

Protein Extract Fortification

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Abstract

The human health depends on the food quality; in turn, the food quality is determined by the composition of foodstuffs, treatment, the physiological needs of the human organism, physical activity, and environmental factors. To improve the balance and the positive effect of the food, additional introduction of nutrients into the foodstuff, i.e. enrichment (fortification) is required. In all countries of the world, fortification of food products is used, which allows solving the problems with acute shortage of essential micronutrients. Studying the possibility of connective tissue protein extracts' fortification with micronutrients, iron and vitamin C is relevant. The paper presents the results of studying the ratios of various types of connective tissues, selecting the optimal processing methods for obtaining an extract of connective proteins. Based on studying the viscosity, the composition of functional groups, organoleptic characteristics of the obtained extract, the possibility of fortification with essential micronutrients, iron and ascorbic acid has been proven. Variants of individual and combined introduction of micronutrients have been suggested. The optimal dosages and the conditions of fortification have been determined. The research has established the possibility of obtaining a protein extract with adjustable physical and mechanical properties as a matrix for iron and vitamin C.

Keywords: research, connective proteins, protein extract, adjustable properties, fortification, vitamin C, iron.

INTRODUCTION

Food is the main factor that provides nutrients to the human organism. Development of the nutrition science promotes discovery of new components that are required for human activity. Currently, in accordance with the optimal nutrition formula, the diet of the modern man should include about 200 various compounds. Traditional food products with high energy and low nutritional density do not provide the conditions for optimal intake of essential substances into the human organism [1].

With the increasing rhythm of life in the urbanized society, there is less time for cooking and eating, which stimulates the demand for quick snacks, including the healthy ones. Therefore, food products must be guaranteed sources of essential nutrients. Most often, this problem is solved by fortification of foodstuffs with essential components, vitamins, minerals, phenolic compounds, etc.

Among the products of the meat industry, the most interesting is the connective tissue, the unique structure of which is mainly determined by collagen. The amino acid composition of this protein is represented by 66% with glycine, alanine, hydroxylysine, proline, and hydroxyproline. When heated in aqueous solutions, individual collagen fibers or their bundles first swell, and then get deformed. An ordered structure of collagen is broken, and the intra- and intermolecular interaction is weakened due to the rupture of a part of the cross-links that stabilize the fiber structure. Protein gets into the amphichroic state and takes random shapes, which results in collagen fibers' shrinking. At the temperature of (55 – 65) °C, shrinkage of collagen occurs, which is accompanied by reparation of a significant part of carbohydrates. Along with changes to the linear dimensions of the collagen fibers, their fibrous structure is disturbed, and fibers become vitreous. Upon collagen shrinkage, the tightly wound triple coils of native collagen rearrange into randomly coned molecules. They become elastic, more accessible to the action of the enzymes of the gastrointestinal tract, and their strength is greatly reduced.

The products of collagen hydrolysis (gluten, gelatin) have the properties of the dietary fiber, stimulate secretion of gastric juice and intestinal motility, and have a positive effect on the state and function of the useful microflora. Enzymatic and chemical treatment of the tissue is used for collagen hydrolysis [2]. Depending on the production method, collagens may have a wide range of properties. They are widely enough used in food production, in cosmetic preparations, in the leather industry, in medicine and pharmacy [3-5].

The advantages of using collagen for the medicinal purposes is the absence of toxic and carcinogenic properties, weak antigenicity, high mechanical strength and resistance to tissue

enzymes, adjustable speed of lysis in the organism, the ability to form complexes with biologically active substances (heparin, chondroitin sulfate, antibiotics, etc.), stimulation of own tissues' regeneration. In particular, collagen is the most promising material for tissue engineering, due to its properties of biocompatibility and biodegradability [6].

However, the capabilities of this protein have not been fully used.

To meet the needs of the human organism, various approaches are used, such as modification of the chemical composition of the products, intravital formation of the qualitative characteristics of raw materials, and the use of biologically active additives [6-13].

The work was aimed at studying the possibility of connective proteins extracts' fortification with micronutrients — iron and vitamin C.

MATERIALS AND METHODS

Cattle tendons and pig skin were used as raw materials. The raw materials were cleaned, hashed, and mixed in the ratio of 2.5:1, respectively. The chemical and fractional compositions of a mixture of connective proteins were studied.

To obtain the protein extract, the specimen was diluted with water (water duty of 1:3), 1 g of citric acid was added, and the mixture was treated at (98 ± 2) °C for 7 hours. After three, five and seven hours, aliquots of protein extracts were filtered, visually studied, and viscosity of the solutions was measured (solutions 1, 2 and 3). The viscous friction of the fluid against the spindle of the Brookfield viscometer was determined by tightening the actuating spring, which was measured by a rotation angle sensor.

The functional composition of the proteins extract was studied in the infrared region. Reference samples were gelatin solutions in various concentrations.

The possibility of fortifying the obtained extract with iron and ascorbic acid was studied. Individual dosages of micronutrients were established, and the possibility of joint admission of vitamins and trace elements was studied.

The physicochemical properties and the fractional composition were determined using the standard and generally adopted methods.

Iron was determined using the method based on measuring the color intensity of the solution of a bivalent iron complex compound with red orthophenanthroline at the wavelength of (490 ± 10) nm.

The content of ascorbic acid was determined by visual titration with the use of ascorbic acid quantitative oxidation with the 2,6-dichlorophenolindophenol sodium solution.

All experiments were repeated 5 times. The obtained data were processed using Excel statistical software package with the use of the Mann-Whitney test. The statistically significant differences $p < 0.05$ were discussed.

RESULTS AND DISCUSSION

The chemical composition of the initial mixture was the following: water (63.0 ± 2.0 %), protein (34.70 ± 1.50 %), ash (0.70 ± 0.03 %), and fat (1.5 ± 0.15 %). Fractional composition of the proteins of the mixture was represented by 82 % by alkali-soluble proteins, mostly collagen, 8 % and 10 % – water and salt-soluble proteins, respectively.

During the heat treatment of the initial mixture, the modes commonly adopted in the meat industry were used. However, after seven hours of treatment, an increased amino nitrogen content was noted, indicating deeper degradation of proteins, and reduced share of alkali-soluble proteins.

After 3, 5, and 7 hours, the viscosity of the studied solutions was measured. The intervals were arbitrary based on visual assessment of the changes in the consistency of the initial solution. The initial stage of gels' formation was accompanied by the appearance of associates of the macromolecules that subsequently combined into a spatial configuration.

After cooling, solutions 2 and 3 acquired the dense structure, while solution 1 remained viscous fluid. In addition, after the prolonged heat treatment, the broth became turbid, and flakes appeared. Considering the duration of thermal treatment (3 hours), the organoleptic characteristics (the broth was transparent), and the extract yield (0.8 liters), solution (experiment) 1 was chosen for further research.

The viscosity of the experimental sample was compared to gelatin solutions of various concentrations. The research was performed at the ambient temperature of (22 ± 2) °C, 20 min (fluid solutions) and 24 hours (viscous solutions) after the heat treatment. In the intervals between measuring viscosity between 20 minutes and 24 hours, the density of the solutions increased due to the formation of the spatial framework by molecules, and the system turned to jelly. By its viscosity, the experimental sample was close to a 1.5 % gelatin solution (Table 1).

The fractional composition of the extract was presented by alkali-soluble proteins, including collagen, by 87.7 %.

In studying the IR spectra of the test sample of gelatin solution, the following functional groups were identified: hydroxyl (up to $3,300 \text{ cm}^{-1}$), azometin and the nitro groups ($1,600\text{-}1,500 \text{ cm}^{-1}$), carbon ($1,900\text{-}1,550 \text{ cm}^{-1}$). A slight difference was observed in the height of peaks, which may indicate differences in the quantitative content of functional groups.

At the next stage of the work, the possibility of fortifying the obtained extract with iron and ascorbic acid was considered.

In choosing the dosage of micronutrients, the authors relied on the physiological norms of consumption approved for the population of Russia. The average intake of vitamin C varies in various countries within (70 – 170) mg/day, and in Russia — (55 – 70) mg/day. The level of physiological needs, as determined for various countries, is (45 – 110) mg/day. The allowed upper consumption level is 2,000 mg/day. The updated physiological need for adults is 90 mg/day. The average consumption of iron in various countries is (10 – 22) mg/day, and in the Russian Federation — 17 mg/day. The upper allowed consumption level is not determined. The physiological need for the adult men is 10 mg/day, and for women — 18 mg/day [14].

Interaction of each micronutrient in the extract was studied individually. The dynamics of vitamin accumulation upon various introduction dosages from 5 mg to 110 mg per 100 cm^3 of the protein extract were studied. After 30 min, pH was measured, and organoleptic assessment of the extracts was performed. With

increasing the amount of ascorbic acid, pH of the extract shifted toward the acid side up to 4.5, and the color of the solution turned yellow. As a result of the research, the variant with adding 45 mg of vitamin C per 100 cm^3 of the extract was chosen, the pH value of the experimental sample was 5.5.

It is known that proteins are biopolymers capable of interacting with positively and negatively charged elements and having a high affinity for metals. In addition, trace elements in the organism are transported by proteins, and most of them are bonded with proteins. Consequently, these are proteins that can act as a matrix of the microelement for targeted delivery to the organism.

Interaction of iron with chemically reactive groups of the protein extract takes time, like any other chemical process. The volume of the solvent is many times more than the volume of the diluted substance — ferric sulfate; therefore, time is required for binding iron. At any temperature different from the absolute zero, continuous movement of particles of the solute and the solvent occurs. This process is spontaneous, as it results in decreasing Gibbs energy due to increasing the degree of disorder, i.e. increasing the entropy of the system. The protein extract is a polyelectrolyte, which is capable of dissociating in the solution, accompanied by the formation of both acidic and basic groups. Its sorption capacity is characterized by the equilibrium amount of sorbate (FeSO_4) absorbed by a unit volume of the extract and depends on the concentration and the temperature. Therefore, diffusion of iron is a slow process, the microelement is oriented at the accessible parts of the protein matrix of the extract.

From the safety point of view, since the resulting protein extract is a favorable medium for the development of microorganisms, the following exposure modes were used: temperature (0 - 4) °C, duration — 24 hours. To determine the degree of binding, the dynamics of iron accumulation were checked every 2 hours.

Matrix saturation with iron was determined upon intake of 80 % of the daily requirement in iron, i.e. 12 mg. The optimal binding time was 10 hours. With increasing the introduced amount of iron, the degree of binding increased slightly, and the content of the free form of the element increased (Fig. 1).

To avoid the presence of free iron in the extract, trace elements were introduced in the dosage of 7.5 mg per 100 cm^3 of the extract. The problem of enriching food systems with microelements lies in the fact that any excess of the element may cause toxic effects. In addition, if iron was introduced at the dosage exceeding 7.5 mg, the color of the extract changed to dark shades of the color.

Given the coordination number of iron (II), one can assume the formation of a dimer molecule as a result of iron binding with two atoms of oxygen and nitrogen. However, the formation of a tetramer is also possible, given the presence of amino groups of amino acids [5].

All studied samples had neutral flavor and aroma. After the introduction of ascorbic acid, a slightly sour flavor was felt.

Variants of joint use of the two micronutrients were considered. It is known that ascorbic acid forms compounds with iron, whereby absorption of the microelement increases, but the activity of the vitamin is lost. In this respect, various sequences of introducing the micronutrients into the protein extract were considered. The following variants were considered: iron and vitamin C simultaneously (Composite 1); first iron and vitamin C after 10 hours (Composite 2) and vice versa, ascorbic acid first and iron 30 minutes later (Composite 3). After the introduction of iron, composites 1 and 3 were kept for 8 hours to bind the trace element with protein. All modes and conditions have been worked out experimentally. During all stages of the fortification the content of micronutrients in the extract was checked (Fig. 2).

Table 1 – Viscosities of solutions

Time, hours	Gelatin solution, PA·s				Experiment, PA·s
	1 %	1.5 %	3 %	5 %	
0.3	0.016	0.5038	0.9018	3.7615	0.4582
24	0.895	11.2067	19.4808	-	10.1879

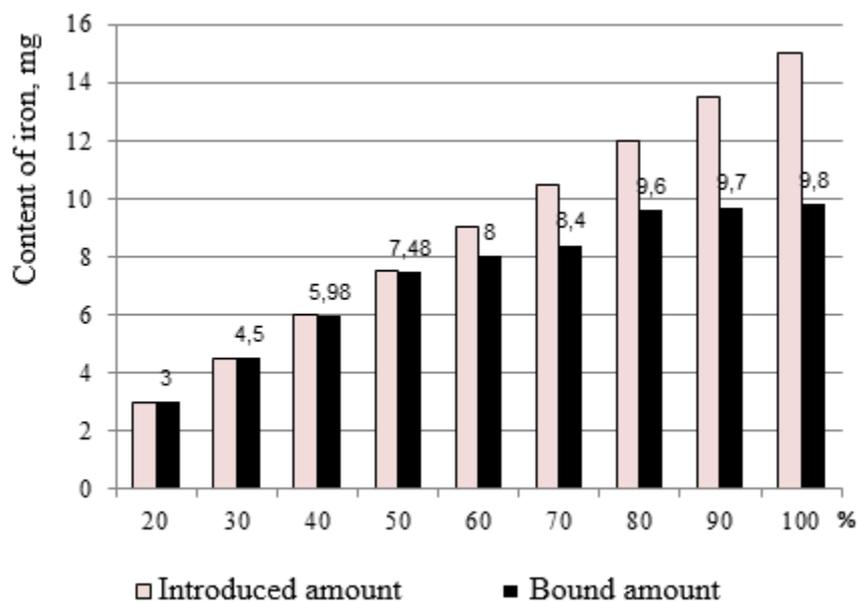


Fig. 1 – The degree of iron binding by the extract

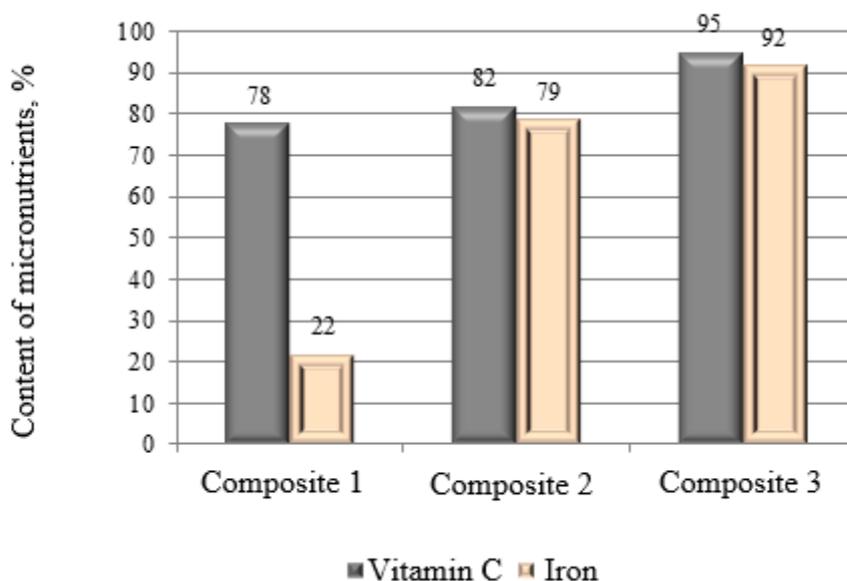


Fig. 2 – The amount of micronutrients in the composites

Composite 3, i.e. first adding ascorbic acid to the extract, and iron 30 minutes later, is the most optimal. In the chosen composite, the content of micronutrients is virtually close to 100 %. By the organoleptic characteristics, the extracts fortified with iron and vitamin had a light-yellow color.

CONCLUSIONS

The research has established the possibility of obtaining a protein extract with adjustable physical and mechanical properties as a matrix for micronutrients. With individual and joint fortification, 100 cm³ of the extract from connective proteins

can provide 50 % of the human organism's daily need in iron and ascorbic acid.

REFERENCES

1. Tutelian, V.A., Zhilinskaya, N.V., Sargsyan, V.A., Kochetkova, A.A., Analysis of the normative-methodical base in the field of specialized food production in the Russian Federation, *Problems of nutrition* 2017, 86(6), 29-35.
2. Ran, X.-G., Wang, L.-Y., Use of ultrasonic and pepsin treatment in tandem for collagen extraction from meat industry by-products, *Journal of the Science of Food and Agriculture* 2014, 94(3), 585-590.

3. Sionkowska, A., Skrzyński, S., Śmiechowski, K., Kołodziejczak, A., The review of versatile application of collagen, *Polymers for Advanced Technologies* 2017, 28(1), 4-9.
4. Parmar, P.A., St-Pierre, J.-P., Chow, L.W., Puetzer, J.L., Stoichevska, V., Peng, Y.Y., Werkmeister, J.A., Ramshaw John, A.M., Stevens, M.M., Harnessing the Versatility of Bacterial Collagen to Improve the Chondrogenic Potential of Porous Collagen Scaffolds, *Advanced Healthcare Materials* 2016, 5(13), 1656-1666.
5. Ryabusheva, A.V., *Development of the technology of iron complexes for enriching meat products: Abstract. diss. ... cand. tech. Sciences*, Ulan-Ude 2008.
6. Yamada, S., Yamamoto, K., Ikeda, T., Yanagiguchi, K., Hayashi, Y., Potency of Fish Collagen as a Scaffold for Regenerative Medicine, *BioMed Research International* 2014, Article ID 302932.
7. Zamora, G., Flores-Urrutia, M.C., Mayén, A.-L., Large-scale fortification of condiments and seasonings as a public health strategy: equity considerations for implementation, *Annals of the New York Academy of Sciences* 2016, 1379(1), 17-27.
8. Dadykin, M.A., Functional foodstuffs based on meat, *All about meat* 2015, 6, 28-29.
9. Lisitsyn, A.B., Chernukha, I.M., Lunina, I.O., Fedulova, L.V., Legislative bases and scientific principles of creating functional food products based on meat, *Bulletin of Altai State Agrarian University* 2016, 12(146(3)), 151-158.
10. González Mejía, E., Aguilera-Gutiérrez, Y., Martín-Cabrejas, M.A., Mejía, L.A., Industrial processing of condiments and seasonings and its implications for micronutrient fortification, *Annals of the New York Academy of Sciences* 2015, 1357(1), 8-28.
11. Wegmüller, R., Zimmermann, M.B., Bühr, V.G., Windhab, E.J., Hurrell, R.F., Development, Stability, and Sensory Testing of Microcapsules Containing Iron, Iodine, and Vitamin A for Use in Food Fortification, *Journal of Food Science* 2006, 71(2), S181-S187.
12. Hurrell, R.F., Preventing Iron Deficiency Through Food Fortification, *Nutrition Reviews* 2009, 55(6), 210-222.
13. Degerud, E.M., Manger, M.S., Dierkes, J., Strand Bioavailability of iron, vitamin A, zinc, and folic acid when added to condiments and seasonings, *Annals of the New York Academy of Sciences* 2015, 1357(1), 29-42.
14. *The norms of physiological needs in energy and nutrients for various population groups of the Russian Federation: Methodical guidelines MR 2.3.1.2432-08*, Federal Center for Hygiene and Epidemiology of Rosпотrebnadzor, Moscow 2009.