A. V. Shchekotova et al /J. Pharm. Sci. & Res. Vol. 10(12), 2018, 3355-3358

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Development of the calcium-and-collagen-rich soft curd cheese technology

A. V. Shchekotova[1], I. S. Khamagaeva[1], E. I. Reshetnik[2], S. I. Artyuhova[3], I. P. Maradudina[1]

[1]East Siberia State University of Technology and Management, Klyuchevskaya Str., 40V, Ulan-Ude, 670013, Russia
[2]Far East State Agrarian University, Politehnicheskaya Str., 86, Blagoveshchensk, 675005, Russia
[3]Omsk State Technical University, Mira Ave., 11, Omsk, 644050, Russia

Abstract:

The article describes the results of the studies on the soft curd cheese technology development based on calcium-containing protein concentrate, with the addition of modified collagen-containing raw materials (cattle rumen), to prevent osteoporosis. It has been established that the introduction of collagen-containing rumen biomodified with lactic bacteria in the amount of 5% of the cheese mass volume yields a cheese with the best consumer properties. It has been noted that the use of collagen-containing raw materials in the production of curd cheese allows obtaining a cheese dough with dense elastic structure without the use of expensive enzymes, emulsifiers and stabilizers. A recommended shelf life of the enriched curd cheese of about 12-14 days has been established. The developed curd cheese technology has provided a therapeutic product with good organoleptic characteristics, containing the significant amount of calcium, biomodified collagen, and viable lactic bacteria cells. Curd cheeses produced as per the technology are essential in the treatment and prevention of osteoporosis and associated diseases.

Keywords: curd cheese, calcium, collagen-containing raw materials, osteoporosis, milk protein concentrate, lactic bacteria.

INTRODUCTION

Due to its high prevalence and medico-social significance osteoporosis is among the top five most pressing diseases for the healthcare of developed countries. Modern prevention and treatment methods allow improving the quality of bones and reducing the incidence of fractures in a significant number of patients. Prevention of osteoporosis includes activities aimed at maximizing bone mass peak in children and young people, as well as maintaining bone mass and preventing falls in people of the older age group [1–4]. The main approach to the prevention of osteoporosis is to increase the intake of calcium, vitamin D and collagen [5-13]. Adequate calcium intake is among the factors necessary to achieve the required bone mass peak, as well as to maintain it throughout life [5, 6, 8-10]. Milk and dairy products (especially cheese) contain the largest volumes of calcium. It should be noted that calcium in dairy products is well digested, regardless of the presence of vitamin D [11, 12]. Collagen helps calcium to stay in the bones and, therefore, increases their elasticity and density [13, 14]. The widespread lack of calcium and collagen consumption with food products dictates the need to develop special complex preparations, dietary supplements and specialized products containing these components.

The world nutrition science recognizes cheese as a highly nutritious, biologically sound and easily digestible product [15-18]. It is an indispensable and obligatory component of the human diet. Curd cheeses make up a special group of cheeses. Biochemical processes occurring during the production of curd cheese result in the formation of peptides and amino acids, giving them the properties of dietary products [15, 18].

The traditional production of high-protein products (cottage cheese and cheese) consists in accumulating the protein-fatty part of milk and transforming these components into compounds, which determine the specific physico-chemical and organoleptic characteristics of the product [15, 19]. Milk components in cheese production are concentrated starting with the coagulation of milk by enzymatic, acidic, acid-enzymatic method or thermo-acid method of protein sedimentation [15]. Thus, destabilization of the colloidal phase of milk, and separation thereof from a part of the dispersed medium is an essential cheese production stage. In contrast to the traditional cheese production methods, the authors chose another approach, i.e. the concentration of skim milk components by thermocalcium coagulation, followed by protein fermentation with proteolytic-active cultures of Lactobacillus helveticus lactic bacteria [20, 21]. The curd cheese produced this way will have a high biological value due to whey proteins and easily digestible calcium in its composition; it will allow more efficient use of valuable components of raw materials; the process of cheese production minimizes labor force, energy costs and production space.

To enrich the curd cheese with collagen, it was decided to add modified collagen-containing raw materials to the calcium-containing concentrated protein mass. Category II byproducts, namely, the rumen rich in collagen proteins (6.8% of the mass of raw tissue) are of particular interest as collagen-containing raw materials (CCRM). Rumen biomodification with probiotic microorganisms will allow to improve the main functional and technological indicators of the collagen-containing raw material and to use it to enrich the curd cheese without deteriorating the organoleptic and other indicators [22-30].

The work is aimed at the development of a soft curd cheese technology based on calcium-containing protein concentrate with the addition of processed CCRM.

MATERIALS AND METHODS

The protein-collagen basis of the curd cheese was developed taking into account the results of the previous studies [20, 21].

Milk-protein concentrates (MPC) were used as a protein basis for soft curd cheese. The MPC was obtained by thermal calcium coagulation of skimmed cow milk, followed by fermentation of proteins with Lactobacillus helveticus H_{17-18} lactic bacteria. The coagulant was introduced into milk in the form of a 20% $CaCl_2$ solution in an amount of 1.5 g/l at of 95 °C. The protein clot was fermented under the following conditions: the dose of the lactic bacteria starter was equal to 5%, the temperature was (40 ± 2) °C, and the duration was 4 hours until reaching the acidity of (100 ± 2) °T. The calcium content in fermented MPC was 265 mg/100 g [20, 21].

The cattle rumen was used as a collagen-containing raw material (CCRM). The raw materials were accepted and prepared as per GOST 32244-13 “Processed meat byproducts. Technical conditions". In order to level the negative features of the structure, properties and composition of the rumen, the collagen-containing raw material was preliminarily subjected to heat treatment. The rumen was cleaned, washed, cut into pieces of 2.5x3 cm and cooked at 100 °C for 2-2.5 hours until softened (the ratio of raw materials to water was 1 : 2). After cooking, the rumen was

crushed on a mincer of 2-3 mm in diameter at the room temperature in order to preserve the fiber-forming ability of dispersed collagen. Bacterial concentrates based on Lactobacillus helveticus H_{17-18} were used as cultures for rumen fermentation. The rumen was fermented under the following conditions: the dose of the lactic bacteria fermentation was 5%, the temperature - (40 ± 2) °C, and the duration - 5 h.

The use of lactic bacteria of the L. helveticus H_{17-18} type for the fermentation of raw materials allows activating proteolytic processes, increases calcium, contributes to the formation of certain organoleptic properties, and also increases the nutritional and biological value of protein and collagen-containing raw materials [30, 31, 20].

Organoleptic and physico-chemical indicators of curd cheese were defined by the standard methods: organoleptic indicators - as per GOST R GOST R ISO 22935-2-2011; mass fraction of moisture - by drying the sample in a drying cabinet as per GOST 3626-73; the mass fraction of protein in the cheese was determined as per GOST R 54662-2011; the mass fraction of calcium was determined by the volumetric method using ammonium oxalate on a Solar M-6 atomic absorption spectrophotometer with a flame atomizer; active acidity was determined by the potentiometric method as per GOST 32892-2014; and titrated acidity was determined by titration as per GOST 3624-92.

The number of lactic bacteria cells was determined by limiting dilutions on a dense agar MRS medium as per TU 10-10-02-789-192-95. E. coli bacteria were determined as per GOST 32901; yeast and mold - as per GOST 10444.12, and GOST 33566.

All experiments were carried out in 3-5 replicates. The data obtained were processed using an Excel statistical software package using the Mann-Whitney test. Statistically significant differences at $p < 0.05$ were discussed.

Results And Discussion

At the first stage of the work, the protein-collagen base of cottage cheese was built, and its composition was experimentally substantiated.

According to literature data [22-29], when enriching foods with various rumen-based additives, special attention should be paid to sensory indicators, since the use of this type of collagen-containing raw material makes it difficult to obtain a product with good organoleptic and physiological properties. Since collagen is not only a high-protein product but also a product with a high degree of hydration that binds water and fat well, which is an excellent emulsifier and stabilizer, besides controlling sensory parameters, particular attention should be paid to the consistency, moisture content and protein in the enriched product [32].

In this regard, in the following series of experiments, the effect of a biomodified collagen-containing rumen on the main organoleptic, physico-chemical and microbiological indicators of cottage cheese based on calcium-containing milk protein concentrate was determined. CCRM were added to calcium-containing milk protein concentrate after self-pressing at a dosage of 5, 10 and 15%. The obtained samples were thoroughly mixed and shaped in the form of a bar and pressed. Curd cheese without collagen served as a control. The results of the assessment of the quality indicators of the studied samples are presented in Table 1.

Analysis of the data in Table 1 has shown that with an increase in the mass fraction of CCRM in the product, the organoleptic properties of the cheese changed, the protein content increased, and the moisture content in the product decreased. It has been noted that the curd cheese enriched with 5% CCRM had the highest organoleptic properties (dense delicate texture, pure sour-milk taste with a faint taste and flavor of CCRM) and acceptable physicochemical indicators (mass fraction of protein 19%, moisture - 72%). The increase in the amount of CCRM to 10-15% leads to compaction of the clot structure followed by an increase in its synergetic properties, as well as a significant deterioration in the product's organoleptic properties (the appearance of a pronounced taste and smell of CCRM). The content of viable lactic bacteria cells in all samples remained at the same level (10^8 CFU/cm^3).

Thus, based on the research, the optimal dose of applying a biomodified collagen-containing rumen in an amount of 5% of the volume of the cheese mass has been chosen, making it possible to produce curd cheese with the best consumer properties. The use of collagen containing raw materials in the production of curd cheese allows obtaining cheese dough with tight elastic structure without the use of expensive enzymes, emulsifiers and stabilizers.

At the next stage of the study, the shelf life of curd cheese enriched with calcium and collagen has been studied.

To determine the shelf life of the product, samples of the enriched curd cheese packaged in consumer packaging (polymer film of 100 g) were placed for storage in a refrigeration chamber at a temperature of (6 ± 2) °C. Samples were taken every 2 days.

Organoleptic characteristics, titratable acidity and microbiological parameters were determined in the studied samples (Table 2).

Table 1 – Indicators of curd cheese samples, depending on the dose of biomodified collagen-containing rumen introduction

Indicators	CCRM dose in samples, % of the protein mass			
	Control (without CCRM)	5	10	15
Consistency	Dense, soft, wet	Dense, tender with the particles of CCRM	Dense, serum sediment is observed	Excessively dense, rubbery, pricking. A significant serum sediment is observed
Taste and smell	Clean, sour milk, characteristic of soft cheeses without ripening	Clean, sour milk, characteristic of soft cheeses without ripening, with a weak taste and smell of CCRM	Clean, sour milk, characteristic of soft cheeses without ripening, with a subtle flavor and smell of CCRM	Clean, sour milk, characteristic of soft cheeses without ripening, with a strong flavor and smell of CCRM
Color	White with a cream shade, uniform throughout the mass	White with a cream shade, uniform throughout the mass	White with a cream shade, uniform throughout the mass	White with a cream shade, uniform throughout the mass
Mass fraction of protein, %	18±0.6	19±0.1	20±0.3	22±0.2
Moisture content, %	70±0.4	72±0.6	78±0.2	83±0.3
Syneresis, ml	1.5±0.05	1.0 ±0.03	0.5 ±0.04	0.4±0.05
The number of viable Lactobacillus helveticus H_{17-18}, cells, CFU/cm^3	$3 \cdot 10^8$	$1 \cdot 10^8$	$3 \cdot 10^8$	$5 \cdot 10^8$

Table 2 – Changes in the quality indicators of the enriched curd cheese during storage

Indicators		Shelf life, days										
		0	2	4	6	8	10	12	14	16	18	20
Organoleptic indicators		Dense, tender consistency with the particles of CCRM. Clean, sour-milk taste, with a subtle flavor and smell of CCRM. White color.								Dense consistency with the particles of CCRM. Sour-milk taste, with a subtle flavor and smell of CCRM and slight bitterness. White color.		
Titratable acidity, ^{0}T		105 ±0.5	106 ±0.2	109 ±0.1	114 ±0.7	117 ±0.6	120 ±0.5	122 ±0.2	124 ±0.2	126 ±0.1	127 ±0.5	130 ±0.1
Microbiological indicators	Number of L. helveticus H_{17-18} cells, CFU/g	$4•10^8$	$3•10^8$	$2•10^8$	$7•10^7$	$5•10^7$	$4•10^7$	$2•10^7$	$2•10^7$	$1•10^7$	$2•10^6$	$2•10^6$
	Coliforms per 0.1 g of product	not detected										
	Yeast, mold, CFU/g	not detected									D-7±1 P-3±2	D-13±2 P-5±1

Table 3 - Qualitative characteristics of curd cheese enriched with CCRM and calcium

Indicator Name	Indicator
Appearance	Sealed product. The presence of a small amount of serum on the product surface is allowed. Shape — low cylinder, or packaging form, or another arbitrary form.
Taste and smell	Clean, sour milk, characteristic of soft cheeses without ripening, with a barely noticeable aroma of CCRM
Consistency	Dense, tender with the particles of CCRM
Color	From white to light cream, uniform throughout the mass
Fat content in dry matter, %, below	5
Moisture content, %, below	75
Mass fraction of milk protein, %, above	6.0
Mass fraction of calcium, mg/100 g, above	200
Mass fraction of modified CCRM, g/100 g, below	5
Mass fraction of salt (sodium chloride), %, below	2.0
Titratable acidity, °T, below	110
Active acidity, pH units, above	4
The number of Lactobacillus helveticus H_{17-18}, cells, CFU/cm^3	above $1•10^6$
E. coli bacteria (coliforms)	Not allowed in 0.01 g
Pathogenic, including salmonella in 25 g	Not allowed
Staphylococcus S. aureus	Not allowed in 0.1 g
Yeast, mold, CFU/g, below	D-100, P-100
Listeria monocytogenes	Not allowed in 125 g (in five samples of 25 g each)

The results indicate that the organoleptic characteristics of the enriched curd cheese remain unchanged during the first 14 days of storage. However, a bitter taste was noted in the samples on the 16th day, which indicated proteolytic processes. Titrated acidity changed slightly during the shelf life under study.

Microbiological studies have shown that throughout the storage period, coliforms were absent in the product, yeast and mold were found in insignificant quantities only at the end of storage (however, their number corresponded to the regulated requirements for microbiological indicators of curd cheese [17]). As for lactic bacteria, it has been found that on the 18th day of storage there was a significant decrease in viable L. helveticus H_{17-18} cells (up to 106 CFU/g).

Thus, the recommended shelf life of the enriched curd cheese at a temperature of (6 ± 2) °C was 12-14 days. The short shelf life is explained by the "naturality" of the cheese and sets apart this product from other brands of curd cheese, the shelf life of which can reach up to 4 months through the use of preservatives, stabilizers or heat treatment.

The experimental data obtained allowed to develop a technology for the production of the curd cheese enriched with CCRM and calcium. A feature of the new type of the curd cheese is the use of calcium-containing milk protein concentrate and biomodified collagen-containing rumen in its production. The technology provides for the preparation of calcium-containing milk protein concentrate and bioprocessed cattle rumen followed by the combination of ingredients, their mixing, salting, shaping, pressing and packaging. The lack of cheese mass ripening will reduce the process of cheese production and speed up the turnover of invested funds.

The qualitative characteristics of the curd cheese are presented in Table 3.

The data in Table 3 show that the curd cheese has good organoleptic characteristics, contains a significant amount of calcium, biomodified collagen and viable lactic bacteria cells. The intake of 100-150 g of the obtained product, containing about 200 mg of calcium once per day, can satisfy the need for calcium by 20% of the daily scale of an adult. The collagen present in the product will contribute to better absorption of calcium, as well as calcium retention in bone tissue and, consequently, an increase in bone mineral density [13, 14].

The production technology of the new curd cheese provides for the implementation of two necessary conditions - the joint presence of digestible calcium and collagen. Fulfilment of these conditions allows obtaining an innovative functional product for the treatment and prevention of osteoporosis and associated diseases.

CONCLUSION

The developed curd cheese technology allows obtaining a product with high biological value and good organoleptic characteristics. The curd cheese produced as per the developed technology is of greater importance in the dietetic nutrition, since it contains a significant amount of medicinal calcium, probiotic microorganisms and protein, being at the same time a low-fat product. Enrichment of cottage cheese with biomodified collagen-containing raw material allows obtaining a therapeutic and prophylactic product to prevent osteoporosis and fractures for all age groups of the population.

The practical value of the research is in the introduction of the developed technology at the milk and meat processing enterprises that are interested in the sustainable use of secondary raw materials and the production of safe, environmentally friendly foods with therapeutic and preventive properties.

REFERENCES

1. Toportsova, N.V., Benevolenskaya, L.I., Osteoporoz: sovremennyy vzglyad na problemu [Osteoporosis: a modern view on the problem], *The attending physician* 2008, 04/08. Retrieved from https://www.lvrach.ru/2008/04/5001283/.
2. Cosman, F., de Beur, S., LeBoff, M., de Beur, S.J., Tanner, B., *Clinician's Guide to Prevention and Treatment of Osteoporosis*, National Osteoporosis Foundation, Washington, 2014.
3. Kanis, J., Bianchi, G., Bilezikian, J., Towards a diagnostic and therapeutic consensus in male osteoporosis, *Osteoporosis International* 2011, 22 (11), 2789-2798.
4. Adler, R., El-Hajj Fuleihan, G., Bauer, D., Managing Osteoporosis in Patients on Long-Term Bisphosphonate Treatment: Report of a Task Force of the American Society for Bone and Mineral Research, *J Bone Miner Res.* 2016, 31(1), 16-35.
5. Lutsenko, A.S., Rozhinskaya, L.Ya., Toroptsova, N.V., Belaya, Zh.E., Rol' i mesto preparatov kal'tsiya i vitamina D dlya profilaktiki i lecheniya osteoporoza [The role and place of calcium and vitamin D preparations for the prevention and treatment of osteoporosis], *Osteoporosis and osteopathy* 2017, 20(2), 69-75.
6. Dawson-Hughes, B., The role of calcium in bone growth and preservation, in *World Congress on Osteoporosis, Osteoarthritis and usculoskeletal Diseases (WCO-IOF-ESCEO 2017): Non-sponsored Symposia Abstracts*, 2017.
7. Carmeliet, G., Dermauw, V., Bouillon, R., Vitamin D signaling in calcium and bone homeostasis: A delicate balance, *Best Practice & Research Clinical Endocrinology & Metabolism.* 2015, 29(4), 621-631.
8. de Assumpção, D., Dias, M.R.M.G., de Azevedo Barros, M.B., Calcium intake by adolescents: a population-based health survey, *J Pediatr (Rio J)* 2016, 92(3), 251-259.
9. Wilczynski, C., Camacho, P., Calcium Use in the Management of Osteoporosis: continuing Questions and Controversies, *Current Osteoporosis Reports* 2014, 12(4), 396-402.
10. Balk, E., Adam, G., Langberg, V., Earley, A., Evidence to support a global map of dietary calcium intake by country and subgroup, in *World Congress on Osteoporosis, Osteoarthritis and usculoskeletal Diseases (WCO-IOF-ESCEO 2017): Non-sponsored Symposia Abstracts*, 2017.
11. Taylor, E.N., Curhan, G.C., Dietary Calcium from Dairy and Nondairy Sources, and Risk of Symptomatic Kidney Stones, *The Journal of Urology* 2013, 190(4), 1255-1259.
12. Bostick, R.M., Kushi, L.H., Wu, Y., Meyer, K.A., Sellers, T.A., Folsom, A.R., Relation of calcium, vitamin D, and dairy food intake to ischemic heart disease mortality among postmenopausal women, *American journal of epidemiology* 1999, 149(2), 151-161.
13. Glukhov, Yu.V., Roli kollagensoderzhashchego pitaniya v profilaktike i lechenii osteoporoza [The role of collagen nutrition in the prevention and treatment of osteoporosis], *Ploklinika* 2010, 2, 122-123.
14. Karmin, A.S., Gromova, O.A., Torshin, I.Yu., Kaltsiy i biosintez kollagena: sistematicheskiy analiz molekulyarnykh mekhanizmov vozdeystviya [Calcium and collagen biosynthesis: a systematic analysis of molecular mechanisms of action], *Russkiy meditsinskiy zhurnal* 2016, 15, 1009-1017.
15. Gostishcheva, E.A., Shramko, M.I., Evdokimov, I.A., Innovatsionnyye tekhnologii tvorozhnykh syrov s primeneniyem mikro- i ultrafiltratsii [Innovative technologies of curd cheese using micro- and ultrafiltration], in *Modern achievements of biotechnology: materials of the international scientific and practical conference*, SKFU, Minsk, 2014, pp. 96-98.
16. Ostroumov, L.A., Smirnova, I.A., Zakharova, L.M., Osobennosti i perspektivy proizvodstva myagkikh syrov [Features and prospects for the production of soft cheeses], *Tekhnika i tekhnologiya pishchevykh proizvodstv* 2015, 4(39), 80-86.
17. *GOST 33480-2015 Curd cheese. General technical conditions*.
18. Suyunchev, O.A., Rudakov, A.S., Slonevskaya, E.A., Ispolzovaniye molochno-belkovykh kontsentratov v kachestve syrya dlya myagkikh syrov [The use of milk protein concentrates as a raw material for soft cheeses], *Pererabotka moloka: tekhnologiya, oborudovaniye, produktsiya* 2006, 3, 19.
19. Gudkov, A.V., *Syrodeliye: tekhnologicheskiye, biologicheskiye i fiziko-khimicheskiye aspekty* [Cheese making: technological, biological and physico-chemical aspects], DeLi print, Moscow, 2007.
20. Shchekotova, A.V., Zambalova, N.A., Boyarintseva, I.V., Maradudina, I.P., Khamagaeva, I.S., The study of the proteolysis of milk proteins obtained by thermal calcium coagulation, *Journal of Pharmaceutical Sciences and Research* 2017, 9(12), 2599-2603.
21. Khamagayeva, I.S., Zherebyatyeva, I.S., Skhekotova, O.A., Shetinina, E.M., Molochno-belkovyy kontsentrat, obogashchennyy kal'tsiyem [Milk protein concentrate, enriched with calcium], *Polzunovskiy vestnik* 2017, 1, 24-29.
22. Khairullin, M.F., O potrebitel'skikh predpochteniyakh pri vybore myasnykh produktov [On consumer preferences when choosing meat products], *Myasnaya industriya* 2011, 12, 15-17.
23. Hashim, P., Mohd Ridzwan, M.S., Bakar, J., Mat Hashim, D., Collagen in food and beverage industries, *International Food Research Journal* 2015, 22(1), 1-8.
24. Neklyudov, A.D., Ivankin, A.N., *Collagen: production, properties and application*, GOY VPO MGUL, Moscow, 2007.
25. Zinina, O.V., *Biotekhnologicheskaya obrabotka myasnogo syr'ya: monografiya* [Biotechnological processing of raw meat: monograph], Novgorod Technopark, Velikiy Novgorod, 2013.
26. Solovyeva, A.A., Zinina, O.V., Rebezov, M.L., Aktualnyye biotekhnologicheskiye resheniya v myasnoy promyshlennosti [Current biotechnological solutions in the meat industry], *Molodoy uchenyy* 2013, 5, 102-105.
27. Rebezov, M.B., Lukin, A.A., Khairullin, M.F., Izmeneniye soyedinitel'noy tkani pod vozdeystviyem fermentnogo preparata i startovykh kul'tur [Changes in the connective tissue under the influence of the enzyme preparation and starter cultures], *Vestnik myasnogo skotovodstva* 2011, 3(64), 78-83.
28. Bolgova, N.V., Perspektivnyye vidy syr'ya pri proizvodstve myasnykh produktov [Perspective types of raw materials in the production of meat products], in *Actual problems of intensive development of livestock: a collection of scientific papers*, BGSKhA, Gorki, 2015, pp. 14-17.
29. Rebezov, M.B., Lukin, A.A., Sravnitel'naya otsenka vozdeystviya fermentnykh preparatov razlichnogo proiskhozhdeniya na kollagensoderzhashcheye syr'ye [Comparative assessment of the impact of enzyme preparations of different origin on collagen-containing raw materials], *Technology and Commodity Science of Innovative Food* 2011, 5, 28-36.
30. Griffiths, M.W., Tellez, A.M., Lactobacillus helveticus: the proteolytic system, *Frontiers in Microbiology* 2013, 4 (30), 124-134.
31. Slattery, L., Invited review Lactobacillus helveticus, *Journal of Dairy Science* 2010, 93(10), 4449-4453.
32. Vorobyev, V.I., Ispolzovaniye rybnogo kollagena i produktov ego gidroliza [The use of fish collagen and its hydrolysis products, *Proceedings of the Kaliningrad State Technical University* 2008, 13, 55-58.