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Biotechnological processing procedures of collagen-containing raw materials for creation of functional foods

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Abstract: The article presents the results of research into the biotechnological processing of collagen-containing raw materials (bovine rumina) to create functional foods. The patterns of biotransformation of collagen-containing raw materials by activated cultures of probiotic microorganisms (*Bifidobacterium longum* B379 M, *Propionibacterium shermanii* KM 186 and *Lactobacillus helveticus* H₁₇₋₁₈) have been studied and theoretically substantiated. It is noted that bovine rumina, following preliminary heat treatment, comprise a good nutrient medium for the development of probiotic cultures. It was revealed that low-molecular compounds formed during the heat treatment of rumina possess prebiotic properties that stimulate the growth of microorganisms. It was established that after 5–8 hours of cultivation, the number of viable cells of the studied cultures in the collagen substrate increases to 10^9 – 10^{10} CFU / g. It is noted that the biomodification of rumina improves their organoleptic properties and consistency, causing it to become succulent, soft and ductile. Biotransformation of collagen-containing raw materials by probiotic microorganisms leads to a significant increase in amino acids in hydrolysates in comparison with the control sample. The greatest increase in amino acids is observed during the fermentation of collagen *Lactobacillus helveticus* H₁₇₋₁₈, which indicates a higher proteolytic activity of this culture. It was shown that the rumen microstructure undergoes changes under the action of probiotic microorganisms in comparison with the initial state (the muscle carcass becomes thinner and looser, as well as undergoing change in terms of the structure of its morphological elements). As a result of the research, a fundamentally new scheme of biotechnological processing of collagen-containing raw materials was developed. The obtained results open up broad prospects for the creation of BAA-synbiotics and food products intended for functional nutrition.

Keywords: collagen-containing raw materials, osteoporosis, rumen, calcium, bifidum bacteria, propionic acid bacteria, lactobacilli

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Исследование процессов биотехнологической обработки коллагенсодержащего сырья для создания функциональных продуктов питания

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Резюме: В статье изложены результаты исследований биотехнологической обработки коллагенсодержащего сырья (рубца крупного рогатого скота) для создания функциональных продуктов питания. Изучены и теоретически обоснованы закономерности биотрансформации коллагенсодержащего сырья активизированными культурами пробиотических микроорганизмов (*Bifidobacterium longum* V379 M, *Propionibacterium shermanii* KM 186 и *Lactobacillus helveticus* H₁₇₋₁₈). Отмечено, что рубец после предварительной термической обработки является хорошей питательной средой для развития пробиотических культур. Выявлено, что низкомолекулярные соединения, образовавшиеся при термической обработке рубца, обладают пребиотическими свойствами и стимулируют рост микроорганизмов. Установлено, что через 5–8 ч культивирования количество жизнеспособных клеток исследуемых культур в субстрате коллагена возрастает до 10^9 – 10^{10} КОЕ/г. Отмечено, что биомодификация рубца улучшает его органолептические свойства и консистенцию, которая становится сочной, мягкой и эластичной. Биотрансформация коллагенсодержащего сырья пробиотическими микроорганизмами приводит к значительному повышению аминокислот в гидролизатах в сравнении с контролем. Наибольшее повышение аминокислот наблюдается при ферментации коллагена *Lactobacillus helveticus* H₁₇₋₁₈, что свидетельствует о более высокой протеолитической активности данной культуры. Показано, что микроструктура рубца под действием пробиотических микроорганизмов претерпевает изменения в сравнении с исходным состоянием (происходит истончение и разрыхление мышечного каркаса, а также изменение структуры его морфологических элементов). В результате проведенных исследований разработана принципиально новая схема биотехнологической обработки коллагенсодержащего сырья. Полученные результаты открывают широкие перспективы для создания БАД-синбиотиков и пищевых продуктов, предназначенных для функционального питания.

Ключевые слова: коллагенсодержащее сырьё, остеопороз, рубец, кальций, бифидобактерии, пропионовокислые бактерии, лактобактерии

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INTRODUCTION

Osteoporosis is referred to today as a "silent epidemic". Epidemic – because at a certain age more than a half of the population suffers from it; and quiet – because the disease develops asymptotically over a long time period. The first symptom of osteoporosis, as a rule, occurs in the form of a fracture. Osteoporosis is a complex problem, with a number of factors contributing to its occurrence, starting with gastrointestinal tract disorders and ending with failures in the immune and endocrine systems. The main cause of the disease is not only the "leaching" of calcium from the inorganic component of the bone tissue, but also collagen deficiency in these tissues. Therefore, in order to increase the elasticity and strength of bones, complex preparations, dietary supplements and collagen-containing products are also needed. Such products act to stimulate anabolic processes in the bone matrix, increasing the amount of collagen it contains [1].

Category 2 animal by-products are of particular interest as a source of collagen. In particular, rumina derived from cattle contain 6.8% of collagen by weight of raw tissue. However, despite its high nutritional value, the use of bovine rumen for food purposes is limited due to its low functional-technological and organoleptic properties.

Data on the use of various methods for modifying collagen-containing raw materials in order to achieve optimal rumen properties are presented in studies [2–13]. Chemical, thermal and enzymatic methods of processing raw materials have been developed and studied. It is noted that the targeted use of biotechnological methods based on the use of various types of microorganisms [2–13] is currently the most promising of existing approaches to the processing collagen-containing raw materials. Among the microorganisms used for this purpose, various types of lactic acid bacteria are predominant. The use of these bacteria contributes to the production of protein collagen-containing semi-finished products having high organoleptic and functional-technological properties. However, there is currently insufficient data on the use of microorganisms with probiotic properties for the bioprocessing of bovine rumen products.

Lactobacillus of the species *Lactobacillus helveticus*, bifidobacteria of the species *Bifidobacterium longum* and propionic acid bacteria of the species *Propionibacterium shermanii* are promising in this respect. *Lactobacillus helveticus* exhibit high proteolytic activity, as well as the ability to inhibit potential pathogens and bind Ca to maintain it in a soluble state. This makes it very attractive for use in the manufacture of products for the prevention

of osteoporosis [14–17]. *Bifidobacterium longum*, which have high antagonistic activity and the ability to destroy toxic metabolites, grow under anaerobic conditions, accumulating aromatic compounds and reducing substances. *Propionibacterium shermanii*, enriched into a product by a variety of biologically active substances (vitamin B₁₂, folic acid, amino acids, enzymes, short chain fatty acids, and others), produce propionic acid, which is capable of inhibiting the growth of pathogenic microorganisms [15, 18].

It is known that the strictly specific proteolytic activity of microorganisms depends on many factors, including the raw materials used. Consequently, the study of the effect of probiotic cultures on the functional and technological properties of collagen-containing raw materials is relevant for their further use as starter cultures for biotreatment of bovine rumen products.

The aim of the study is to research the processes of biotechnological processing of collagen-containing raw materials with probiotic microorganisms in order to create functional foods.

EXPERIMENTAL PART

Experimental studies were carried out at the Dairy Produce Technology. Commodity Research and Examination of Goods department of the FSBEI HE East Siberia State University of Technology and Management (ESSUTM).

The objects of research were the following probiotic cultures: *Bifidobacterium longum* B379 M, *Propionibacterium shermanii* KM 186 and *Lactobacillus helveticus* H₁₇₋₁₈. Cultures were provided by the Research Institute of Genetics and Selection of Industrial Microorganisms (Moscow). The *Bifidobacterium longum* B379 M and *Propionibacterium shermanii* KM 186 strains were activated by a unique biotechnological method developed at ESSUTM [19].

A bovine rumen product meeting the requirements of GOST 32244-13 was used as a collagen-containing raw material. Preparation of raw materials was carried out according to the method described by L.V. Antipova [20]. Preliminary heat treatment of the collagen-containing raw material was carried out in order to reduce the negative structural and compositional properties of the rumen. The rumen was cleaned, washed, cut into pieces of 2.5 × 3 cm and cooked at 100 °C for 2–2.5 hours until softening (the ratio of raw materials to water was 1:2). After boiling, the rumen was minced in a grinder with a diameter of 2–3 mm at room temperature in order to preserve the fibre-forming ability of dispersed collagen. 5% whey was added to the obtained rumen substrate as a source of carbohydrates.

Probiotic microorganisms were introduced into the prepared raw materials in the form of bacterial concentrates [19, 21]. Biotechnological processing of collagen-containing raw materials was carried out at optimum cultivation temperatures: *Lactobacillus helveticus* H₁₇₋₁₈ – 40±2 °C; *Bifidobacterium longum* B

379 M – 37±2 °C, and *Propionibacterium shermanii* KM 186 – 30±2 °C.

Controlled parameters of collagen-containing raw materials were determined by standard methods: organoleptic indicators – according to GOST 9959-2015; moisture-binding ability – according to the method of L.V. Antipova [20]; active acidity was determined by a potentiometric method according to GOST R 51478-99 (ISO 2917-74); the mass moisture fraction – by drying the sample in a drying cabinet according to GOST 33319-2015.

The amino acid composition of collagen substances was determined according to the described method (M-04-38-2009), using a Capel-105M capillary electrophoresis system. This method is based on the decomposition of samples by acid or alkaline (only for tryptophan) hydrolysis with the conversion of amino acids into free forms, obtaining FTC-derivatives, as well as their further separation and quantitative determination by capillary electrophoresis.

The changes occurring in the tissues under the influence of biotechnological processing were established by the method of histological examination of samples according to GOST 19496-2013 "Meat and meat products. Method of histological examination".

Microbiological indicators were determined in accordance with the regulatory base: the number of lactobacillus cells was determined by limiting dilution method on a dense agar medium MRS; the number of cells of bifidobacteria and propionic acid bacteria on a dense agar medium MMC [19, 21]. The microstructure of the samples was examined on a JSM-6510LV JEOL scanning electron microscope.

All experiments were repeated 3–5 times. The obtained data were processed using the Excel statistical software package using the Mann-Whitney test. Statistically significant differences are discussed where $p < 0.05$.

RESULTS AND DISCUSSION

At the first stage of the research, the ability of probiotic microorganisms to develop in collagen-containing raw materials was studied. Fermentation was carried out after heat treatment of the rumen by introducing the studied cultures to the prepared whey and liquid bacterial concentrate raw materials in the amounts of 3, 5 and 7%. The fermentation process was controlled by the number of viable microbial cells in the substrate. The growth of viable cells of microorganisms in the substrate is represented in Table 1.

Analysis of the data presented in Table 1 showed that the studied cultures actively develop in the substrate of the rumen. It is known that, during heat treatment, long-term heating and mincing, low molecular weight collagen disaggregation products are formed, which probably stimulate the growth of probiotic microorganisms.

Table 1
Counting of microorganisms during the fermentation of collagen-containing raw materials

Таблица 1
Количественный учет микроорганизмов в процессе ферментации коллагенсодержащего сырья

Type of cultures used	Dose of ferment, %	Duration of fermentation, h							
		1	2	3	4	5	6	7	8
<i>L. helveticus</i> <i>H₁₇₋₁₈</i>	3	2·10 ⁴	6·10 ⁴	3·10 ⁵	4·10 ⁶	6·10 ⁹	7·10 ⁹	2·10 ⁹	5·10 ¹⁰
	5	6·10 ⁴	3·10 ⁶	4·10 ⁷	4·10 ⁸	2·10 ¹⁰	3·10 ¹⁰	6·10 ¹⁰	8·10 ¹⁰
	7	9·10 ⁴	1·10 ⁶	8·10 ⁸	2·10 ⁹	3·10 ¹⁰	5·10 ¹⁰	7·10 ¹⁰	9·10 ¹⁰
<i>B. longum</i> <i>B379M</i>	3	7·10 ³	2·10 ⁴	2·10 ⁵	7·10 ⁶	2·10 ⁸	8·10 ⁸	2·10 ⁹	7·10 ⁹
	5	5·10 ³	7·10 ⁴	4·10 ⁵	3·10 ⁶	1·10 ⁸	7·10 ⁸	6·10 ⁹	8·10 ⁹
	7	3·10 ⁴	2·10 ⁵	1·10 ⁶	2·10 ⁸	3·10 ⁹	6·10 ⁹	4·10 ¹⁰	7·10 ¹⁰
<i>P. shermanii</i> <i>KM 186</i>	3	3·10 ³	2·10 ⁵	9·10 ⁶	2·10 ⁷	8·10 ⁸	7·10 ⁸	5·10 ⁹	4·10 ⁹
	5	8·10 ³	7·10 ⁶	5·10 ⁷	4·10 ⁸	4·10 ⁹	3·10 ⁹	2·10 ¹⁰	6·10 ¹⁰
	7	5·10 ⁴	3·10 ⁶	4·10 ⁷	2·10 ⁹	5·10 ⁹	8·10 ⁹	3·10 ¹⁰	8·10 ¹⁰

It should be noted that, following 7–8 hours of cultivation on a collagen substrate, the number of viable cells is *Bifidobacterium longum* B379M and *Propionibacterium shermanii* KM 186 reaches 10⁹–10¹⁰ CEUs, which confirms the prebiotic properties of low molecular weight compounds in the collagen hydrolysate. As well as being resistant to hydrolysis in the upper gastrointestinal tract, these prebiotic fibres are not sensitive to proteases of the gastrointestinal tract, making them similar to prebiotic dietary fibres such as oligosaccharides [22]. The presence of low molecular weight compounds in the collagen hydrolysate provides a synbiotic effect by means of creating favourable conditions for the development of bifidobacteria and propionic acid bacteria when it is used.

In contrast to bifidobacteria and propionic acid bacteria, *Lactobacillus helveticus* H₁₇₋₁₈ is a strong acid-forming agent [14, 16, 17] and more actively ferments in the substrate. Along with

this, the number of lactobacilli cells reaches 10⁹–10¹⁰ CFU/g after 5 hours of cultivation. Research results showed that increasing the dose of bacterial concentrates up to 7% does not lead to a noticeable growth of microorganism cells (see Table 1).

The combination of the research indicates that the optimal dose of bacterial concentrates applied is 5%. The duration of the fermentation of collagen-containing raw materials is: when using *Lactobacillus helveticus* *Lactobacillus helveticus* H₁₇₋₁₈ – 5 h, *B. longum* B379 M and *P. shermanii* KM 186 – 7–8 h.

According to the published data [3–11], the fermentation process can have a positive effect on the organoleptic properties and physicochemical properties of collagen-containing raw materials. In this regard, in further experiments, the organoleptic and basic physicochemical properties of the samples studied were evaluated before and after biotreatment (Table 2).

Table 2
Evaluation of organoleptic and physical-chemical properties of fermented collagen containing raw material

Таблица 2
Оценка органолептических и физико-химических свойств ферментированного коллагенсодержащего сырья

Indicator	Control	Test specimen, fermented		
		<i>Lactobacillus helveticus</i> H ₁₇₋₁₈	<i>Bifidobacterium longum</i> B379M	<i>Propionibacterium shermanii</i> KM 186
Outward appearance	Homogeneous fine fibre mass			
Colour, taste, smell	Inherent to this product, with a pronounced specific smell	Colour and taste inherent to this product slight lactic acid smell		
Consistency	Elastic, hard	Soft, gentle	Soft	Soft
Moisture-binding ability, %	58 ± 0.70	89 ± 0.40	87 ± 0.30	86 ± 0.50
Active acidity, pH	6.7 ± 0.08	5.9 ± 0.03	6.4 ± 0.01	6.1 ± 0.03
Moisture content, %	70 ± 0.50	76 ± 0.70	78 ± 0.20	77 ± 0.30

The data in Table 2 shows that the biotechnological treatment of the rumen helps to improve the flavouring characteristics of the test samples compared to the control: the consistency of the sample has changed, the specific smell has almost disappeared; instead, it has a pleasant lactic acid smell. Probably, the improvement in organoleptic properties is associated with the accumulation of fermentation products of probiotic cultures (in particular, lactic or propionic acids). The biomodification of the rumen allowed the main functional and technological parameters of the collagen-containing raw material to be improved. The moisture-binding capacity in products increased, which affected the moisture content of fermented samples. The increase in moisture binding ability has a positive effect on the consistency of collagen-containing raw materials making it more succulent, soft and ductile. The marked increase in the moisture binding capacity of collagen during fermentation is associated both with its preliminary heat treatment and with the effect of proteolytic enzymes of starter microorganisms. A decrease in the pH of the medium and swelling of the collagen led to a disintegration of polypeptide chains with the formation of a large number of hydrophilic groups and the binding of additional water molecules.

At the next stage of the study, structural changes in collagen substances in the biomodified rumen were studied.

According to the published data [23–26], the unusual mechanical properties of collagens are associated with their primary and spatial structures. Collagen molecules consist of three polypeptide chains, called α -chains. The composition of collagens can include three identical or different chains. The primary structure of α -chains of

collagen is unusual, since every third amino acid in the polypeptide chain is represented by glycine, about ¼ of amino acid residues are proline or 4-hydroxyproline and about 11% is alanine. Collagen lacks such amino acids as cysteine and tryptophan. Histidine, methionine and tyrosine are found only in very small amounts [23–26].

Comparative characteristics of the amino acid composition of the samples before and after fermentation are presented in Table 3.

The analysis of data in Table 3 showed that, following fermentation, the content of almost all amino acids (except arginine) in collagen substances increased 2–4 times; this indicates a rather high proteolytic activity of microorganisms. It should be noted that the studied fermented samples are characterised by varying contents of individual amino acids. This indicates differences in the structure, and, consequently, in the properties of the resulting collagen substances.

A characteristic feature of collagen proteins is the presence and high content of amino acids such as proline and glycine [25, 26]. These particular amino acids provide the formation of a specific secondary structure of collagens in the form of triple-stranded helix [26]. The highest value of these amino acids in the sample fermented by *Lactobacillus helveticus* H₁₇₋₁₈ (see Table 3) is noted, which indicates a deeper hydrolysis of proteins. It should be noted that *Lactobacillus helveticus* H₁₇₋₁₈ has a higher proteolytic system and has a developed complex of peptidases and proteases [14, 16, 17].

It is believed that amino acids such as alanine, serine, and tyrosine may be involved in the synthesis of collagen [23–26]. A significant content of these amino acids is observed in all studied samples (see Table 3).

**Comparative characteristics of the amino acid composition
of collagen substances before and after fermentation**

Table 3

**Сравнительная характеристика аминокислотного состава коллагеновых
субстанций до и после ферментации**

Таблица 3

Amino acid	Concentration, mg/l			
	Control	Test specimen, fermented		
		<i>Lactobacillus helveticus</i> H ₁₇₋₁₈	<i>Bifidobacterium longum</i> B379M	<i>Propionibacterium shermanii</i> KM 186
Arginine	22.50 ± 0.004	11.60 ± 0.001	18.10 ± 0.009	15.60 ± 0.001
Lysine	24.40 ± 0.010	48.90 ± 0.004	37.00 ± 0.001	39.20 ± 0.002
Tyrosine	1.36 ± 0.005	4.39 ± 0.001	2.06 ± 0.005	3.06 ± 0.004
Phenylalanine	2.33 ± 0.001	6.52 ± 0.002	3.35 ± 0.007	4.47 ± 0.005
Histidine	1.63 ± 0.004	2.12 ± 0.007	4.21 ± 0.001	3.76 ± 0.002
Leucine + isoleucine	10.50 ± 0.006	20.70 ± 0.005	19.10 ± 0.002	19.80 ± 0.009
Methionine	1.13 ± 0.009	2.54 ± 0.001	2.15 ± 0.004	2.45 ± 0.001
Valin	2.27 ± 0.002	6.43 ± 0.007	8.20 ± 0.009	9.65 ± 0.002
Proline	4.15 ± 0.005	7.51 ± 0.002	4.73 ± 0.001	5.12 ± 0.005
Threonine	2.70 ± 0.001	7.05 ± 0.007	3.26 ± 0.004	4.66 ± 0.001
Serine	2.71 ± 0.007	9.92 ± 0.009	6.99 ± 0.002	7.11 ± 0.004
Alanine	11.40 ± 0.004	24.80 ± 0.002	16.90 ± 0.009	18.30 ± 0.002

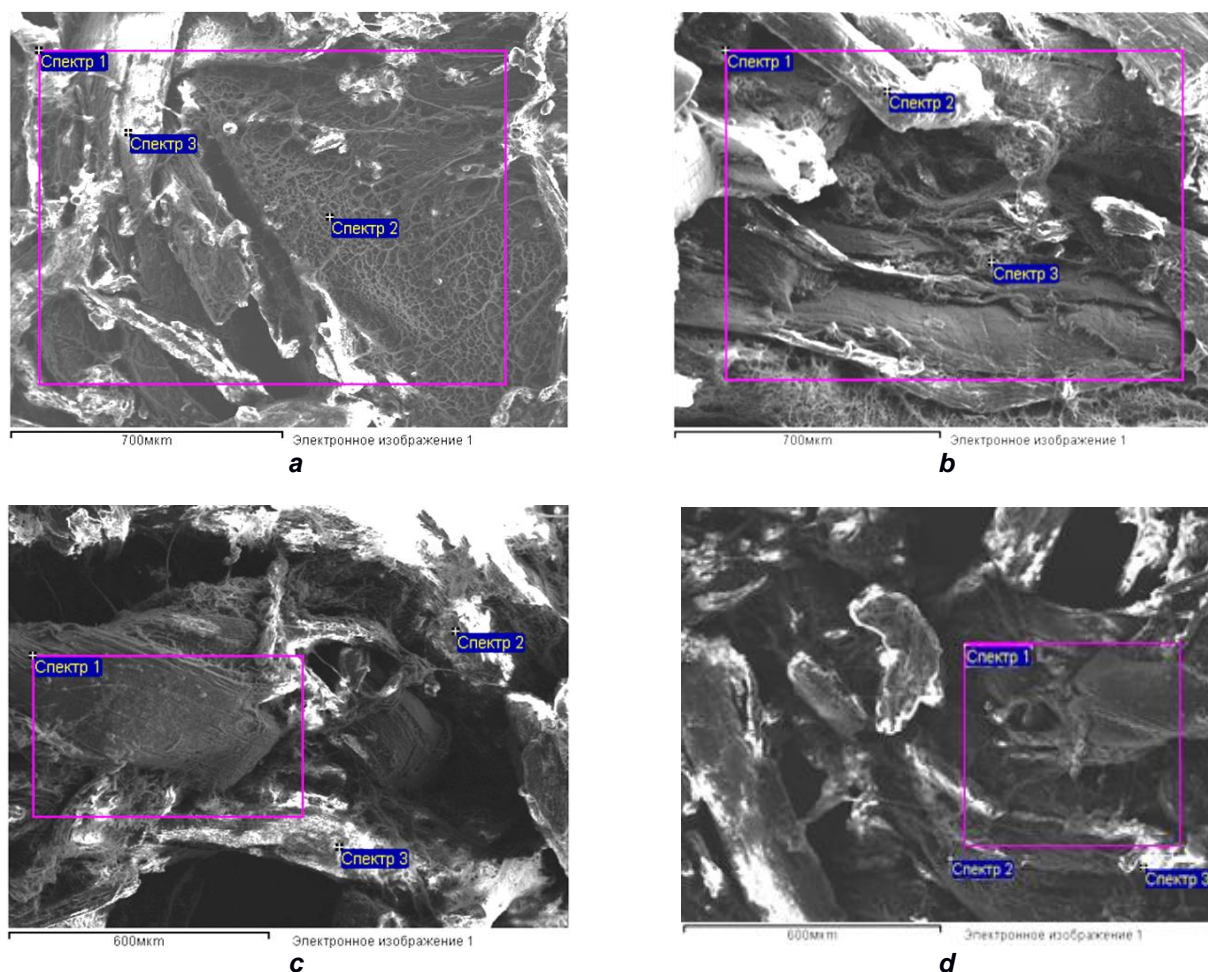


Fig. Microstructure of collagen-containing raw material (CCRM):

a – control; b – CCRM, fermented with *L. helveticus* H 17-18,

c – CCRM, fermented with *B. longum* B379M,

d – CCRM, fermented with *P. shermanii* KM 186

Рис. Микроструктура коллагенсодержащего сырья (КСС):

a – контроль; b – КСС, ферментированные *L. helveticus* H₁₇₋₁₈,

c – КСС, ферментированные *B. longum* B379M,

d – КСС, ферментированные *P. shermanii* KM 186

Further, the effect of fermentation on the change in the histological structure of the rumen was studied. Histosections stained with haematoxylin-eosin revealed changes following biomodification of individual structural elements with a magnification of $\times 700$ and $\times 600$. The microstructure of the studied samples is presented in Figure.

In the control sample of the rumen (image a), the mucous membrane is represented by the papillae of various shapes, covered with multi-layered epithelium. On the histosections of the rumen fragments following treatment of the samples with the starters of probiotic cultures (images b – d) it can be clearly seen that the collagen fibres and mucous elastin layer have been stretched and in the longitudinal section have a pronounced striation. The intermuscular space of bioprocessing specimens is also stretched; in some places the surface

layer is thinned; in others, some loosening of its structure is also noticeable. All this indicates that the rumen biotreatment with bacterial concentrates of probiotic cultures leads to changes in the structure of its morphological elements. The distribution profile of the elemental composition of the studied samples (see Figure) is presented in Table 4.

From the data presented in Table 4, it can be seen that, following fermentation, the elemental composition of the collagen-containing raw material is replenished with calcium and potassium. Evidently, these elements were introduced into the collagen-containing raw material preparation along with the whey. The presented research results confirm the fact that probiotic cultures are able to accumulate a significant quantity of calcium ions on their surface in various forms [15], thereby creating a kind of "depot" of this cation.

Table 4
Elemental composition of collagen-containing raw materials before and after fermentation

Таблица 4
Элементный состав коллагенсодержащего сырья до и после ферментации

Investigated specimen range	The elemental composition, in weight %								
	C	O	Na	P	S	Cl	Ca	K	Total
<i>Control</i>									
Spectrum 1	64.40	34.66	0.14	0.15	0.57	0.08	–	–	100.00
Spectrum 2	61.61	37.17	0.26	0.13	0.67	0.17	–	–	100.00
Spectrum 3	73.71	25.29	0.26	0.26	0.93	0.09	–	–	100.00
Average value	66.39	32.37	0.22	0.18	0.73	0.011	–	–	100.00
Standard deviation	6.04	6.26	0.07	0.07	0.18	0.05	–	–	–
Max.	73.17	37.17	0.26	0.26	0.93	0.17	–	–	–
<i>CCRMM, fermented L. helveticus H₁₇₋₁₈</i>									
Spectrum 1	40.68	57.25	0.35	0.34	0.87	0.15	0.26	0.10	100.00
Spectrum 2	44.56	48.24	0.61	0.94	2.58	1.44	0.53	1.10	100.00
Spectrum 3	40.99	54.25	0.74	0.36	2.47	0.17	0.33	0.69	100.00
Average value	42.08	53.25	0.57	0.55	1.97	0.59	0.37	0.63	100.00
Standard deviation	2.15	4.59	0.20	0.34	0.96	0.74	0.14	0.50	–
Max.	44.56	57.25	0.74	0.94	2.58	1.44	0.53	1.10	–
<i>CCRMM, fermented B. longum B379M</i>									
Spectrum 1	63.32	35.35	0.19	0.18	0.69	0.12	0.10	–	100.00
Spectrum 2	74.16	22.58	0.31	0.12	2.09	0.27	0.30	–	100.00
Spectrum 3	76.36	21.33	0.30	0.71	0.84	0.02	0.09	–	100.00
Average value	71.28	26.42	0.27	0.19	1.20	0.14	0.16	–	100.00
Standard deviation	6.98	7.76	0.07	0.32	0.77	0.13	0.12	–	–
Max.	76.36	35.35	0.31	0.71	2.09	0.27	0.30	–	–
<i>CCRMM, fermented P. shermanii KM 186</i>									
Spectrum 1	58.68	39.25	0.37	0.28	0.92	0.19	0.15	0.16	100.00
Spectrum 2	57.79	38.58	1.26	0.30	1.41	0.14	0.12	0.40	100.00
Spectrum 3	48.31	48.56	1.00	0.71	0.81	0.29	0.18	0.14	100.00
Average	54.93	42.13	0.88	0.43	1.05	0.21	0.15	0.23	100.00
Standard deviation	5.75	5.58	0.46	0.24	0.32	0.08	0.03	0.14	–
Max.	58.68	48.56	1.26	0.71	1.41	0.29	0.18	0.40	–

Thus, as a result of the research, a technological scheme for the production of collagen hydrolysate has been developed, which includes: preparation of raw materials; thermal hydrolysis at a temperature of 100 °C for 2 hours; mincing and adding 5% whey; cooling to fermentation temperature; biotechnological processing of bacterial concentrates of probiotic microorganisms. The duration of fermentation for bifidobacteria and propionic acid bacteria was 7–8 hours; for lactobacteria – 5 hours. Collagen hydrolysate had good organoleptic properties and contained a high number of viable cells of probiotic microorganisms (10^9 – 10^{10} CFU/g).

The proposed biotechnological methods of processing secondary collagen-containing raw materials not only allowed its organoleptic and functional-technological properties to be improved, but also opened up broad prospects for creating a number of useful collagen- and calcium-containing products for various purposes, including the prevention of osteoporosis.

CONCLUSION

As a result of the research, a fundamentally new scheme for the biotechnological processing of collagen-containing raw materials has been developed, allowing a collagen hydrolysate with synbiotic properties to be obtained.

It is established that, following preliminary heat treatment, bovine rumen comprises a good nutrient medium for the development of probiotic cultures. It is revealed that low molecular weight products of collagen disaggregation have prebiotic properties and thus stimulate the growth of microorganisms.

In the process of biotransformation of the rumen, the flavouring characteristics are improved, the specific smell of the rumen disappears, it acquires a pleasant lactic acid taste, and the consistency becomes more succulent, soft and ductile.

Fermentation of collagen-containing raw materials with probiotic microorganisms increases the content of amino acids in hydrolysates. This is especially true for *Lactobacillus helveticus*, which is characterised by high proteolytic activity. It is noted that fermentation changes the structure of the rumen and its morphological elements.

Biotechnological treatment of the rumen following thermal hydrolysis by activated cultures of probiotic microorganisms significantly reduces the process of obtaining collagen hydrolysate and improves the quality of the finished product. The total duration of the described rumen treatment, including thermal and biotechnological, is 10–11 hours, which increases the economic efficiency of biotechnological processing in comparison with known analogues.

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Contribution

Anna V. Shchekotova, Irina S. Khamagaeva, Vladimir Zh. Tsyrenov, Natal'ya V. Darbakova, Sof'ya N. Khazagaeva carried out the experimental work, on the basis of the results summarized the material and wrote the manuscript. Anna V. Shchekotova, Irina S. Khamagaeva, Vladimir Zh. Tsyrenov, Natal'ya V. Darbakova, Sof'ya N. Khazagaeva have equal author's rights and bear equal responsibility for plagiarism.

Conflict of interests

The authors declare no conflict of interests regarding the publication of this article.

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Щёкотова А.В., Хамагаева И.С., Цыренов В.Ж., Дарбакова Н.В., Хазагаева С.Н. выполнили экспериментальную работу, на основании полученных результатов провели обобщение и написали рукопись. Щёкотова А.В., Хамагаева И.С., Цыренов В.Ж., Дарбакова Н.В., Хазагаева С.Н. имеют на статью равные авторские права и несут равную ответственность за плагиат.

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