particles gets thicker, the concentration of proteins in solution of interlayers decreases, their viscosity, structural strength and the values of structural and mechanical properties decrease also [20]. At the same time, this leads to an increase in the yield of products, reduces the load on all elements of processing machines.

The process of thickening of liquid layers is inhibited by the reverse process — swelling of muscle fibers, increase of their surface and binding of moisture, which contributes to increase in strength. The cumulative effect of these processes in general leads to minor decrease in strength of structure of minced meat.

Pressure. As a rule, the structural and mechanical properties of food products are determined at atmospheric pressure, which does not correspond to the conditions under which the product in processed and transported. In most cases the processes of forming / molding, pressing, transportation, etc. take place under conditions of all-round compression; therefore, to obtain a finished product of high quality, it is necessary to take into account the effect of pressure on its structural and mechanical properties.

Density is an essential characteristic when calculating a number of machines and processes. Like in many liquid products, the density of viscous-plastic food products increases with increasing of pressure.

For a range of sausage mince production compositions (milk sausages, milk sausages, diet sausages, table sausages, diabetic sausages), the density, depending on pressure, changes according to the logarithmic law and can be determined from the following dependence

$$p = a_1 - (a_2\varphi + a_3 U) + a_4 \lg Y \tag{1}$$

where

a_1 — is the coefficient, kg/m^3, numerically equal to
1052 at $0 < p \cdot 10^{-5}$, Pa $\leq 2{,}5$,
1107 at $2{,}5 < p \cdot 10^{-5}$, Pa ≤ 16 ;

$Y = (p \cdot 10^{-5} + 1)$ — modulus (numerical value) of absolute pressure;

a_2, a_3 — empirical coefficients, kg/m^3, respectively equal to 270 and 10,5;

a_4 — coefficient, kg / m^3, numerically equal to
143 at $0 < p \cdot 10^{-5}$, Па $\leq 2{,}5$,
47 at $2{,}5 < p \cdot 10^{-5}$, Па ≤ 16;

φ — fat content of minced meat (kg. of fat per 1 kg. of minced meat);

U — moisture content of minced meat (kg. of moisture per 1 kg. of dry residue).

The equation (1) is valid at pressure $(0 \div 16) \cdot 10^5$ Pa.

It was found that the highest compaction of minced meat is observed within the pressure range $p = (0 \div 16) \cdot 10^5$ Pa. At high pressures the density of minced meat increases only insignificantly. The obtained data allows determining the volume of the product in a compressed state and after discharge of pressure.

With increasing pressure, the values of the structural and mechanical characteristics of the minced meat also increase — the ultimate shear stress θ_0 Pa, effective viscosities at unit velocity B Pa·s and unit gradient of velocity B_0^* Pa·s, rate of structure destruction m. Plastic viscosity η_{pl} Pa·s practically does not depend on pressure.

The change in main values at different pressures happens mainly due to the reorientation of particles and their denser compaction, with subsequent increase of interaction in contact surfaces of the coagulation structure of the minced meat, and to destroy this system more intense external influences are required.

Temperature and viscosity. Meat and meat products are complicated substances in their chemical composition and have a complex of various properties that together make up the quality of products. In this case the structural and mechanical properties stipulate the behavior of meat and meat products in a stressed state, which main parameters under applied force are stress, magnitude and rate of deformation. Awareness of these parameters as well as defining of their dependence on various factors have great practical importance for obtaining objective information about the state of the product at various stages of its processing. This information is also needed for development of new technologies and methods of control over technological processes, and for technological design of machines and devices [19].

In production of sausages, which occupy a leading place among other meat products, with the help of devices that allow measuring structural and mechanical properties, it is possible to control the technological parameters of raw materials and minced meat, quality of product at any stage of minced meat processing (from ripening of meat to stuffing of minced meat into sausage casing or mold), as well as the chewy texture of finished products [18].

During production in majority of cases technological processes occur in conditions of changing temperature. Therefore it is important to know the influence of temperature on structural and mechanical properties of the processed meat mass, which will make it possible to determine the optimal operating modes of the sausage forming equipment.

Structural and mechanical properties (ultimate shear stress (θ_0, Pa), effective viscosity (η_{ef}, Pa·s) and plastic viscosity (η_{pl}, Pa·s), effective viscosity at unit velocity (V), effective viscosity at unit gradient of velocity (B^*_0), the rate of the structure destruction (m) was determined within the range of temperature from 275 till 313 °K (2–40 °C) for minced meat of industrial production composition — milk sausages, diet sausage, milk sausage, diabetic sausage, special sausage.

When processing the experimental data, it was found that an increase in temperature leads to decrease in values of investigated structural and mechanical properties. This is caused by the fact that an increase in temperature leads to a weakening of bonds in the introductory-protein-salt layers due to decrease in the solvent viscosity and a more intense thermal motion of molecules. This mechanism of the temperature effect on the product leads to a weakening of the strength of the structure as a whole.

To calculate the effective viscosity (η_{ef_t}) at different temperatures the generalized equation is suggested

$$\eta_{ef_t} = AB^*_{0_{red}} \cdot e^{E/RT} \cdot (\dot{\gamma}/\dot{\gamma}_1)^{-m}, \qquad (2)$$

where

A — is pre-exponential factor;

$B^*_{0_{red}}$ — is the reduced effective viscosity at a unit velocity gradient, Pa·s;

E — is the energy of activation, kJ / kmol;

R — is the universal gas constant, (8,32 kJ / kmol · K));

T — is the absolute temperature, ^{0}K;

$\dot{\gamma}/\dot{\gamma}_1 = \dot{\gamma}_*$ — is the numerical value of the velocity gradient;

$\dot{\gamma}_1$ — is the unit value of the velocity gradient;

m — is the rate of destruction of structure;

$B^*_{0_t} = AB^*_{0_{np}} \cdot e^{E/RT}$ — is the effective viscosity at the unit velocity gradient and at any temperature.

The coefficients A and E have the following values depending on the temperature:

Within the temperature range (275–299,5) ^{0}K — $A = 0{,}055$, $E = 7074$;

Within the temperature range (305–313) ^{0}K — $A = 0{,}022$, $E = 6428$.

For practical application of results of this research a graphic analytic method for determination of the effective viscosity at any values of velocity and temperature gradient within the studied ranges is proposed (Figure 1) [21].

Each section of the straight line in the figure "1 b" corresponds to its own value of the activation energy. Moreover, the change in the activation energy does not occur continuously, but occurs in the form of activated bounces, which is caused by melting of fat contained in sausage meat.

The first inflection point at a temperature of 299.5 ^{0}K corresponds to the beginning of fats melting and, apparently, is the limit of a quantitative change of structure. In section 2–3 the crystallization structure begins to break down spontaneously with a simultaneous increase in fluidity, while most of the components turn into a liquid state. The melting point of 305.5 ^{0}K, corresponding to the second inflection point, can be considered as qualitative boundary of state change, which features the transition of minced meat from the crystallization structure to the coagulation structure.

The temperature of about 311 ^{0}K corresponds to the beginning of the manifestation of denaturation processes and the transition of minced meat from a plastic-viscous state to an elastic one. The effect of the onset of denaturation must be taken into account when considering a number of technological processes.

The value of the effective viscosity at different temperatures according to the graphs shown in Figure 1 is determined in two stages. According to the graph (Figure 1 b) at a given temperature, in the specified range, we find the value of the effective viscosity at a unit gradient of velocity ($B^*_{0_t}$). Then, according to the graph (Figure 1 a), we determine the effective viscosity (η_{ef_t}) according to the value of the velocity gradient obtained as a result of viscometric studies.

Considering that even small changes in temperature cause significant changes in viscosity and other structural and mechanical properties, it is necessary to strictly comply with the temperature modes for production of minced meat and ready-to-eat food products, made of it.

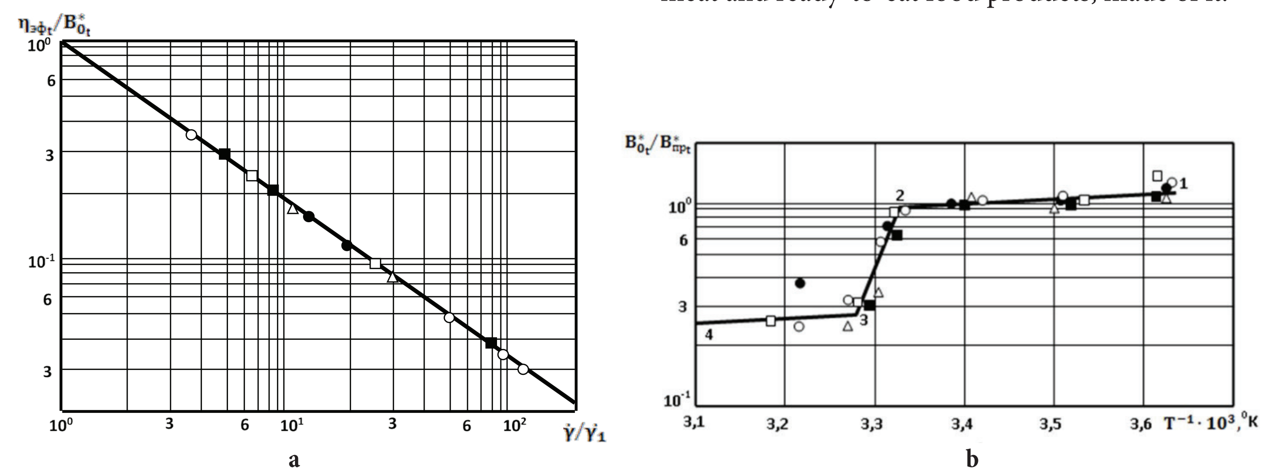

Figure 1. Dependence of the effective viscosity on the gradient of velocity (a) and on temperature (b) for minced meat: ○ — dairy sausages; □ — special sausage; Δ — diabetic sausage; ● — milk sausages; ■ — diet sausages

The flow of food masses in circular channels

When shaping sausages, one of the final stages before filling the sausage casing is the movement of the minced meat along the channel of the sausage stuffer, which is a circular channel of a certain length.

Currently a wide range of theoretical and empirical equations are known for calculation the process of food product movement along the circular channels, since they are used in a device for molding of food masses, as interoperative transport, and also in capillary viscometry [22]. The mathematical description of the product behavior is a prerequisite for the engineering design of the flow process.

Since the capillary diameter can reach several tens of millimeters for high-viscosity non-Newtonian fluids and plastic-viscous systems, it is important to define the distribution of velocities and deformations over the cross section of the mass flow during the movement of the food product.

The speed of the minced meat flowing out of the stuffer horn has a significant effect on both the state of the minced meat and the productivity of the stuffing machine. The larger is the diameter of the stuffer horn, the lower is the flow rate, the less pronounced changes in the structural and mechanical properties of the minced meat are observed and the higher is the productivity of the equipment [20].

In the majority cases all types of minced meat used for the production of sausage products can be attributed to viscous-plastic materials, and an increase in the shear rate creates not only a conventional shear stress, but also leads to normal stresses that lead to swelling of the meat flow in the moment of its exit from a stuffer horn or capillary (Barus effect). This phenomenon was established when studying the change in pressure along the length of the minced meat line at different flow rates of minced meat and is very important in assessing the applicability of minced meat for processing.

When flowing through short channels, it is possible to determine the pressure loss by estimating the difference between the pressure in the food mass reservoir at the inlet point to the stuffer horn and the ambient pressure at the outlet from the stuffer horn. The measured pressure difference is greatly influenced by the so-called input effects caused by non-laminar flow in the inlet area and unsteady flow conditions. To minimize the end effects (at the inlet and outlet of the channel) on pressure loss, they use capillaries with a high capillary length ratio (stuffer horn) L to its diameter D ($L/D = 20/1$ or higher) [23].

In theory of flow of any media, the basis for calculating of pipelines parameters is definition of the type of function. It allows calculating the velocity and the velocity gradient over the cross-section of the live flow.

The velocity profile is quite comprehensively characterized by the ratio u_{max}/w. In theory this ratio varies from one to three, depending on the flow mode. The consideration of the "power-law fluid" motion model significantly expands the understanding of flow modes.

Theoretical velocity distribution profiles for round pipe, composed with different fluid flow index, according to the following equation

$$u/w = (1+3n)/(1+n)\left[1-(r/R)^{\frac{n+1}{n}}\right] \tag{3}$$

and theoretical velocity gradients distribution profiles, composed accordingly upon the following equation

$$\gamma^{\cdot} = \partial u/\partial r = -w/R\big((1+3n)/n\big)\big((r/R)^{1/n}\big) \tag{4}$$

are in good agreement with the experimental distribution profiles of velocities and velocity gradients [24] and thus they indicate the presence of two zones — a wall layer, i. e. annular layer with a thickness of $\delta = R - R_1$, and the main flow with radius R_1 (Fig. 2).

In the equations 3 and 4 above the following notations are used: u — velocity of elementary layer, located at a distance r from the pipe axis, m/s; w — average velocity of flow, m/s; n — index of flow; R — inner radius of a pipe, m.

As can be seen from Figure 2 below, the sausage meat is exposed to the greatest deformations in the wall layer, where there is a sharp change in speed. The core of the flow moves in the form of a quasi-solid rod.

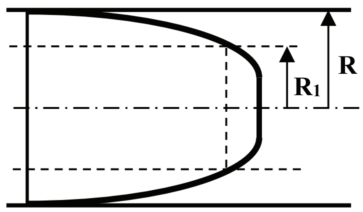

Figure 2. Diagram of flow of minced sausage meat in a round pipe

Analyzing the experimental and theoretical profiles of distributions of velocities and velocity gradients, it can be noted that for any flow index, the greatest change in velocity within 0.8 of the maximum velocity occurs within the wall layer. It allows determining the thickness of the wall layer when the sausage meat moves in a round pipe. Basing on this it is possible to write down the following

$$\dot{\gamma}(R_1) = 0{,}2\,\dot{\gamma}_{max} \tag{5}$$

where $\dot{\gamma}(R_1)$ — in the gradient of velocity in the flow layer at the distance R_1 from the axis, s^{-1}.

The maximal gradient of velocity has the following form

$$\dot{\gamma}_{max} = -w\cdot\big((3n+1)/n\big)\cdot 1/R, \tag{6}$$

And the gradient of velocity in the layer of flow at a distance R_1

$$\dot{\gamma}(R_1) = -w\cdot\big((3n+1)/n\big)\cdot 1/R\cdot(R_1/R)^{1/n}, \tag{7}$$

where w — is the average velocity, m/s; n — index of flow.

So the equation 5 has the following form, taking into consideration the equations 6 and 7

$$(R_1/R)^{1/n} = 0{,}2 \text{ or } R_1 = 0{,}2^n R. \tag{8}$$

At the known value of R_1 the thickness of the wall layer δ any index of flow can be determined by the dependence

$$\delta = R - R_1 \text{ or } \delta = R(1 - 0{,}2^n R). \tag{9}$$

To determine the velocity gradient in the wall layer and the relative thickness of the gradient layer, the graphs of their dependence on the flow index are plotted below (Figure 3).

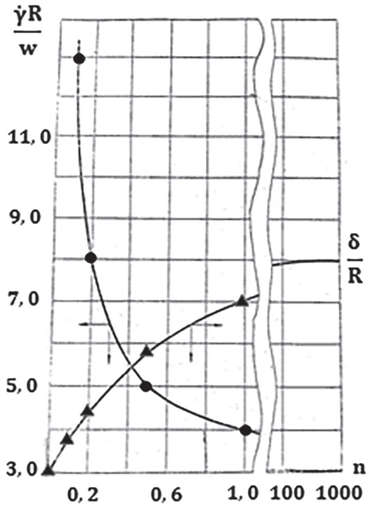

Figure 3. Dependences of maximal gradients of velocity and thickness of the gradient layer on flow index

Determining the effectiveness of the equipment

For the successful operation of any type of company, it is necessary to objectively assess the actual efficiency of using the available equipment. The availability of this information will eliminate a range of problems that arise during the operation of technological equipment, improve its technical and economic performance indices, and develop a system of measures to improve its maintenance and repair. As an assessment of the efficient operation of equipment, the concept of Overall Equipment Effectiveness (OEE) can be proposed, which allows to determine all losses of working time that affect the operation of the equipment (Figure 4) [6, 9, 25].

In accordance with Fig. 4, the overall equipment effectiveness (OEE), expressed as a percentage, is calculated by the following formula

$$OEE = (B/A) \cdot (D/C) \cdot (F/E) \cdot 100, \qquad (10)$$

where

B/A — availability of equipment (loss of time due to equipment downtime);

D/C — productivity (loss in speed due to short-term shutdown of equipment and, as a result, a decrease in throughput capacity);

F/E — quality of product (loss in quality due to the production of products below the quality standards).

Waste reduces overall production efficiency. In international practice, it is considered to be a bad OEE of less than 65%, satisfactory — from 65% to 75%, good — more than 75% [25].

When assessing the operation of equipment, six types of losses are usually defined, which can be classified as follows:

Availability (readiness of the equipment):

Downtime — equipment breakdown (the most common cause of equipment failures), stops for unscheduled tooling changes and unscheduled maintenance, failure of auxiliary equipment, stops not provided for in the production chart or schedule;

Changeover / adjustment — dismantling / installation of equipment, planned change of equipment, running of preparatory work, restrictions for consumables;

Performance:

Short-term equipment shutdown — can occur due to minor malfunctions, interruptions in delivery of materials. However these stops, which can last from a few seconds to 5 minutes, result in loss of productivity and reduced rate of productivity;

Decrease in processing speed — caused by equipment wear, decrease in power, increase in loading time, irregular operation of equipment, human error factor;

Quality:

Rejection and rework of defect product — producing of substandard products during the production process that do not meet the requirements of consumers. When reworking substandard products and eliminating defects, losses occur due to the waste of resources and the time spent on these production works;

Equipment start-up losses — rejects produced during start-up, warm-up or other initial stages of equipment operation.

In various companies other types of losses may be added to the above classification of losses arising from the certain operations.

An important element in determining the efficiency of equipment operation is the arrangement and monitoring of the production process in order to collect information on losses that cause the greatest problems in the process of technological equipment operation. To process the obtained data, it is recommended to use Pareto analysis, which allows identifying a few but important factors that have the greatest impact on losses [10, 11, 13, 25]. Ongoing monitoring of the overall efficiency of the equipment will reveal not only downtime due to breakdowns, but also losses due to inef-

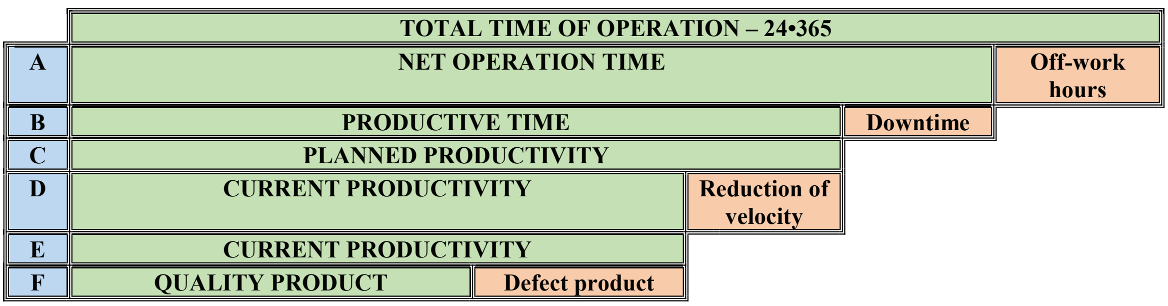

Figure 4. Elements of OEE and losses related to the peculiar features of the equipment operation

fective equipment setup, reduced productivity or time losses while waiting for materials to be delivered.

In order to identify losses associated with the operation of the equipment, the operation of vacuum filler for stuffing sausages, installed at one of the small company of the city, was studied and analyzed.

Based on the results of the studies and processing of data on types of losses that arose during the operation of a vacuum stuffer for sausages stuffing, the following parameters included in ratio were obtained (10). An example of calculating the coefficient of overall equipment effectiveness (OEE) for a vacuum filler is shown in Figures 5, 6, 7 [26].

Availability (readiness) of equipment

Shift duration (SD) – 480 min.

A	Net working hours (NWH)	Off-work time (OFT)

Off-work time (OFT):
Short breaks (ShB) 2 times per 15 min. – 30 min.;
Lunch (L) 1 times per 60 min. – 60 min.
OFT = ShB + L = 30 min. + 60 min. = 90 min.
Planned time of work (PTW)
PTW = SD – OFT = 480 min. – 90 min. = 390 min.

B	Working hours (WH)	Downtime (Dt)

Downtime (Dt) – **ПР = 82 min.**:
Readjustment of equipment;
Interruptions in the supply of raw materials.
WH = PTW – Dt = 390 min. – 82 min. = 308 min.
Availability (time lost due to equipment downtime)
$\mathbf{B/A = PTW/WH = 308/390 = 0,79}$.

Figure 5. Calculation of readiness (availability) of equipment

Productivity of equipment

C	Planned productivity (PP)

Due to the limited volume of production, the planned productivity is: **PP = 800 kg.**

D	Current productivity (CP)	Loss of speed (LS)

The loss in speed during the operation of the equipment led to a decrease in productivity by 192 kg., i.e. **SD = 192 kg.**

Loss of speed:
Idle operation with short-term stops of the equipment;
Running of the equipment at a decreased speed.

Current productivity (CP) is equal to
CP = PP – SD = 800 kg. – 192 kg. = 608 kg.

Performance (loss in speed due to short-term downtime of equipment and, as a result, a decrease in throughput capacity)
$\mathbf{D/C = CP/PP = 608/800 = 0,76}$.

Figure 6. Calculation of productivity rate of the equipment

Quality of product

E	Current productivity (CP)

As it was defined by the performance rate of the equipment, current productivity равна **CP = 608 kg.**

F	Quality product (QP)	Losses due to defect product (LDP)

The losses from defect products caused by the disruption of the production process and the reduction in the production volume amounted to LDP = 30 kg.
Below is the calculation for the quality product rate
QP = CP – LDP = 608 kg. – 30 kg. = 578 kg.

Quality (quality losses due to the production of substandard products)
$\mathbf{F/E = QP/CP = 578/608 = 0,95}$.

Overall equipment effectiveness (OEE)

Taking into account the data obtained on the availability, productivity rate of equipment and rate of quality product, the overall equipment effectiveness (OEE), expressed as a percentage, is equal to
$\mathbf{OEE = (B/A) \cdot (D/C) \cdot (F/E) \cdot 100 = 0,79 \cdot 0,76 \cdot 0,95 \cdot 100 = 57\%}$.

Figure 7. Calculation of product quality, overall equipment effectiveness

The overall equipment effectiveness is shown in Figure 8 below.

As it is obvious from the above data, the low efficiency of equipment during its operation was largely caused by a decrease in both the availability (readiness) of the equipment to work, and the parameter related to the productivity of the equipment. This fact indicates the necessity to find ways to reduce and control the downtime of equipment in order to eliminate losses during the operation of technological equipment, i. e. a key reserve for OEE growth is the elimination of losses. Similar studies can be run for each piece of equipment included in the production line for any producing task.

Conclusion

The inevitable requirements for increase in volume and range of semi-finished products, the most rational use of resources, and constant increase of nutritional value of food products determine the need for optimization and intensification of technological processes, as well as assess the quality of semi-finished products. The mechanization and automation of production of semi-finished food products is impossible without deeper understanding of the properties and parameters of raw materials used for their production, and the influence of various technological factors. In this regard, the demand for modern equipment for production of semi-finished food products with high productivity and reliability in operation is increasing.

In view of some reference target value based on the best data for availability, productivity and quality of the analyzed equipment, it is possible to get information on how to improve equipment operation by reducing downtime of this equipment.

Using the OEE concept and collecting the statistical data on causes of equipment failures, we are able to classify the factors that reduce the efficiency of equipment. In addition, information on factors influencing the loss of time during the operation of equipment will allow creating a system of equipment maintenance and repair (MRO), which can be used for planning of preventive maintenance and timely provision of spare parts and materials. For any fairly large and modern production it is crucially important.

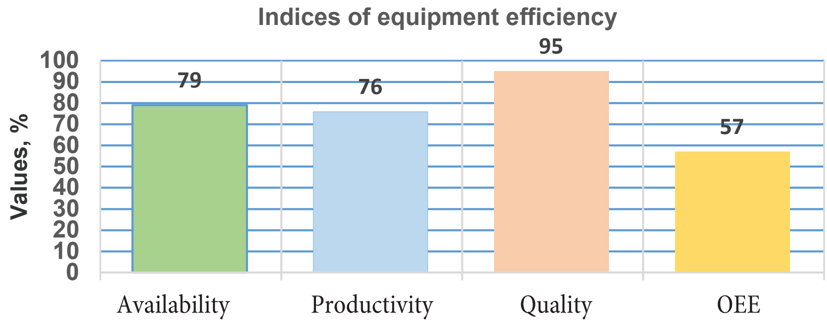

Figure 8. Indices of the overall efficiency of the equipment

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THE EFFECT OF ESSENTIAL OILS ON QUALITY AND SAFETY PARAMETERS OF MEAT DURING ITS STORAGE

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Keywords: *essential oils, autolytic processes, specific electrical conductivity, histological structural changes, bactericidal efficiency*

Abstract
This article researches the basic principles of mechanism for finding the concentrations of essential oils (EO) which are efficient for antimicrobial action, when used as a natural alternative to synthetic analogues. The effect of EO of rosemary, sage, garlic, laurel, cloves, basil and thyme on the structural components of meat, its physical and chemical, organoleptic and microbiological quality indices was studied. The effect of EO on duration of microorganisms' growth phases is analyzed. It is shown that the reasonable concentration of the essential-oil mixture (EOM) is equal to 0.1%, since at this concentration the optimal inhibition of saprophytic microflora was provided with satisfactory organoleptic quality indices ("taste peculiar for this type of product", "pleasant fragrance", "spicy aroma"). The analysis of histological structural changes in muscle tissue showed that application of EOM slows down autolytic processes, which correlates with the data obtained in research of the specific electrical conductivity in water extracts from the muscle tissue. The autolysis process in the control samples was more intensive in comparison with the experimental samples stored in the EOM. Thus, by 84th hour of storage in the control sample the basophilic staining of the samples slices was observed, as well as almost complete disappearance of the striation of muscle fibers. In the muscle tissue slices the disintegration of individual fragments into myofibrils, and myofibrils into sarcomeres, was observed in form of a granular mass, sometimes enclosed in endomysium. The meat fibers were deformed and their separation was observed. The same changes with the studied samples stored in the EOM occurred only after 204 hours of storage. The above studies of changes in the specific electrical conductivity of water extract from meat during storage showed the presence of complexes of EOM components formed with proteins and lipids of muscle tissue. The obtained data are confirmed by histological studies. Thus, the use of EOM allowed elongation of the cold storage period of semi-finished natural lump products by 2.6 times, which proves the practical benefits of its use in the meat-processing industry.

Introduction

The annual increase in consumption of meat and meat products makes it necessary to develop logistics, transport networks and new technologies that ensure the due quality of the product throughout the entire chain of its life cycle. Meat losses after production are estimated at 10% approximately. It should be noted that microbiological spoilage is the main contributor to these losses, along with lipid oxidation and enzymatic spoilage. Despite the improvement of production methods, despite introduction and implementation of hygienic and control measures in the food industry, every year there are cases of food diseases caused by food that does not meet the safety requirements. According to WHO [1] data, every year 600 million people, or almost one of ten people in the world, get food-poisoned after eating contaminated food, 420,000 of them die, including 125,000 children under age of five. 15% of such cases are caused by the use of substandard quality meat products.

This way, the relatively short shelf life and the growing demand for high-quality and safe meat products lead to the need for new technologies which are able to extend the shelf life while maintaining a high level of quality. In this regard, in order to increase the shelf life of meat products, many synthetic and natural components are used nowadays. The growing interest of manufacturers and consumers in natural food additives contributes to the practical value of this study and to the search for a natural alternative to synthetic analogues. These components must form the necessary parameters, indices and factors of meat products quality and meet the food safety requirements.

One possible solution to this problem is application of EO as natural preservatives in manufacture of food products, in particular: meat and meat products. It is known [2] that essential oils feature the properties of natural additives with a wide spectrum of action, for example, they feature antimicrobial, antiviral, antiparasitic, antifungal, antioxidant etc. properties in relation to many pathogens, such as *Pseudomonas, Acinetobacter-Moraxella, Enterobacteriaceae, Fusarium, Mucor, Candida, Torulopsis, etc.*, which were previously studied by a wide range of researchers

The authors [3] studied and considered the issue of assessing the antimicrobial effect of essential oils in certain combinations on food pathogens. The research shows that the active components of essential oils can be divided into four groups according to their chemical structure: terpenes, such as lemon EO; terpenoids, such as jasmine essential EO; phenylpropenes, such as vanilla EO; and others, such as mustard EO, which contains allyl isothiocyanate as the active agent. At the same time, mustard EO showed the

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highest antimicrobial activity against all tested pathogens with the largest diameter of the growth inhibition zone.

It was shown in research [4] that the combination of EO of rosemary and eucalyptus can be considered as the most efficient against pathogenic microorganisms when used in the food industry and medicine. It is also shown that the EO concentration of 40% is the most efficient and the inhibition zone increases along with the increase of concentration.

In the review [5] the main mechanisms of EO components effect on the microbial cell were considered. Special attention is paid to the effect of phenolic compounds on permeability of microbial cells. Phenolic compounds damage the cytoplasmic membrane, and disrupt the mechanism of ATP generation and driving proton force.

The authors [6] showed that EO components, like carvacrol and thymol, have noticeable disintegration action on cells walls, as evidenced by their enhancing effect on absorption of 1-N-phenylnaphthylamine and on release of lipopolysaccharides, as well as increasing of sensitivity to detergents. The breakdown of cytoplasmic membrane integrity leads to excessive loss of metabolites and enzymes from the cell and loss of its viability. These compounds also inhibit bacterial growth at the same concentrations which are necessary for cell wall disintegration, and increase the permeability of the ATP cytoplasmic membrane.

In [7], the destruction of membrane proteins of pathogenic cells *Bacillus cereus* under the effect of carvacrol was studied in detail. Carvacrol is the main active antibacterial component in EO of thyme and oregano. It increases permeability of the cell membrane to potassium ions. Potassium ion is an important cation for vegetative bacterial cell; it is involved in several key biochemical processes of bacterial cells. This ion plays an important role in activating cytoplasmic enzymes, maintaining turgor pressure, and regulating pH of cell. The loss of potassium ions is the first indicator of bacteria membrane damage. Inhibition of range of enzymes due to loss of basic ions leads to loss of cell turgor, violation of DNA synthesis, decrease of cell metabolic activity, etc.

As described by the authors of the research [8], carvacrol makes the cell membrane excessively permeable to potassium and hydrogen ions, and inhibits the synthesis of ATP due to violation of chemiosmosis process, which can lead to loss of activity of cellular transport of granulation and coagulation of cytoplasm.

The activity of EO varies within wide range depending on initial plant raw material, chemical composition, extraction methods, and many other factors. The composition, concentration, and ratio of the chemical components also determine the biological effect of EO.

The interaction between antimicrobial substances in their combination can have three different results: synergistic, additive, or antagonistic. Synergy occurs when a mixture of two antimicrobial compounds has antimicrobial activity greater than the sum of the activities of the individual components. An additive effect is obtained when a mixture of antimicrobial substances has a combined effect equal to the sum of the individual activities of the compounds. Antagonism occurs when a mixture of antimicrobial compounds has a combined effect less than with a separate application.

Despite the large amount of literature which covers the study of the antimicrobial properties of essential oils, the results *in vitro* are not easy to compare with results obtained *in vivo* when using them as preservatives for meat. However, studies of rosemary, cinnamon, lavender, sage, garlic, oregano and ginger essential oils have shown positive results regarding their ability to act as food preservatives [2,9].

Essential oils tend to be more efficient when they come into direct contact with organisms, so the practical interest is to create a mechanism for finding concentrations of essential oils that are efficient to achieve an antimicrobial action. However, direct contact with meat can affect its organoleptic quality indices through structural and chemical reactions or interactions. Essential oils tend to have an intense aroma even at low concentrations and, if used incorrectly, can negatively affect the consumer properties of food products.

Due to the possible interaction of essential oil components with the cellular structures of meat, a higher concentration of essential oils is usually required to achieve an antimicrobial effect. Along with that the application of EO in high concentrations to achieve the required antimicrobial activity causes negative changes in the quality, taste and smell of meat.

In industrial production scale, EO as a meat preservative is very difficult and expensive to be used directly, while ensuring sufficient control of their quality and quantity. The use of active packaging is promising perspective, in which EO can either be incorporated into the packaging materials and then diffuse into the meat, or released on the meat by evaporation in the free space between the packaging and the meat. Thus, the use of *Rosmarinus officinalis* essential oil, as part of the active packaging in amount of 4%, inhibits the development of putrid processes caused by bacteria (including *Enterobacteriaceae* and *Brochothrix thermosphacta*), and formation of such substances like cadaverine [2].

The authors [10] proved that use of special coatings with EO in packaging can reduce the undesirable effects on the taste of the product. Use of such active packaging also allows extending the EO effect on the product due to its slow release effect.

The research [11] shows the efficiency of application of chitosan and whey protein films soaked with nanocapsulated 2% garlic essential oil for extension of the shelf life of chilled sausage in vacuum packaging. Based on the analysis of microbiological and lipid stability of sausages, these active films slowed down the oxidation of lipids and the growth of main groups of microorganisms that cause spoilage.

To assess the effect of EO on quality of meat it is necessary to use methods that allow assessment of the physical and chemical state of meat raw materials.

It is proved that the study of specific electrical conductivity of muscle tissue by both direct and indirect methods is promising way for assessing the condition of pork during its storage and subsequent processing [12]. The authors proved the effect of morphological composition, various methods of technological processing, as well as the effect of food additives use on specific electrical conductivity [13].

The value of the specific electrical conductivity is directly related to the concentration of ions in the solution, to their nature and mobility. The change in electrical conductivity is also an indicator of the cell membranes breakdown in muscle tissue cells that retain fluid within and outside of the cells. In muscle tissue with intact cell membranes, a low electrical conductivity value is observed, which increases with an increase of water content in the intercellular space [14].

However, nowadays essential oils and their compounds are used in meat products only to limited extent. One of the reasons for this is the insufficient study of the essential oils effect on meat during its storage.

Therefore, in order to assess the degree of EO effect on meat, it was considered appropriate to conduct researches on EOM efficiency, as well as histological and structural, microbiological and physical and chemical parameters of meat during its storage.

Materials and methods

Raw meat. Chilled pork cutlet meat was used as a raw material. The sample was taken within the production environment of a meat processing enterprise in the Republic of Belarus. Sampling and preparation of samples for study was carried out according to GOST 7269–2015[1] and GOST R51447–99[2].

To prepare the samples, a liner soaked with essential-oils mixture (EOM), was placed on the bottom of a sealed glass container. The required amount of raw meat was placed in such a way as to ensure that it did not come into direct contact with the liner. The container was hermetically sealed.

Containers with meat samples were stored at a temperature of 0 °C to 4 °C and relative humidity of 85%. The samples were examined on 0, 36, 60, 84, 156, 204th hour of meat storage.

Determination of threshold concentration of EOM. The threshold concentration of EOM was selected on the grounds of results obtained from analysis of data on minimum inhibitory concentration (MIC) of EOM and the determination of threshold concentration of EOM by organoleptic parameters.

For analysis of the MIC EOM, a bacterial suspension was prepared with a concentration of microbial cells of 1×10^8 CFU/ml, (0.5 units according to the McFarland turbidity standard). The concentration of microbial cells was determined indirectly using the PE-5400 VI photoelectric colorimeter measuring the optical density of solutions, which was equal to 0.08–0.1. The obtained suspension was diluted with meat-peptone broth (MPB) to obtain a concentration of microbial cells of 1×10^6 CFU/ml, which corresponds to the indices of old cutlet meat according to the Unified Sanitary-Epidemiological and Hygienic Requirements for food products (commodities) subject to sanitary-epidemiological supervision (control)[3].

100 cm^3 of the prepared bacterial suspension was added to the sterile flasks and EOM was added until a certain concentration was reached (0.01%; 0.05%; 0.1%; 0.5%). The flasks were hermetically sealed and shaken on a SHR-1D laboratory shaker for 6 hours at 20 °C. At the end of shaking, 0.1 cm^3 of the suspension was inoculated on sterile GRM-agar culture media from each flask and evenly distributed over the surface of medium. Petri dishes with crops were incubated at 37 °C for 24 hours. After counting the grown colonies of microorganisms, the number of colony-forming units CFU/ml was determined and the results were compared with the value of the concentration of microbial cells in the control sample (1×10^6 CFU/ml).

In order to determine the threshold concentration of EOM in a food product on the organoleptic basis, samples of pork cutlets were prepared in accordance with the collection of recipes with application of OEM in following amounts: 0% (control sample), 0.01% and 0.1%. Upon reaching the culinary (edible) readiness, the samples were evaluated according to the following organoleptic parameters: taste peculiar for this type of product, medicinal aroma, spicy fragrance, pleasant smell, hot taste, sour aftertaste (GOST 31986–2012[4]).

Definition of total viable count — i. e. quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM).

Definition of quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM) and bacterioscopic examination of meat was carried out according to GOST 10444.15–94[5].

Study of the histological structure of meat. Histological examination of meat samples was carried out in accordance with the GOST R31479–2012[6] and GOST 19496–2013[7].

[1] GOST 2015–7269 "Meat. Methods of sampling and organoleptic methods of freshness test" Moscow: Standartinform, 2019. — 14 p. (In Russian)

[2] GOST R51447–99 "Meat and meat products. Methods of primary sampling" Moscow: Standartinform, 2014. — 8 p. (In Russian)

[3] Unified sanitary-epidemiological and hygienic requirements for products (goods) subject to sanitary-epidemiological supervision (control). Decision of the Council of the Eurasian economic Commission of October 28, 2010, № 299. Moscow, 2010. (In Russian)

[4] GOST 31986–2012 "Public catering service. Method of sensory evaluation of catering products" Moscow: Standartinform, 2019. — 15 p. (In Russian)

[5] GOST 10444.15–94." Food products. Methods for determination of quantity of mesophilic aerobes and facultative anaerobes" Moscow: Standartinform, 2010. — 7 p.

[6] GOST R31479–2012 "Meat and meat products. Method of histological identification of composition" Moscow: Standartinform, 2019. — 11 p.

[7] GOST 19496–2013 "Meat and meat products. The method of histological investigation" Moscow: Standartinform, 2019. — 12 p.

Determination of the specific electrical conductivity. To determine the value of the specific electrical conductivity of meat, an aqueous extract was prepared in a ratio of 1:10, for which the sample was carefully chopped, placed in a cylindrical vessel of 100 ml and extracted with distilled water on a laboratory shaker SHR-1D for 30 minutes at a temperature of 20 °C. The resulting extract was filtered through a folded paper filter. The electrical conductivity of 10% of the water extract of meat was determined at a fixed temperature by Orion-StarA112 conductivity meter with Orion 011050MD cell.

Results and discussion

As the essential oils included in the EOM may have different inhibitory activity, it was considered appropriate to determine the inhibitory activity of each EO separately and as part of an essential-oil mixture at a concentration of 0.1% EOM was compiled on the basis of organoleptic quality indices that are most suitable for its application in production of meat products (Figure 1).

From the data presented in Figure 1, it is obvious that the efficiency of EO in its pure form in amount of 0.1% widely varied (from 40.00% for EO of garlic to 99.76% for EO of thyme). For most of the studied EOs, the bactericidal efficiency ranged from 69.06% (for EO of clove) to 82.09% (for EO of basil). The bactericidal efficiency of EOM exceeded 70%. According to the obtained results, it is reasonable to state the additive effect of EO as mixture.

In order to determine the concentration of EOM in the modified gas medium at a meat storage temperature from 0 °C to 4 °C, studies were conducted to determine the minimum inhibitory concentration (MIC) and organoleptic parameters of meat quality.

When determining the MIC, the method of serial dilutions was used. Essential oils, that are part of EOM, were used as inhibitory agents.

The following EOM concentrations were used for serial dilution: 0%, 0.01%, 0.05%, 0.1%, and 0.5%. The dependence of the number of colony forming units on concentration of EOM is shown in the Figure 2 below.

From the data presented in Figure 2, it is obvious that MIC50 (concentration of EOM, to which 50% of the studied strains are sensitive) and MIC90 (concentration of EOM, to which 90% of the studied strains are sensitive) for a mixture of essential oils varied within the range of 0.1–0.5%. The EOM concentration of 0.1% ensured complete inhibition of the growth of bacteria *P. Salmonella* on *Bismuth-sulfite-GRM* based agar.

Due to the well pronounced taste and smell of EOM, the task was to determine the maximum possible content of essential oils in finished meat products based on their organoleptic quality parameters. The results of researches are shown in Figure 3. Samples of cutlets were prepared with different EOM concentration (0.01%, 0.1% and 1% by weight of minced meat). Upon reaching culinary (edible) readiness, the samples were evaluated according to the following organoleptic parameters: taste peculiar for this type of product, medicinal aroma, spicy fragrance, pleasant smell, hot taste, sour aftertaste.

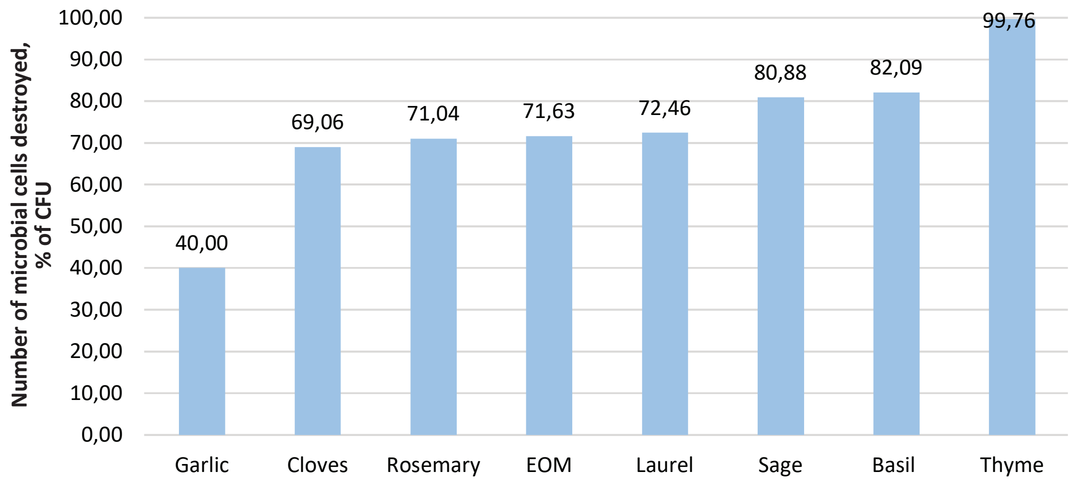


Figure 1. Bactericidal efficiency of essential oils and EOM at concentration of 0.1%, % of CFU

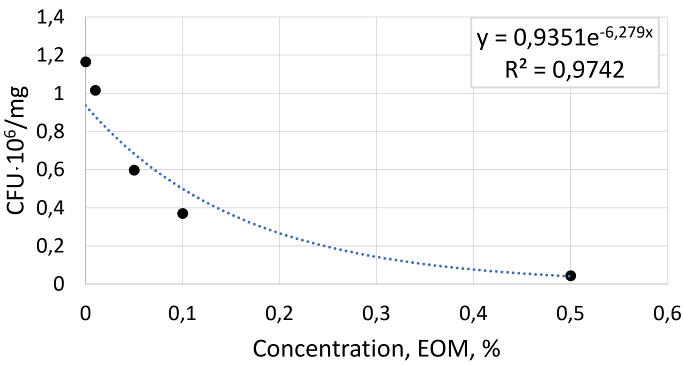


Figure 2. Inhibition of microbial cells by essential-oils mixture

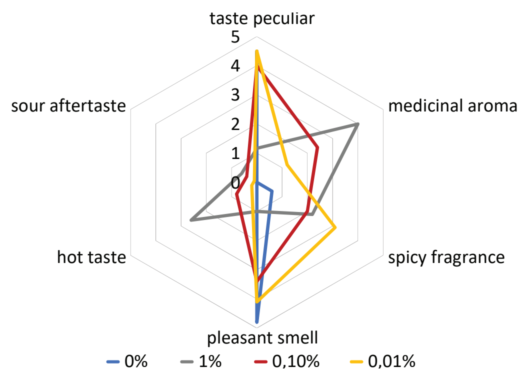


Figure 3. Profilogram of organoleptic parameters of quality

As can be seen from the data of the profilogram shown above in Figure 3, the samples of cutlets with an EOM concentration in amount of 0.01% of minced meat weight on the parameter "taste, peculiar for this type of product" were estimated by experts at 4.5 points, which is a good index for the product. This product had a pleasant spicy fragrance (3.1 points), no pronounced medicinal aroma, sour aftertaste and hot taste. In samples with 0.1% EOM content, the parameter "taste peculiar for this type of product" was estimated at 4 points, which is a satisfactory rating for the product. However, such organoleptic parameters as "medicinal aroma", "hot taste" and "sour aftertaste" were higher than similar parameters of samples with 0.01% EOM by 50%, 25% and 25% respectively.

The samples with 1% concentration of EOM featured by a pronounced medicinal aroma (4 points) and a hot taste (2.6 points). According to the parameter "taste peculiar for this type of product", it was rated at 1.17 points, which is unsatisfactory for the food. Based on the results of the organoleptic evaluation of the quality parameters and indices, it was determined that the threshold concentration of EOM is 0.1%. This concentration is the maximum one, since at this concentration EOM organoleptic quality indices ("taste peculiar for this type of product", "pleasant smell", "spicy fragrance") are considered as satisfactory rating.

Thus, the optimal concentration for conducting experiments was determined to be 0.1%, since at this EOM concentration, optimal inhibition of saprophytic microflora was provided (the residual content is equal to 2.3×10^5 CFU/ml), which corresponds to the safety indices of fresh meat, according to the Unified Sanitary and Epidemiological and Hygienic Requirements for food products (goods) subject to sanitary and epidemiological supervision (control), as well as satisfactory organoleptic indices of quality.

QMAFAnM is important specified parameter of food safety. According to the Unified Sanitary and Epidemiological and Hygienic Requirements for goods subject to sanitary-epidemiological supervision (control), the permissible level of QMAFAnM for small-batch semi-finished products shall not be more than 1×10^6 CFU/g. No coliforms, pathogenic microorganisms (including *salmonella*), *L. monocytogenes* are allowed. Therefore, the changes in the quantitative and qualitative composition of the microflora were further investigated.

At the beginning of the experiment the microflora of meat was represented by the following microorganisms:

- *Micrococcus sp.;*
- *Bacillus sp.;*
- *Pseudomonas sp.;*
- *Flavimonas sp.;*
- *Sphaerotilus sp.*

During the first day of storage of control samples a slight decrease in QMAFAnM was observed due to cooling treatment of meat (at a storage temperature of 0 °C to 4 °C) from 4.15×10^4 to 3.1×10^4 CFU/g by the 36th hour of storage. After 60 hours of storage the QMAFAnM (3.3×10^4 CFU/g) increased. By the 84th hour of storage the QMAFAnM in series of control samples exceeded the standard value by 2.3 times. By the 156th hour this parameter exceeded the normalized index by 40 times. QMAFAnM was not measured after that.

In the meat samples with EOM, the significant reduction of lag phase duration, and the rapid onset of the exponential growth phase of microorganisms were observed due to action of essential oils on these samples (4.15×104 CFU/g at the beginning of the experiment and 4.43×104 CFU/g in 36 hours). The stationary phase of growth is also reduced, as can be seen from the rapid onset of die-off phase (1.57×104 CFU/g, 60 hours of storage). Starting from the 60th hours, there is an increase in quantity of microorganisms. However, QMAFAnM did not exceed the standard value by the 84th hour of storage (2×105 CFU/g) and the 156th hour of storage (2.2×105 CFU/g). However, in 8.5 days of storage, there was a 1.34-fold excess of the standard QMAFAnM value (Figure 4).

Based on the obtained data it is obvious that the shelf life of the control samples was equal to 60 hours approximately, and the shelf life of the samples with EOM — about 156 hours. Thus, EOM allowed extension of the period of cold storage of semi-finished natural meat lump (cutlet pork) by 2.6 times.

The average time of microorganisms generation on control samples was 9.83 hours, for the samples with EOM — 27.59 hours.

Histological studies have shown that use of essential oils mixture slows down autolytic processes. So on the first day of research, the microstructure of the muscle tissue of the prototypes corresponded to the classical course of autolysis with a clearly defined core structure, striation and uniform color. After 36 hours of storage the differences in microstructural changes became more apparent. In the sections obtained from meat stored in a gas environment, the fiber thickness was lower in comparison with the control samples. The fibers of the muscle tissue were smooth, tightly fitting to each other, and in some places there were transverse and slit-like violations of fiber integrity. While in the control sample the fragmentation of individual muscle fibers and sometimes shadow-like coloration of the nuclei were noted (Figure 5).

Further the autolysis process in the control samples ran more intensively in comparison with the samples stored in an essential-oil mixture (Figure 6). By the 84th of storage of the control sample, there was a complete disappearance of the striation of the muscle fibers; the surface of the cut slice of the muscle tissue got a basophilic color. In the loose connective tissue of the superficial fasciae in the perimysium and endomysium, the diffuse overlays were predominantly the rod-shaped microflora. The slices revealed the disintegration of individual fragments into myofibrils, and disintegration of myofibrils into sarcomeres in the form of a granular mass, sometimes enclosed in endomysium. The fibers were deformed and separation of fibers was observed.

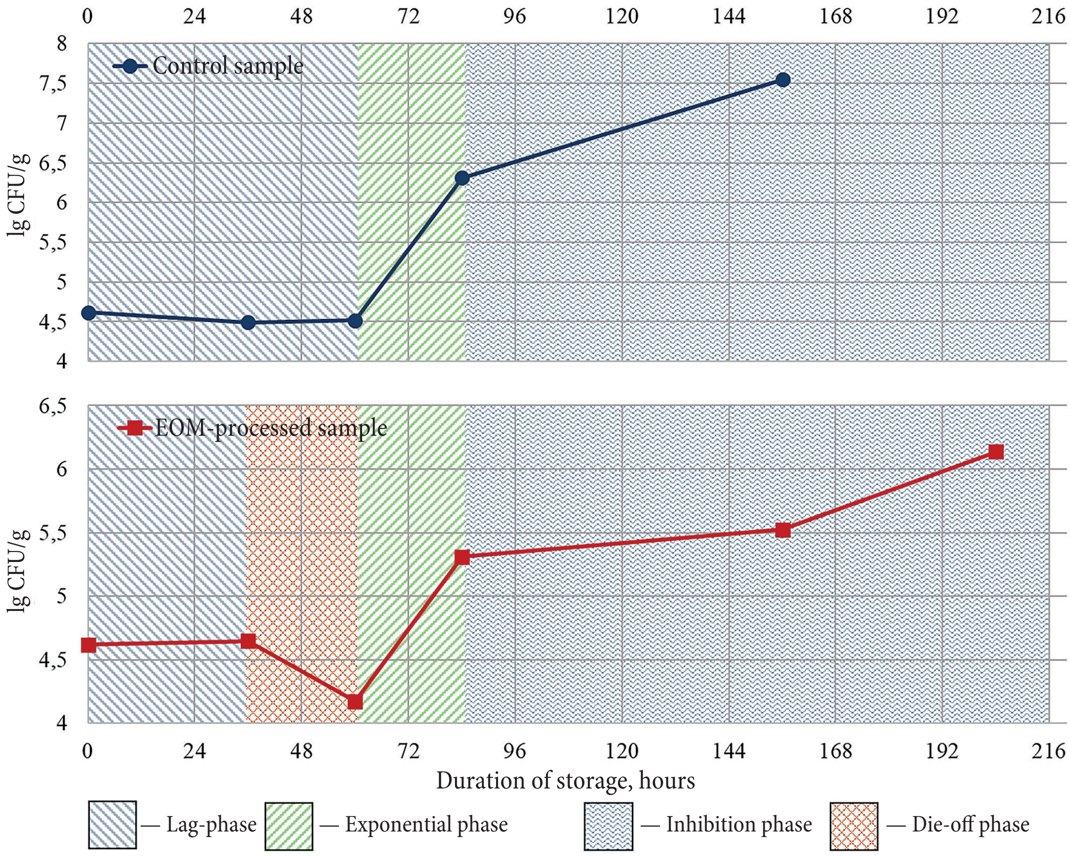
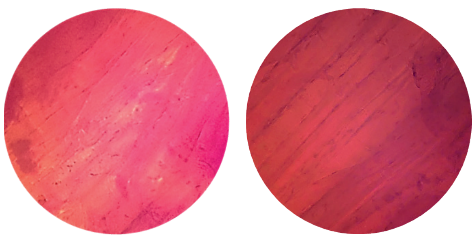
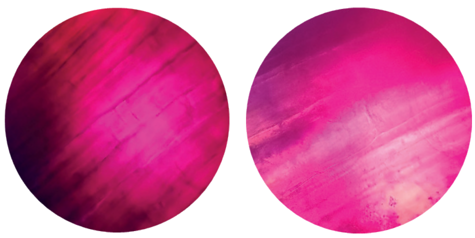


Figure 4. Change of QMAFAnM in meat during its storage



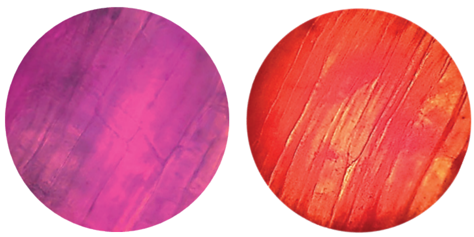
Control sample Sample with EOM

Figure 5. Histological specimen of longitudinal slices of pork cutlet meat after 36 hours of storage



Control sample Sample with EOM

Figure 6. Histological specimen of longitudinal slices of pork cutlet meat after 84 hours of storage



Control sample Sample with EOM

Figure 7. Histological specimen of longitudinal slices of pork cutlet meat after 204 hours of storage

Meat has become non-technologically unfeasible and unsuitable for its consumption in terms of safety parameters and indices. Similar changes occurred with samples stored in the EOM by the 204th hour of storage (Figure 7).

The difference in intensity of proteolytic processes was also confirmed by results of study of specific electrical conductivity. Changes in specific electrical conductivity of extracts from muscle tissue (Figure 8) occurred as a result of increase in quantity of charged particles due to the hydrolysis of proteins. Along with the decomposition of high-molecular compounds, the specific electrical conductivity of the control samples increased from 870 μS/cm to 910 μS/cm. The average rate of increase of specific electrical conductivity was 11.5 (μS / cm)/day. The further decrease of specific electrical conductivity from the 84th hour of storage of the control sample to 902 μS/cm may be caused by development of microflora, which both actively consumes protein and low-molecular substances, and accelerates the decomposition of high-molecular components, which leads to formation of interionic associates or ion pairs, to increasing of solution viscosity, and to other effects that reduce the mobility of ions and cause decrease of specific electrical conductivity [14].

The higher values of specific electrical conductivity of solutions of aqueous extracts obtained from muscle tissue with EOM compared to the control sample can be explained by the fact that essential oils interact with proteins and lipids of muscle tissue and create an additional electric charge. At the same time, the microflora has only insignificant effect on change of specific electrical conductivity value. The

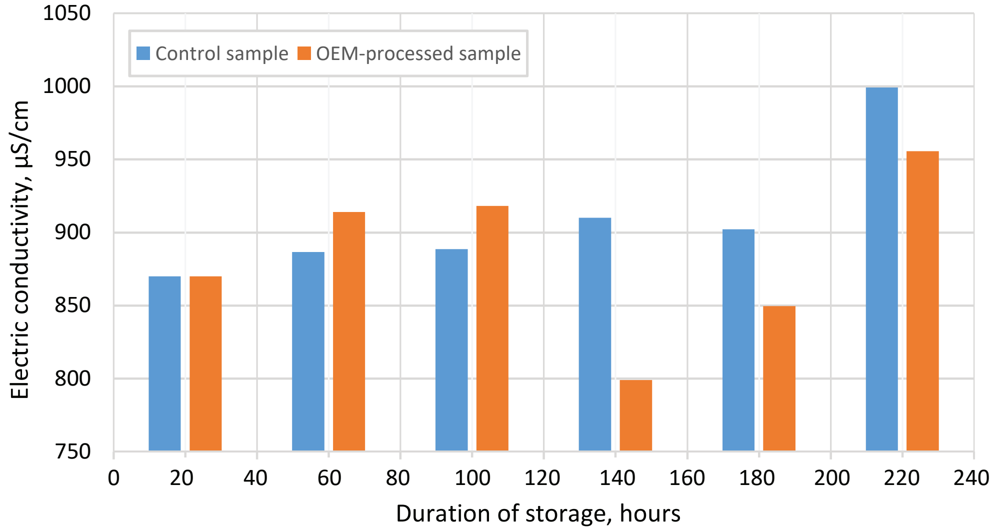


FIgure 8. Change in the value of the specific electrical conductivity of meat samples

subsequent decrease of specific electrical conductivity value (down to 799 μS/cm) is associated with formation of complex compounds of the essential oils components with proteins and lipids of muscle tissue, which formation results to the cell structures strengthening, as the cells walls become less permeable and more elastic, and the thickness of the muscle fiber slightly reduces in comparison with the control sample. These results are confirmed by studies of histological structural changes which occur in meat. This change of morphological properties of muscle tissue prov+ides a smaller outlet of muscle fibers contents into intercellular space, thereby reducing specific electrical conductivity value [14].

Conclusion

In result of the conducted studies it is possible to conclude that application of EOM in gas mixture composition at concentration of 0.1% for the storage of meat semi-finished food products has highly promising prospects, since application of this OEM increases the shelf life of food product by 2.6 times. The analysis of histological structural and electrochemical changes in muscle tissue showed differences in the process of autolysis in meat during its storage in a gas environment with EOM added.

It should also be noted that EOM allows regulation of flavor (taste and smell) properties of meat. However, EOM in composition of gas medium for meat storage must be used along with components that allow regulation of color and strength parameters, as essential oils are able to cause changes in color and texture of a food product, when interacting with proteins and lipids of meat.

Thus, the application of EOM in food systems reduces the amount of synthetic preservatives to be used, and can serve as an alternative solution to meet the growing need of consumers for food safety of meat and meat products.

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