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Method of obtaining preventive orientation curd product

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Abstract. Research on the use of components of pike-perch (*Sander lucioperca*) scales in the form of fibrous collagen-containing additive (CA) and powder additive containing mainly calcium hydroxyapatite (HAp) as components of formulas, when obtaining curd products, has been carried out. Experimental and control samples of curd products and milk whey separated at their reception, according to three similar formulations, differing in that to their main components (milk and kefir) in the formulation No. 1 was added calcium chloride powder (control), in the formulation No. 2 – a solution of CA in lemon juice, in the formulation No. 3 – a solution of HAp in lemon juice. The results of the study showed that CA used in formulation No. 2 increases the yield of curd product (220 g) compared to the control (217 g) and the experimental sample with HAp (194 g). The curd product with the addition of CA has an increased mass fraction of protein, fat, and more optimal ratio of calcium and phosphorus for their assimilation by humans, and separated whey has a reduced mass fraction of protein (1.73%) compared to the control (2.69%) and experimental sample with HAp (4.32%). The curd product with the addition of CA also has a more elastic, elastic and dense consistency without perceptible particles of milk protein compared to the control and experimental sample with HAp, sour-milk saturated taste and smell, without extraneous flavours and odours, white colour with a creamy tinge. On the basis of empirical data obtained with the use of CA, a method of obtaining curd product of preventive orientation was developed.

Keywords: pike-perch scales, collagen-containing additive, calcium hydroxyapatite, curd product, milk whey

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Научная статья

Способ получения творожного продукта профилактической направленности

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Аннотация. Проведены исследования по использованию составляющих чешуи судака (*Sander lucioperca*) в виде волокнистой коллагенсодержащей добавки (КД) и порошковой добавки, содержащей преимущественно гидроксиапатит кальция (ГАП), в качестве компонентов рецептур при получении творожной продукции. Были приготовлены опытные и контрольные образцы творожных продуктов и отделенных при их получении молочных сывороток, согласно трем аналогичным рецептурам, отличающимся тем, что к основным их компонентам (молоко и кефир) в рецептуре № 1 был добавлен порошок кальция хлорида (контроль), в рецептуре № 2 – раствор КД в лимонном соке, в рецептуре № 3 – раствор ГАП в лимонном соке. Результаты исследования показали, что КД, используемая в рецептуре № 2, способствует увеличению выхода творожного продукта (220 г) по сравнению с контролем (217 г) и опытным образцом с ГАП (194 г). Творожный продукт с добавлением КД имеет повышенную массовую долю белка, жира и более оптимальное соотношение кальция и фосфора для их усвоения человеком, а отделенная молочная сыворотка – пониженную массовую долю белка (1,73 %) по сравнению с контролем (2,69 %) и опытным образцом с ГАП (4,32 %). Творожный продукт с добавлением КД также имеет более эластичную, упругую и плотную консистенцию, без ощутимых частиц молочного белка, по сравнению с контролем и опытным образцом с ГАП, кисломолочный насыщенный вкус и запах, без посторонних привкусов и запахов, белый цвет с кремовым оттенком. На основании эмпирических данных, полученных с применением КД, был разработан способ получения творожного продукта профилактической направленности.

Ключевые слова: чешуя судака, коллагенсодержащая добавка, гидроксиапатит кальция, творожный продукт, молочная сыворотка

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Introduction

The population of most countries consumes insufficient amounts of calcium (daily physiological requirement for children (400-1 200 mg), adults (1 000 mg) and the elderly (1 200 mg) with food products, which is one of the main reasons (along with collagen deficiency, with a recommended intake of 5-10 g/day) for the risk of widespread osteoporosis and osteoarthritis [1, 2].

Milk and its products, whose consumption per capita in the world tends to decrease, as well as hydrobionts are the main sources of calcium intake [2, 3].

To prevent calcium deficiency in the human diet, it is necessary to enrich food products (including dairy products) with calcium salts.

The problem of introduction of calcium ions into dairy products is associated with poor solubility of traditionally used inorganic salts ($CaCl_2$, $CaCO_3$) and their interaction with proteins during thermal processing, leading to coagulation and reduction of nutritional value of finished products, so research is being conducted to find the most acceptable forms of calcium for use [3, 4].

Calcium hydroxyapatite nanoparticles (HAp) with an optimal mass ratio of phosphorus and calcium 1.0 : 1.67 are the main mineral component of teeth, bones, and are also found in the blood of mammals, which indicates its significant biological value [5].

It is known that HAp is the equivalent of natural nanoparticles of colloidal calcium phosphate contained in milk localised mainly inside casein micelles and linking individual casein molecules into a globule, which indicates their structuring function [6, 7].

During the production of dairy products (curd, cheese, etc.) hydroxyapatite nanoparticles do not dissociate in solution (unlike $CaCl_2$), but are centres of aggregation of paracasein molecules, which provides a change in the structure of the milk clot, accompanied by a change in its physicochemical properties, in addition, they contribute to the binding of whey proteins and accelerate the process of syneresis of the gel [7].

As a cheap natural source of HAp and collagen in dairy products can be used components of fish scales; collagen-containing additive in the form of fibre and additive containing gyroxyapatite in the form of powder [8].

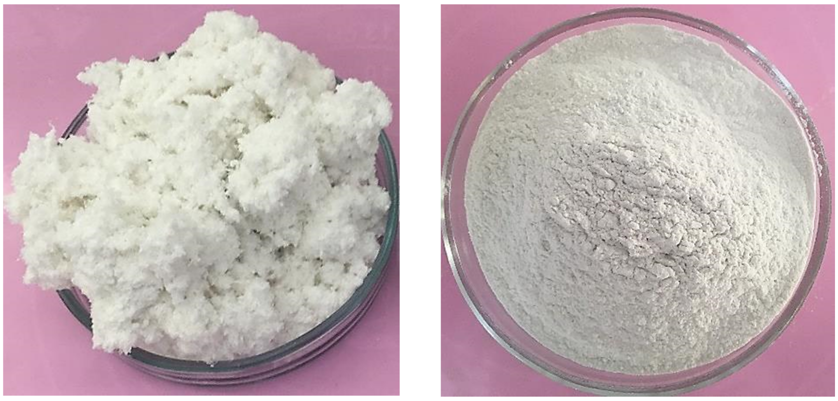
It is of interest to use HAp and collagen additives from little-used scales formed in significant quantities during the processing of fish raw materials to obtain curd products of functional orientation.

Purpose of the study: evaluation of the possibility of using components of fish scales in the form of additives, in obtaining curd products used for preventive purposes.

Materials and methods

The objects of research were curd products, as well as milk whey formed during their production, according to three different recipes. As components of the recipes were used: milk “Nezhinskoye” according to ISS 31450-2013 “Drinking milk. Technical conditions” fat content of 2.5%; kefir “Nezhinsky” according to ISS 31454-2012 “Kefir. Technical conditions” with fat content of 2.5%; collagen-containing additive (CA) obtained from pikeperch scales (*Sander lucioperca*); calcium-containing additive mainly in the form of HAp, obtained from pikeperch scales; calcium chloride (substance) for the production of medicines JSC “Karpov Chemical Plant” (Mendeleevsk); lemon juice.

The components of CA and HAp formulations were obtained in laboratory conditions of the Chemistry Department by researchers of Kaliningrad State Technical University (Kaliningrad) from fish scales, according to the previously developed technology [8, 9]. The appearance of CA and HAp from pike-perch scales is presented in Fig. 1.



a *b*

Fig. 1. Appearance of CA (*a*) and HAp (*b*) additives obtained from pikeperch scales

The total chemical composition of CA and HAp (including calcium and phosphorus) from pikeperch scales, is presented in Table 1.

Table 1

Total chemical composition of the components of the formulations of cottage cheese products CA and HAp from pikeperch scales (including calcium and phosphorus)

Components of recipes	Mass fraction of substance, %					
	water	protein	fat	ash	Ca	P
CA	9.20 ± 0.7	67.40 ± 0.28	3.75 ± 0.56	22.40 ± 0.01	6.56 ± 1.11	3.77 ± 0.63
HAp	6.90 ± 0.7	25.78 ± 0.28	2.42 ± 0.49	63.58 ± 0.01	17.41 ± 2.96	3.71 ± 0.62

Table 1 shows that CA has a mass fraction of protein 67.4% and ash 22.40%, while HAp contains 25.78% protein and ash 63.68%.

Production of experimental and control samples of curd products, as well as their physical and chemical analyses were carried out in the conditions of the Department of Chemistry and certified laboratory "Kaliningrad Testing Centre" (Kaliningrad). The total chemical composition (including phosphorus and calcium) was determined according to the current normative documentation. Mass fraction of moisture was determined according to ISS R 54668-2011 "Milk and dairy products. Methods of determining the mass fraction of moisture and dry matter", fat according to ISS 5867-90 "Milk and dairy products. Methods of determination of fat", protein ISS 34454-2018 "Determination of the mass fraction of protein by the Kjeldahl method", total ash according to ISS R 54607.10-2017 "Determination of the mass fraction of total ash", carbohydrates according to MG No. 4237-86 from 29.12.86 (calculation method), calcium according to ISS R 55331-2012 "Titrimetric method for determination of calcium content", phosphorus according to ISS 31584-2012 (ISO 9874:2006) "Milk. Spectrophotometric method for the determination of the mass fraction of total phosphorus", acidity according to ISS R 54669-2011 "Milk and milk products. Methods of determination of acidity", hydrogen pH indicator according to ISS 26188-2016 "Fruit and vegetable processing products, canned meat and meats. Method of determination of pH".

Recipes of curd products are presented in Table 2.

Table 2

Recipes of curd products

Components of curd product recipes	Recipes		
	No. 1 (control)	No. 2 (experience)	No. 3 (experience)
Milk "Nezhinskoye", fat content 2.5%, ml	1 000		
Kefir "Nezhinsky", fat content 2.5%, ml	200		
Collagen-containing additive (CA), g	–	10	–
Calcium-containing additive (HAp), g		–	10
Calcium chloride, g	4.4		–
Lemon juice, ml	–	40	40

As can be seen from Table 2, the formulations differ from each other only by the introduction of calcium and collagen-containing additives in dry or liquid (pre-dissolved in lemon juice) form.

The process of obtaining curd products was carried out as follows. To develop a control sample (recipe No. 1) milk "Nezhinskoye" fat content of 2.5% in the amount of 1 000 ml poured into a container and heated on a gas cooker to 36-37 ºC. To the heated milk was added kefir "Nezhinskiy" with fat content of 2.5% in the amount of 200 ml and 4.4 g of calcium chloride powder ($CaCl_2$), then the obtained mixture was heated up to 90 ºC by stirring and in hot form was poured into a lavsan bag for separation of liquid part (milk whey) in the process of self-pressing of the formed clot (curd product) and its cooling within 2-3 hours, the pressed curd product in the amount of 217 g was placed in the refrigerator (4 ± 2 °C). The mass of separated whey was 1 003 g.

The process of obtaining curd products according to the recipes No. 2 and 3 was similar to the control (formulation 1), except that instead of using calcium chloride in the recipe No. 2, a solution of CA (10 g) in 40 ml of lemon juice, pre-cooked and heated to 60 ºC, which was introduced into the heated mixture of milk and kefir, was used. In recipe No. 3, a solution of HAp (10 g) in 40 ml of lemon juice was prepared, which was pre-incubated (due to the very poor solubility of calcium hydroxyapatite) for 7 days in a refrigerator at 4 ºC, then it was heated to 60 ºC and added to the heated mixture of milk and kefir.

Results and discussion

It is known that calcium chloride has a significant industrial application in cottage cheese production, where it is used to increase the yield of finished products [10, 11]. On this basis, when obtaining experimental samples of products, as a comparison (control) used samples of recipe No. 1, where calcium chloride was added to the milk.

The total chemical composition of curd product and whey (including Ca and P), as well as their acidity and pH obtained with the use of calcium chloride in the form of powder (control), according to the presented recipe No. 1, is shown in Table 3.

Table 3

Total chemical composition of curd product and whey (including Ca and P), as well as their acidity and pH obtained with calcium chloride powder (control)

Indicator to be determined	Results	
	Curd product	Whey
Mass fraction of water, %	60.9 ± 0.3	93.8 ± 0.8
Mass fraction of protein, %	15.21 ± 0.15	2.69 ± 0.1
Mass fraction of fat, %	16.00 ± 0.3	less than 0.1
Mass fraction of carbohydrates, %	6.1 ± 0.8	3.2 ± 1.0
Mass fraction of ash, %	1.809 ± 0.009	0.295 ± 0.009
Mass fraction of calcium, %	0,390 ± 0.005	0.132 ± 0.005
Mass fraction of phosphorus, %	0.319 ± 0.005	0.045 ± 0.005
Acidity, ºT	92.0 ± 3.5	31.0 ± 1.9
Hydrogen index / pH, units pH	5.69 ± 0.24	5.77 ± 0.24

As can be seen from Table 3, the mass ratio of calcium to phosphorus (0.390 : 0.319) in the curd product is 1.22.

The yield of the finished curd product in the control was 217 g, while the whey separated during its self-pressing process was 1 003 g.

The total chemical composition of curd product and whey (including Ca and P), as well as their acidity and pH obtained with the use of CA in the form of fibrous particles, according to the presented recipe No. 2, is shown in Table 4.

Table 4

Total chemical composition of curd product and whey (including Ca and P), as well as their acidity and pH obtained with fibre particle CA

Indicator to be determined	Results	
	Curd product	Whey
Mass fraction of water, %	60.7 ± 0.3	93.3 ± 0.8
Mass fraction of protein, %	17.69 ± 0.15	1.73 ± 0.1
Mass fraction of fat, %	16.50 ± 0.3	less than 0.1
Mass fraction of carbohydrates, %	4.1 ± 0.8	4.3 ± 0.9
Mass fraction of ash, %	0.987 ± 0.009	0.637 ± 0.009
Mass fraction of calcium, %	0.397 ± 0.005	0.144 ± 0.005
Mass fraction of phosphorus, %	0.222 ± 0.005	0.071 ± 0.005
Acidity, °T	114.0 ± 3.5	53.0 ± 1.9
Hydrogen index / pH, units pH	5.24 ± 0.24	4.93 ± 0.24

As can be seen from Table 4, the experimental sample of curd product at approximately the same moisture content as the control (Table 3), had an increased mass fraction of protein, fat and reduced carbohydrates and total ash, with almost the same calcium content, although the amount of added minerals in the curd product in the experimental sample of recipe No. 2, was less (2.24 g based on the chemical composition of CA) than in the control – 4.4 g. It is important to note that the ratio of calcium to phosphorus (0.379 : 0.222) in the obtained curd product is 1.788, which is closer to the optimum 1.67 for human assimilation, compared to the control (1.222), which will contribute to better assimilation and functionality of its mineral component. The separated milk whey of the experimental sample No. 2 is more transparent and has a lower mass fraction of protein (due to binding products of collagen hydrolysis of whey proteins and fat), with an increased content of carbohydrates and total ash, compared with the control, which also confirms the higher nutritional value and functional properties of the proposed curd product. The yield of the finished curd product was 220 g, and the resulting milk whey was 1 026 g.

The total chemical composition of curd product and whey (including Ca and P), as well as their acidity and pH obtained with the addition of HAp in the form of powder, according to the presented recipe No. 3, is given in Table 5.

Table 5

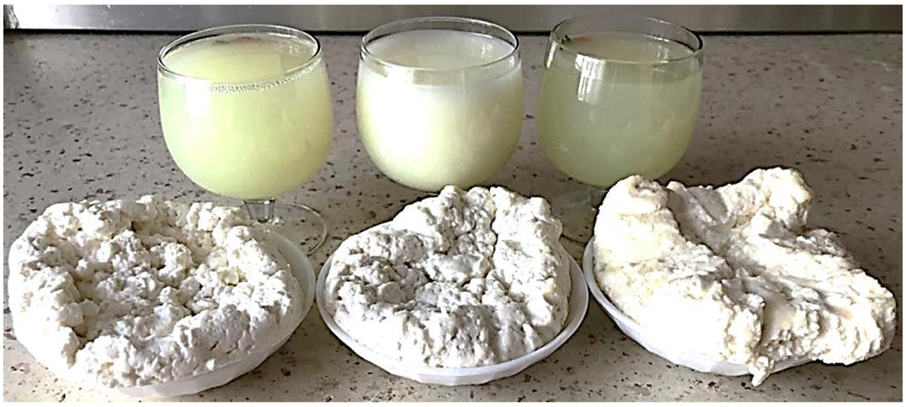
Total chemical composition of curd product and whey (including Ca and P) and their acidity and pH obtained with the addition of HAp in powder form

Indicator to be determined	Results	
	Curd product	Whey
Mass fraction of water, %	59.7 ± 0.3	93.5 ± 0.8
Mass fraction of protein, %	15.87 ± 0.15	4.32 ± 0.1
Mass fraction of fat, %	16.00 ± 0.3	0.2
Mass fraction of carbohydrates, %	4.1 ± 0.8	4.3 ± 0.9
Mass fraction of ash, %	4.543 ± 0.009	0.526 ± 0.009
Mass fraction of calcium, %	0.395 ± 0.005	0.159 ± 0.005
Mass fraction of phosphorus, %	0.717 ± 0.005	0.049 ± 0.005
Acidity, °T	112.0 ± 3.5	35.0 ± 1.9
Hydrogen index / pH, units pH	5.59 ± 0.24	5.83 ± 0.24

According to Table 5, the experimental sample of curd product has similar values of mass fraction of water, protein and fat with reduced carbohydrate content and increased total ash compared to the control. Milk whey has increased mass fraction of protein, fat, carbohydrates and total ash as compared to control.

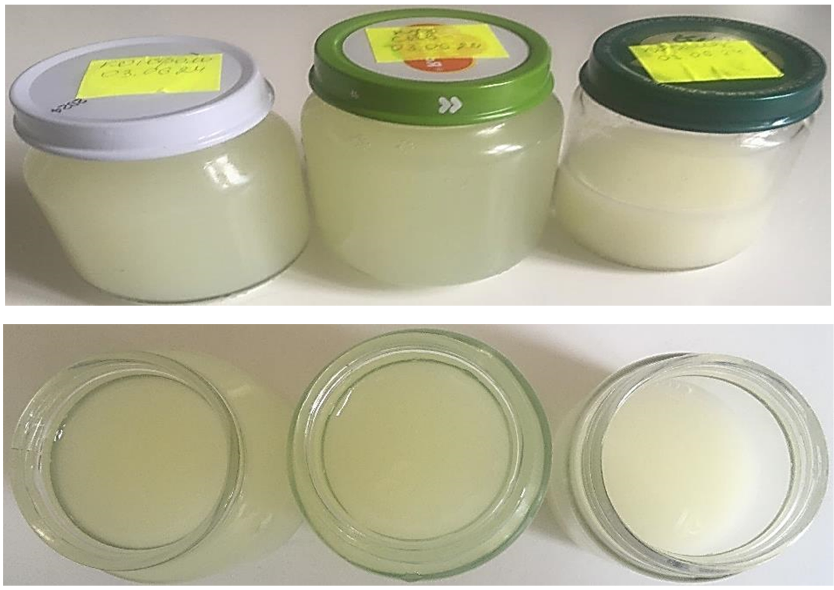
The yield of finished curd product was 194 g, which had less elastic, elastic and dense consistency compared to experimental sample No. 2. The separated milk whey (1 039 g) was opaque and had a pronounced whitish colour, indicating the presence of suspension of calcium particles in the liquid.

The appearance of the obtained samples of curd products, as well as milk whey separated during their obtaining, is presented in Fig. 2 and 3.



a *b* *c*

Fig. 2. Appearance of obtained curd products with separated whey (glasses):
a – control (with addition of $CaCl_2$); *b* – with addition of HAp; *c* – with addition of CA



a *b* *c*

Fig. 3. Appearance of separated milk whey at obtaining curd products:
a – control (with addition of $CaCl_2$); *b* – with addition of CA; *c* – with addition of HAp

From Fig. 2 and 3, it can be seen that the experimental sample of curd product obtained with the addition of CA has a more dense, elastic and elastic consistency with less crumb as compared to the control and experimental sample with HAp, and the separated whey is more transparent.

The conducted studies have shown that CA used in obtaining curd product (recipe No. 2) contributes to an increase in the yield of curd product (220 g) compared with the control (217 g) and experimental sample with HAp (194 g). Curd product with addition of CA has increased mass fraction of protein, fat, and more optimal ratio of calcium and phosphorus, and separated whey has reduced mass fraction of protein (1.73%) in comparison with control (2.69%) and experimental sample with HAp (4.32%).

The curd product with the addition of CA, has a more elastic, elastic and dense consistency without perceptible particles of milk protein compared to the control and experimental sample with HAp, sour-milk saturated taste and smell, without extraneous flavours and odours, white colour with a creamy tinge throughout the mass.

This allows us to conclude that the use of CA in curd product production contributes to increasing its nutritional value and giving it preventive (functional) properties due to enrichment with collagen and products of its hydrolysis formed during heating of milk mixture, as well as HAp of natural origin, while expanding the range of curd products. 100 g of curd product (see Table 4) contains 397 mg of calcium (39.7% of daily human requirement) and about 2.45 g (24.5-49.0% of the recommended daily allowance) of collagen determined by calculation method (100 g of curd product contains 3.06 g of fish protein (6.74 g of protein (CA) : 2.2), where approximately 80% is collagen). The curd product of preventive orientation is recommended for middle-aged and elderly people.

The obtained control and experimental samples are related to curd products, as they are produced using cheap starter microflora of kefir fungi (in the form of kefir, which is a probiotic and one of the main components of the therapeutic diet), containing lactic acid microorganisms (lactococci, lactobacilli, leuconostocci), acetic acid bacteria and yeast [12-14]. Kefir fungi are known for numerous functional properties which produce microbial polysaccharides that promote the immune system and have radioprotective, antigenotoxic, antimutagenic, antioxidant, hypotensive, hypoglycaemic, anti-inflammatory and wound healing, antimicrobial, anti-allergic and other effects [15, 16]. The introduction of kefir as a cheap starter with high biological potential and functionality, as well as colloidal solution of CA particles in lemon juice (increasing the acidity of the mixture), heated to 60 °C, contribute to the acceleration of the process of clot formation and better separation of the formed whey from it.

The amount of lemon juice required was determined empirically, where the main criterion was the complete immersion of fibrous particles of CA in the liquid, taken in mass ratios of 1 : 4.1 respectively.

Lemon juice, acting on CA when heated to 60 °C, transforms it into a partially soluble (colloidal) state before introduction into the milk mixture. Experimentally, it was found that the temperature of 60 °C is the optimal temperature for heating, providing the transition of suspension into a colloidal solution, at which fibrous particles of CA become insusceptible when chewing the finished product.

The obvious advantage of the proposed method is the use of only natural components in obtaining curd product, as well as the fact that its implementation will contribute to the involvement of significant amounts of virtually unused scales of hydrobionts formed in the process of fish processing in the industrial production of food products, while reducing the negative ecological load on the environment.

Conclusion

The possibility of using CA obtained from pike-perch scales in the form of fibrous particles in obtaining curd product of preventive purpose has been established.

It was determined that the use of CA having a mass fraction of protein 67.4% (mainly collagen) and total ash 22.4% (mainly consisting of calcium hydroxyapatite) in obtaining curd product, contributes to an increase in its mass fraction of protein, fat and a decrease in carbohydrates and total ash, with almost the same content of calcium and moisture in the finished product, compared to the control (curd with the addition of calcium chloride ($CaCl_2$).

It is noted that the ratio of calcium to phosphorus (0.379 : 0.222) in the obtained curd product is 1.788, which is closer to the optimal 1.67 for human assimilation, compared to the control (1.222), which contributes to better assimilation of its mineral component. The yield of curd product is slightly higher than in the control.

It is revealed that the waste whey was more transparent and had a reduced mass fraction of protein (1.73%), with increased carbohydrates (4.3%), compared with the control, respectively, 2.64 and 3.2%.

It is shown that the use of HAp obtained from pike-perch scales in obtaining curd product is inexpedient, due to insignificant yield of finished products compared with the control and, accordingly, significant losses of raw materials together with waste milk whey.

As a result of research, developed a method of obtaining curd product of preventive orientation, where its obvious advantage is the simplicity of obtaining the finished product using cheap natural components

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