1 Introduction

Most food products have quite a limited shelf life due to the activity of microorganisms and enzymes. One of the methods to suppress the microorganisms growth and enzymatic activity in the product is to remove the moisture drying the it in an artificial or natural way. Mold fungi and bacteria develop in products with a moisture content of 10% to 30% (Bianchini & Bullerman, 2009). To increase the raw material shelf life, it is necessary to bring the moisture content of the product to a level that prevents the development of microorganisms.

Raw materials of animal origin, by contrast, have less moisture and more dry matter.

Animal proteins are natural products, whose production is based on thermal (degreasing, dehydration) and mechanical (grinding) processes.

Animal proteins are obtained from various raw materials: pork skins, pork veins, beef veins, pork or beef blood plasma, whey, and so on.

It should be noted that collagen is a group of proteins of natural origin obtained from the muscle and connective tissues of animals. This is the main component of connective tissue and the most common protein in mammals, comprising 25-35% of the proteins in the whole body. Collagen, in the form of elongated fibrils, is mainly found in fibrous connective tissues such as ligaments, tendons and skin, as well as in large quantities in the cornea, cartilage, bones, blood vessels, intestines, and intervertebral discs.

Due to their chemical composition and functional properties, animal proteins are an alternative to soy isolated proteins and can be used in the production of meat products as a substitute for meat, increase nutritional and biological value, improve organoleptic properties, enhance meat taste, and reduce the cost of meat products (Vidal et al., 2018).

Using animal proteins obtained from collagen-containing raw materials allows to enrich meat products with dietary fiber, significantly improving the rheological properties of food products, especially the texture. The high functional properties of animal proteins are manifested in their water-holding capacity (Araujo et al., 2018).

The problem of preserving the quality of the obtained raw materials has always remained critical, so new methods of preserving it are being developed. At a later stage, we looked into the problem of obtaining collagen hydrolysate by biomodification of frozen cattle lips and ears. The preparation of lyophilized collagen hydrolysate was carried out as follows: the lips and ears were preliminary washed in running tap water for 20 minutes in order to remove mucus and impurities, muscle and adipose tissue was also removed; after draining (15-20 minutes), the raw material was ground on a top with a grid diameter of 2-3 mm. At the next stage, the crushed lips and ears were treated with a solution of Protepsin enzyme preparation in the ratio of the raw material to solution 1:2 and kept for 0.5-1.5 hours at a concentration of the enzyme preparation of 0.06% by weight of the raw material, while stirring periodically (at 17-22 °C). The filtrate from the product was separated on a sieve. Processed as described above, the raw material was subjected to freeze-drying. Evaluation of the temperature and duration of freeze-drying of collagen hydrolysate was carried out at a drying layer thickness of 5.0 mm and different drying temperatures: 20 °C, 30 °C, 40 °C, and 50 °C.

Keywords: enzyme preparation; freeze-drying; functional and technological properties; collagen-containing raw material; meat processing industry.

Practical Application: Processing of secondary collagen-containing products of the meat industry.
problem of the maximum preservation of organoleptic, physical, chemical, functional and technological indicators, as well as ensuring long shelf life of processed raw materials.

Drying seems the most promising among many ways to preserve raw materials.

There are many ways of drying raw materials of plant and animal origin: cabinet drying, drum and convection drying, freeze-drying (lyophilization), vacuum-pulse, infrared, microwave drying, etc.

Among the hundreds of engineering solutions and processes used nowadays, there are two principal directions: atmospheric drying and vacuum drying. Atmospheric drying has a significant drawback, i.e. it implies a long-term high-temperature contact of the product with the oxygen in the air (Wenfeng et al., 2013). This leads to intense oxidative reactions and, as a consequence, to the low quality of many dry products. Therefore, at present, vacuum drying at pressures below the triple point of water (freeze-drying), or vacuum moisture evaporation, is becoming more widespread (Haseley & Detjen, 2017).

The analysis of modern drying methods allows us to conclude that the most gentle method of dehydrating biological objects is freeze-drying (lyophilization) (Nireesha et al., 2013).

The products produced with the freeze-drying technology are stored and transported at room temperature, thus no cold chains are needed. Compared with food products produced with the heated-air drying, vacuum drying, and spray drying methods, those produced with the freeze-drying method has brighter color and higher nutritional value, and is easier to digest, preserving the nutrients and color, flavor and taste of the meat to the greatest extent (Huan et al., 2005; Kotwaliwale et al., 2007). Therefore, it is especially suitable for drying non-staple food which is extremely sensitive to heat and easy to be oxidized. Freeze drying does not damage the protein in meat and causes no loss of fat-soluble vitamin such as Vitamin A and Vitamin D (Juan & Yuanzhi, 2012; Chen, 2008). By-products and meat processed in this way can totally replace fresh food in terms of nutrition supply.

The principle of lyophilization is to dehydrate biological objects in a frozen state under vacuum. During freeze-drying, the moisture is removed by an ice-steam phase transition. The major amount of moisture (75-90%) is removed when ice sublimes at temperatures below 0 °C, and only the residual moisture is removed when the material is heated to 40-60 °C (Papavasiliou et al., 2008).

Freeze-drying is carried out in three steps. The first step is the freezing of the biological material, during which 10-15% of all moisture evaporates from the object as the heat is released due to ice melting when water freezes. The second step (sublimation) is characterized by a constant drying rate of the object. At this time, the major moisture is removed. The third step is residual moisture removal, characterized by a falling drying rate, when the temperature of the object becomes positive. At this step, the bound moisture that is not frozen in the object is removed. Drying rate depends on the intensity of heat supply. The temperature of the object gradually increases to the environment one.

Freeze-drying somewhat changes the properties and quality of the product. However, other methods of preservation change the properties of the raw materials more dramatically. The main advantages of freeze-dried foods are: high palatability and biological full-value; fast restorability; low weight; long-term storage under various conditions without the use of cold, which largely determines the purpose of these products and makes this type of drying preferable (Fissore & Pisano, 2015).

The purpose of this study is to investigate and develop the manufacturing technology of lyophilized collagen hydrolysate (LCH) and to study functional and technological properties of model forcemeat systems with LCH.

2 Materials and methods

The study subjects in this paper are:

- enzyme preparation Protepsin (standard proteolytic activity is 100 units/g) produced by ZAO Zavod Endokrinnykh Fermentov, Rzhavki settlement, Solnechnogorsky district, Moscow region, in accordance with TU 9219-005-42789257-2005, Hygiene Certificate No. 50.16.01.000.M.000638.10.07 dated October 19, 2007 (State Committee for Standards and Product Quality Management, 1989);
- collagen from the connective tissue of the cattle lips and ears;
- collagen hydrolysate (CH);
- lyophilized collagen hydrolysate (LCH);
- forcemeat systems.

At the first stage, CH properties were studied using a collagen-containing raw material obtained from the cattle lips and ears. CH from the lips and ears was obtained as follows. The lips and ears were preliminary washed in running tap water for 20 minutes in order to remove mucus and impurities, muscle and adipose tissue was also removed; after draining (15-20 minutes), they were ground on a top with a grid diameter of 2-3 mm (Singh et al., 2018). At the next stage, the crushed lips and ears were treated with a solution of Protepsin enzyme preparation in the ratio of raw materials to solution 1:2 and kept for 0.5-1.5 hours at a concentration of the enzyme preparation of 0.06% by weight of the raw material, while stirring periodically (at 17-22 °C). The filtrate from the product was separated on a sieve.

Processed as described above, the raw material was subjected to freeze-drying on Bio-Rus-7FD.

The design includes two systems: vacuum treatment and cold supply. The first of them allows to implement the traditional vacuum-freeze-drying by “ice-steam” phase transition, the second – dehydration by vacuum evaporation. These two modes can also be combined within a single drying cycle, with any combination of the duration of each of them.

Bio-Rus-7FD has the following technical parameters: evaporation capacity – up to 3.5 kg/cycle; total area of work shelves – 0.48 m²; heating temperature of electric heaters – up to 300 °C; desublimator plates cooling temperature – up to...
-40 °C; the residual chamber pressure (depending on the surface temperature of the desublimator and the capacity of the heat sink to the raw material to be dried) for freeze-drying – from 0.1 to 0.5 mm Hg (13.33-66.65 Pa).

The raw material was placed on metal sheets, which were then placed in a freezer with a temperature of –18 ± 1 °C and natural air circulation.

At this stage, the freeze drying parameters (temperature and layer thickness) of the CH were set.

**Functional and technological properties** (moisture-retaining capacity (MRC), fat-retaining capacity (FRC), emulsifying capacity (EC)) of model forcemeat systems were determined according to recommendations (Skurikhin & Tutelyan, 1998). Moisture-binding capacity (MBC) was evaluated by Grau and Hamm method modified by V.P. Volovinskon and B.I. Kelman (Skurikhin & Tutelyan, 1998).

**Dynamic viscosity** (η, Pa.s) of model forcemeat systems was determined on RHEOTEST 2.1 viscometer (Germany) according to the device manual, using the viscometer rotor. Dynamic viscosity in all samples was determined at 20 °C.

**The mass fraction of hygroscopic moisture** in the raw material was determined by drying the samples at 100-105 °C for 3 hours.

**Mass fraction of fat** in the model forcemeat was determined using the Soxhlet extractor.

**The mass fraction of minerals** was determined after burning organic matter in a muffle furnace at 500 °C for 5-6 hours to constant weight.

**The mass fraction of protein** was determined by the photometric method.

**Adhesion properties** of model forcemeat systems were determined in a laboratory unit according to the method of S.I. Tyshkevich (Skurikhin & Tutelyan, 1998). The method determines the amount of force required to separate the two surfaces connected by the forcemeat under test.

**Moisture retaining capacity** (MRC) is estimated by determining the difference between the mass fraction of moisture in the forcemeat and the amount of moisture separated during the heat treatment.

**Fat retaining capacity** (FRC) is defined as the difference between the mass fraction of fat in the forcemeat and the amount of fat separated during the heat treatment.

**Emulsifying capacity** (EC) is the ratio of the emulsified oil volume to the total oil volume in the system. This definition includes the concept of emulsion stability which manifests itself over the period from the end of emulsification to the time of measuring under fixed experimental conditions.

### 2.1 Experimental design and statistical analysis

All analyses were carried out in triplicate unless otherwise stated and the average values were calculated. The results were expressed as mean value ± standard deviation. Significant differences between mean values at significance level p < 0.05 were established using the One-way analysis of variance and Student's test. Microsoft Excel version 2010 was used as the statistical analysis software.

### 3 Results and discussion

Evaluation of the temperature and duration of CH freeze-drying was carried out at a drying layer thickness of 5.0 mm and different drying temperatures: 20 °C, 30 °C, 40 °C, and 50 °C. The curves of CH lyophilization at different heating temperatures are shown in Figure 1.

Figure 1 suggests that an increase in heating temperature results in a decrease in drying time and moisture content of CH: at 20 °C, 30 °C, and 40 °C freeze-drying lasted for about 270 minutes, with 14.0% mass fraction of moisture in the final

![Figure 1. Lyophilization curves of CH at temperatures: 1-20 °C; 2-30 °C; 3-40 °C; 4-50 °C.](image-url)
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The muscle tissue in meat has a significant impact on the FTP, since it consists of a complex of structurally different proteins. In the aspect of functional properties during obtaining meat products, the combination of muscle proteins is responsible for the efficiency of meat emulsions formation. The quantity of protein in the system, its qualitative composition, and environmental conditions predetermine the stability of the resulting meat systems, influencing structural, mechanical, and organoleptic characteristics (Neklyudov & Ivankin, 2007).

Functional and technological properties (FTP) of a meat raw material are understood as a set of indicators characterizing the levels of MBC, MRC, FRC, EC, structural and mechanical properties (stickiness, viscosity, plasticity, etc.), sensory characteristics (color, taste, smell), the yield and loss during heat treatment of various types of raw materials and meat systems. These indicators have a priority in determining acceptability of meat for food production.

The functional properties of isolated proteins are commonly understood as a wide range of physical and chemical characteristics that determine their behavior during processing and storage, providing the desired structure, technological and consumer properties of finished products (Aylangan & Öztan, 2008).

Functional and technological indicators of model forcemeat systems before heat treatment are given in Figure 3.

The data obtained indicate an increase in MBC and plasticity of model meat systems. MBC values ranged from 79.78% to 85.13%. Probably, there occurred a change in the spatial availability of charged groups of proteins, which caused loosening of the protein structure, since repulsion of like-charged amino acid residues led to the expansion of the space between polypeptide chains and increases the availability of active centers of the protein macromolecule, which allowed them to attract dipoles of water molecules, forming a solvation (hydration) shell. The forcemeat in the system became more yielding, due to the increase in MBC and plasticity.

[Figure 2: LCH Curves at heating temperature of 50 °C and different thickness of product layer: 1-5.0 mm; 2-10.0 mm; 3-15.0 mm; 4-20.0 mm.]

Forcemeat is a complex heterogeneous system, the functional properties of which depend on the ratio of tissues, and the content of specific proteins, fats, water, and morphological components in such tissues.
to the interaction of dispersed collagen molecules with water. MRC of the formula compositions with the added LCH ranged from 90.11% to 93.56%. The data obtained indicate that there was no apparent dependence of the MRC upon the amount of LCH introduced into the test sample.

The FRC indicators also remained at the same level in the test samples, which is explained by the way the ground meat components were laid in the cutter. However, the test sample with 15% replacement of raw meat with LCH showed the maximum FRC value of 73.95%.

EC indicators in all test samples were in the same percentage. By nature, forcemeat has a certain viscosity. The rheological properties of such systems vary depending on shear stress, forcemeat properties and other factors (Bhaskar et al., 2008). The change in the plasticity of forcemeat mainly depends on the formula components in the system. The main characteristics of any forcemeat is a combination of elastic, plastic or viscous-plastic properties.

Due to the interaction of van der Waals cohesive forces, coagulatory bonds in disperse systems are formed by the interaction between particles and molecules through the layers of the dispersion sphere. The thickness of the layers is characterized to a certain extent by the content of the dispersion medium (Bobuș, 2010). With increasing water content, the values of the shear properties gradually decreased, as indicated by the data presented in Figure 4.

The diagram confirms that an increase in LCH amount in the system led to a shift in the ultimate shear stress. The resulting LCH contributed to the formation of structures in the protein-fat system, thereby contributing to an increase in the tenderness of model forcemeat systems compared to the samples produced without the food additive.

Model forcemeat with the addition of 20% LCH instead of raw meat had a smearing consistency close to the one of paste forcemeat. The addition of 15% LCH to the forcemeat formula was considered to be the best choice.

The introduction of a high proportion of LCH caused an insignificant decrease in the mass fraction of protein in the model forcemeat systems under study, which is explained by the low content of free peptides and amino acids in the chemical composition of LCH compared to beef and pork forcemeat.

«Lyubitelskoye» boiled sausage with added LHC was used as the basic formula (Table 1).

Figure 5 shows the properties of forcemeats of model meat systems obtained in accordance with the specified options.

At the maximum values of mass fraction of fat and ash in the control sample (10.99 and 0.98%), the following trend was observed: with an increase in LCH replacing beef and pork in the model forcemeat systems, there occurred their slight decrease. In the test sample these values are 8.9 and 0.91, respectively.

The water-to-protein ratio increased (from 3.98 in the control, to 4.37 in 20% replacement option) with an increase in the amount of LCH in the system. According to the literature data, the water-to-protein ratio in the resulting composition is somewhat high with respect to the optimal, ranging from 3.0 to 3.5 (Antipova et al., 2008).

During heat treatment, the amount of moisture and protein will be lost due to evaporation.

In the process of cutting sausage meat, there may form an adhering layer on the surface of the cutter and the main cutting mechanisms. Reducing the adhesion properties of forcemeat is a major task in the meat production, because of the products losses due to an adhering forcemeat layer on the equipment walls (Dieterich et al., 2014; Spohner et al., 2015). Laboratory studies of the test samples adhesion properties (Figure 6) showed that the formulas with prevailing pork are characterized by minimal adhesion indices. This is primarily due to the high content of adipose tissue, which contributes to the formation of a coagulation
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Table 1. Formula of control and test samples.

| Raw material                        | Boiled sausage «Lyubitskoye» | Boiled sausage «Lyubitskoye» with added LHC |
|-------------------------------------|------------------------------|-------------------------------------------|
| Trimmed beef, grade 1              | 35.0                         | 30.0                                      |
| Semi-fat trimmed pork              | 40.0                         | 30.0                                      |
| Pork mid-back fat                  | 25.0                         | 25.0                                      |
| LHC                                 | –                            | 15.0                                      |
| Sodium nitrite                     | 5.6                          | 5.6                                       |
| Granulated sugar                   | 110.0                        | 110.0                                     |
| Ground black pepper                | 85.0                         | 85.0                                      |
| Nutmeg                              | 55.0                         | 55.0                                      |
| Salt                                | 2500.0                       | 2500.0                                    |

Figure 4. The change in the ultimate shear stress of model forcemeat.

Figure 5. Physical and chemical indicators of model meat systems.
The obtained results suggest that this type of drying has a positive effect on the properties of the raw material, while maintaining all its quality characteristics, without damaging its original structure. The results of the study are of great practical importance, since freeze-drying today is a unique technology that helps preserve the biological full-value of food products and ensure a long shelf life.

In the course of the work, the following parameters were set for CH freeze-drying: heating temperature – 50 °С; drying time – 260 minutes; drying layer thickness – 5.0 mm. MRC of formulas with added LCH ranged from 90.11% to 93.56%. The FRC indicators also remained at the same level in the test samples, which is explained by the way the ground meat components were laid in the cutter. However, the test sample with 15% LCH replacement of raw meat showed a maximum FRC value of 73.95%.

The study of LHC applicability in the sausage production was necessary to determine the most rational amount of the obtained LHC to be introduced into the model forcemeat systems. The amount of LHC introduced instead of the forcemeat was 5%, 10%, 15%, and 20%.

The model forcemeat with 20% LCH instead of raw meat had a smearing consistency close to the one of paste forcemeat. 15% LCH added to the forcemeat formula was considered to be the best option.

It follows from the above that the use of LHC in sausage technology not only improves the FTP, but also creates biologically active complexes for the enrichment of meat products with protein, so as to save the raw material.

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