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TECHNOLOGICAL ASPECTS OF PRODUCING PASTEURIZED MILK WITH A BIOACTIVE MINERAL-PEPTIDE COMPLEX

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Abstract.

Calcium and vitamin D deficiencies in Ukraine and many European countries are associated with impaired musculoskeletal health. Pasteurized milk is a suitable basis for enrichment with bioactive components; however, the combination of Ca with vitamin D_3 and dairy peptides requires validated technological solutions that preserve product stability and sensory quality over 6 days. To develop and substantiate a technology for pasteurized milk enriched with calcium citrate, vitamin D_3, and casein phosphopeptides (CPP), ensuring homogeneity, colloidal stability, and acceptable sensory properties. Materials and Methods. Base milk: 2.5% fat. Variants: (1) calcium citrate 0.15% + vitamin D_3 2.5 μg/100 g; (2) same + CPP 0.05%. Measured parameters: pH, titratable acidity, viscosity, soluble Ca fraction, sediment after centrifugation, vitamin D_3 retention (6 days, 4 ± 1 °C), and sensory score (5-point). Pasteurization: 88–90 °C/50–60 s; homogenization: 15 MPa. At the technologically justified concentration of 0.15% calcium citrate, the physicochemical parameters of the samples remained within acceptable technological limits; in the finished product, pH was 6.61 and titratable acidity was 19 °T. In the final samples, sediment did not exceed 0.5%. With 0.05% CPP (at 0.15% calcium citrate), the soluble Ca fraction increased from 31.7% (without CPP) to 34.2% (with CPP), while sediment decreased (0.26% vs 0.32%). Pasteurization did not change total Ca (≤1%) but reduced soluble Ca by 5–6%; in CPP-fortified milk the absolute level of soluble Ca after pasteurization remained higher than in the Ca+D_3 variant. At 2.5 μg/100 g, vitamin D_3 retention at day 6 was 86% in the Ca+D_3 sample and 89% in the Ca+D_3+CPP sample in light-protected packaging; CPP reduced vitamin losses by about 3–4% over storage. Sensory scores remained high: 4.7 (Ca+D_3) and 4.8 (Ca+D_3+CPP) vs 4.9 in control. The viscosity of the finished product was 1.98 mPa·s and remained close to this level up to day 6 of storage. The combination of 0.15% calcium citrate, 2.5 μg/100 g vitamin D_3, and 0.05% CPP made it possible to obtain stable, homogeneous pasteurized milk with high sensory scores, while increasing the soluble Ca fraction and ensuring adequate D_3 retention over 6 days. Technologically, CPP addition after pasteurization at 35–40 °C is advisable under hygienic conditions. Vitamin D_3 may be added after pasteurization as a pre-dispersed preparation or before homogenization, taking into account possible thermal losses.

Keywords: pasteurized milk; calcium citrate; vitamin D_3; casein phosphopeptides; calcium solubility; colloidal stability; sensory quality.

Introduction. Formulation of the problem

Calcium and vitamin D deficiency remains a relevant nutritional problem in Ukraine and in many European countries. Insufficient consumption of dairy products, limited sun exposure during the winter–spring period, characteristics of modern diets, and age-related changes in calcium metabolism increase the risk of osteoporosis, impaired bone health, muscle weakness, and reduced immune function.

Pasteurized milk is a commonly consumed product and a suitable basis for enrichment with bioactive components. Unlike ultra-pasteurized or sterilized products, it is subjected to milder heat treatment, which contributes to better preservation of the natural structure of the protein–mineral complex. At the same time, the natural levels of calcium and vitamin D in such milk are insufficient to meet the daily requirements of most consumers, which substantiates the feasibility of its enrichment.

A promising approach is the use of a bioactive mineral–peptide complex comprising calcium citrate, vitamin D_3, and casein phosphopeptides (CPP). CPP may contribute to maintaining calcium in a soluble or colloidally available form and stabilizing the calcium–casein equilibrium, thereby reducing the risk of coagulation and sediment formation in the protein–mineral system. Such a combination may potentially enhance calcium solubility without impairing the sensory and rheological properties of the product.

At the same time, the addition of mineral salts and peptides may affect pH, viscosity, sediment formation, vitamin D_3 stability, and microbiological indicators during storage. This necessitates the establishment of optimal dosages and stages of component addition that would ensure a balance between nutritional value and technological stability of the product.

Therefore, there is a need to analyze current scientific data on the properties of calcium salts, vitamin D_3, and casein phosphopeptides, as well as to assess the possibility of their rational combination specifically in pasteurized milk with a short shelf life, for which the available scientific data remain limited.

Analysis of recent research and publications

Calcium and vitamin D are among the most important micronutrients for human health. They affect not only the condition of bones and muscles, but also the functioning of the immune, nervous, and cardiovascular systems [1–5]. According to numerous review papers, in many European countries the intake of calcium, vitamin D, and magnesium is lower than that recommended by scientific and medical organizations [1, 6, 7]. Milk is traditionally regarded as a major source of calcium; however, its natural content is not always sufficient to meet the daily requirement, which substantiates the feasibility of its enrichment [1–3].

The form of calcium added to a product significantly affects its solubility and behavior in a milk system. According to the literature, calcium citrate is considered a promising form for the enrichment of foods and, in particular, certain dairy systems, due to its technologically acceptable solubility and milder effect on colloidal equilibrium compared with some other calcium salts [2, 8]. However, an excessively high concentration of Ca^{2+} ions may disturb the natural protein–mineral balance: casein micelles may aggregate, viscosity may increase, and the risk of sediment formation may arise, especially during heating [2, 8, 11]. Therefore, it is important to select both the appropriate calcium form and the stage at which it is added to milk, i.e., before or after homogenization and pasteurization.

Vitamin D_3 plays a key role in the regulation of calcium metabolism, as it activates proteins responsible for Ca^{2+} transport in the intestine and participates in the formation and renewal of bone tissue [2, 3, 5]. It has been demonstrated that milk enrichment with vitamin D_3 is an effective approach to increasing its nutritional value and may contribute to improving the vitamin D status of consumers [1, 2]. However, vitamin D_3 is sensitive to light, oxygen, elevated temperature, and storage conditions [2, 3, 11]. The stability of vitamin D_3 in dairy structures is also affected by product composition and packaging conditions. For example, Jafari et al. reported changes in vitamin D_3 content during storage of fortified yoghurt and yoghurt drink, confirming the need to control processing and packaging conditions for vitamin-D-fortified dairy products [12]. Studies have shown that LED lighting may be even more aggressive than fluorescent lighting in promoting light-induced changes in milk [3, 11, 13]. This explains why the use of light-impermeable packaging, antioxidants, or vitamin microencapsulation technology is recommended for fortified dairy products [3, 11, 13].

One of the most promising approaches to improving calcium absorption is the addition of milk-derived peptides, particularly casein phosphopeptides (CPP). They contain phosphoserine groups capable of binding Ca^{2+} ions and maintaining them in solution, thereby preventing the formation of insoluble compounds in the gastrointestinal tract [9, 10, 15–16]. In vitro and clinical studies indicate that casein phosphopeptides can increase calcium availability and improve its absorption [15, 16]. CPP are relatively heat-stable compared with other peptides, which makes them promising for use in pasteurized products [9, 17]. In addition, they possess antioxidant and antihypertensive properties, which allows them to be considered as an additional functional component in dairy products [9, 18].

The combination of calcium, vitamin D_3, and CPP may be regarded as a complementary technological and nutritional approach: vitamin D_3 is involved in the regulation of calcium metabolism; CPP retain Ca^{2+} ions in solution and reduce the risk of sediment formation; and calcium in a soluble or technologically available form contributes to increasing the mineral value of the product [2, 15, 16]. At the same time, the stability of peptides and vitamin D_3 in pasteurized milk may be lower than in dry or UHT products due to the presence of oxygen, residual enzymatic activity, and storage conditions [2, 3, 11, 14, 17]. Therefore, the stage of peptide and vitamin addition is critical, and their incorporation after pasteurization or during cooling is generally considered preferable in order to minimize the

loss of activity while maintaining acceptable sensory and rheological properties of the product [2, 11, 14].

Most of the available studies have been carried out using model solutions or ultra-pasteurized milk, whereas there are considerably fewer studies devoted specifically to fresh pasteurized milk with a short shelf life [2, 3, 11, 17, 20]. In such products, the main challenges remain the stability of the colloidal system, taste, color, and light stability of active substances. Therefore, there is currently a need to study the optimal forms, dosages, and technological conditions for the addition of calcium, vitamin D_3, and CPP specifically to pasteurized milk.

The literature also emphasizes the role of magnesium in the activation of vitamin D and the regulation of calcium metabolism [6, 7]. Magnesium deficiency may reduce the effectiveness of vitamin D even when its intake is sufficient [5–7]. However, in liquid dairy products the addition of magnesium may be complicated by undesirable sensory effects, including bitterness and metallic taste notes; therefore, this direction is still considered promising but experimental [6, 7, 21].

Certain publications describe the combination of calcium, vitamin D_3, and casein phosphopeptides in cow's milk; however, such studies are mainly focused on biological effects and osteoporosis models rather than on production technology. Detailed pasteurization parameters, the behavior of the protein–mineral system, the stability of active substances, and sensory characteristics under real production conditions are virtually not represented. Therefore, the combination of calcium citrate, vitamin D_3, and CPP in fresh pasteurized milk with a short shelf life remains insufficiently covered from the technological point of view.

Thus, the compatibility of calcium citrate, vitamin D_3, and CPP in pasteurized milk, as well as the effect of this combination on stability, sediment formation, rheological properties, and storage, remains insufficiently studied and requires targeted research.

Study Aim and Objectives

The aim of the study was to experimentally substantiate technological solutions for the production of pasteurized milk enriched with a bioactive mineral–peptide complex based on calcium, vitamin D_3, and casein phosphopeptides, which promotes increased calcium solubility and preservation of the physicochemical and sensory properties of the product.

To achieve this aim, the following objectives were defined:

1. To investigate the effect of calcium citrate concentration on the colloidal stability of pasteurized milk.
2. To evaluate the stability (retention) of vitamin D_3 in the protein–mineral system of pasteurized milk.
3. To analyze the role of casein phosphopeptides in the stabilization of mineral complexes and the possibility of their use as a natural component that increases calcium solubility and stability.
4. To determine the technological sequence of Ca, D_3, and CPP addition operations in order to ensure uniform distribution and stability during storage.
5. To experimentally verify the effect of the bioactive complex on the quality parameters of pasteurized milk (pH, viscosity, colloidal stability, and sensory properties).
6. To develop a technological flowchart for the production of the developed product.

Research materials and methods

The study was conducted under laboratory conditions at the Department of Technology of Milk, Oil and Fat Products, and Beauty Industry of Odesa National University of Technology.

Cow's milk standardized to 2.5% fat was used as the main raw material. The following fortifying ingredients were applied:

— calcium citrate, $Ca_3(C_6H_5O_7)_2$ (Khimfarm LLC, Ukraine);

— vitamin D_3 in the form of a food-grade oil solution, NOW Foods Liquid Vitamin D_3 (USA; 10 μg in 4 drops);

— casein phosphopeptides (CPP), powdered concentrate CPP-95 (Shanghai Piochem Biotech Co., Ltd, China).

Preparation of samples

Three groups of samples were studied:

— control (without fortification);

— milk with 0.15% calcium citrate and vitamin D_3 at 2.5 μg/100 g;

— milk with 0.15% calcium citrate, vitamin D_3 at 2.5 μg/100 g, and 0.05% CPP.

Calcium citrate was added before homogenization. Homogenization was carried out at a pressure of 15 MPa, and pasteurization was performed at 88–90 °C for 50–60 s. After cooling to 35–40 °C, vitamin D_3 and pre-hydrated CPP (1:10) were added, followed by mixing for 5 min. The product was then cooled to 4 ± 1 °C, packaged in light-impermeable containers, and stored for 8 days. In addition, the effect of calcium citrate concentration, CPP concentration, and the stage of component addition on milk stability parameters was evaluated, including the case of CPP addition before pasteurization.

Methods of analysis

pH and titratable acidity were determined by potentiometric and titrimetric methods, respectively (DSTU 8550:2015).

Total and soluble calcium were determined by the photometric method using Arsenazo III reagent (λ = 650–660 nm):

— total calcium, after sample mineralization;

— soluble calcium, in the supernatant after centrifugation (3000 rpm, 10 min).

Vitamin D_3 content was assessed spectrophotometrically after extraction of the fat-soluble fraction into isooctane. Quantitative evaluation was performed using a calibration curve constructed with a standard vitamin D_3 solution under identical sample preparation and measurement conditions. The method was used for the comparative assessment of vitamin D_3 retention in the experimental samples after processing and during storage. For each sample, no fewer than three parallel determinations were performed, and the results were expressed as the mean value in μg/100 g of product.

The content of added casein phosphopeptides (CPP) was assessed spectrophotometrically at $\lambda = 215$ nm using a calibration curve constructed with a standard CPP solution. The analysis was performed in parallel replicates under identical sample preparation conditions. The method was applied for the comparative assessment of changes in the level of added CPP before and after heat treatment. The results were expressed as the mean value of repeated measurements in mg/100 g of product.

Viscosity was determined using a rotational viscometer at 20 °C.

Sediment formation was assessed by the mass of sediment after centrifugation (3000 rpm, 10 min).

Sensory analysis was conducted in accordance with DSTU ISO 6658:2005 by a tasting panel consisting of 5 specialists.

Microbiological indicators, including total viable count (TVC) and coliform bacteria, were determined according to DSTU 7357:2013.

All analyses were carried out in at least three replicates. Identical sample preparation and measurement conditions were used for all analytical determinations. The result was taken as the mean value of parallel determinations. Reproducibility was evaluated based on the consistency of the obtained results.

Results of the research and their discussion

Among the various calcium salts considered for the enrichment of pasteurized milk, calcium citrate was selected. According to the literature, it is characterized by technologically acceptable solubility, stability of the casein system, a low risk of coagulation during heat treatment, and the absence of a pronounced negative effect on taste. Unlike other calcium salts, particularly carbonate, chloride, and gluconate, citrate does not cause a sharp decrease in pH, is better compatible with the protein–mineral system of milk, and may contribute to maintaining the proportion of soluble calcium during storage [2, 19]. This makes it possible to regard calcium citrate as a technologically appropriate form of calcium for dairy systems, which was also confirmed by the results of our own studies (Table 1).

Scientific justification of the selected concentrations

Several experimental samples were prepared to evaluate the effect of the bioactive mineral–peptide complex on the properties of pasteurized milk. The control sample consisted of standardized milk (2.5% fat) without the addition of calcium, CPP, or vitamin D_3.

The following samples were studied:

• samples with different concentrations of calcium citrate: 0, 0.10, 0.15, and 0.20%;

• samples with the addition of CPP to calcium-enriched milk: 0, 0.05, and 0.10%;

• samples containing the combinations Ca + D_3 and Ca + D_3 + CPP;

• comparison of two CPP addition schemes: before pasteurization and after pasteurization (at 35–40 °C).

The ranges of calcium citrate (0.10–0.20%) and CPP (0.05–0.10%) were selected on the basis of literature data, within which pronounced sediment formation, substantial viscosity increase, and undesirable sensory effects are not expected [2, 10, 15, 19].

Effect of calcium citrate concentration on the properties of milk before pasteurization. The results presented in Table 1 show that calcium citrate at doses up to 0.15% does not cause substantial sediment formation (0.2% according to centrifugation data, with no visible sediment), while pH and titratable acidity remain within the technological norm.

Calcium citrate concentrations of 0.10–0.15% are technologically acceptable in terms of colloidal stability. A calcium citrate concentration of 0.20% marks the beginning of exceeding the stability limit (1% sediment). Therefore, the concentration of 0.15% was selected as technologically appropriate for further studies.

Effect of CPP on viscosity and calcium solubility before pasteurization. According to the data presented in Table 2, CPP increase the proportion of soluble calcium from 31.7 to 34.2% at a dose of 0.05%, whereas a further increase in the dose to 0.10% raises this value to 35.6%, but is accompanied by a decrease in sensory score and a slight increase in viscosity.

Table 1 – Effect of calcium citrate concentration on the physicochemical and sensory properties of milk before pasteurization

Calcium citrate concentration, %	pH	Titratable acidity, °T	Sediment after centrifugation, %	Sensory score (5-point scale)
0 (control)	6.68	17	0.0	4.9
0.10	6.66	17	0.0	4.8
0.15	6.64	17	0.2	4.9
0.20	6.62	18	1.0	4.6

Table 2 – Effect of casein phosphopeptides on calcium solubility and milk viscosity before pasteurization (at 0.15% calcium citrate)

CPP, %	Soluble Ca^{2+}, % of total	Viscosity, mPa·s	Sensory score
0.00	31.7	1.84	4.9
0.05	34.2	1.88	4.8
0.10	35.6	1.92	4.6

Thus, a CPP concentration of 0.05% was considered technologically appropriate, as it promotes an increase in the proportion of soluble calcium without negatively affecting the structure and taste of milk.

Effect of pasteurization on calcium, CPP, and colloidal stability. In the present study, an elevated pasteurization regime of 88–90 °C / 50–60 s was selected as the baseline for the enriched product, in which it is important to ensure appropriate microbiological safety and stability of the quality parameters during storage. The investigation of alternative regimes (for example, 76–78 °C / 15–20 s or 80–85 °C / 20–30 s) in terms of their effect on colloidal stability and vitamin D_3 retention is a promising direction for further research, but falls outside the scope of the present study.

The effect of pasteurization on calcium-binding components and the stability parameters of milk samples is presented in Table 3.

Pasteurization had virtually no effect on total calcium content, but was accompanied by a certain decrease in its soluble fraction. The addition of CPP contributed to maintaining calcium in the dissolved state, reducing sediment formation, and was not accompanied by significant changes in viscosity.

To assess the effect of heat treatment on calcium-binding components, samples in which CPP and vitamin D_3 were added before pasteurization were additionally studied. At a CPP concentration of 0.05%, their content after pasteurization decreased by approximately 6%, which is likely associated not with peptide degradation, but with a partial transition into a form less accessible for analysis in the protein–mineral system.

Taken together, the obtained data indicate that the addition of CPP after pasteurization at 35–40 °C is technologically more appropriate, since it ensures minimal analytical losses of peptides and contributes to the stability of the protein–mineral system during storage.

Vitamin D_3 stability during storage. The study of vitamin D_3 stability during storage showed a gradual decrease in its content, which may be associated with storage conditions and the characteristics of the protein–mineral component of the product. At the initial dose of 2.5 μg/100 g, 86% of vitamin D_3 was retained in the Ca + D_3 sample and 89% in the Ca + D_3 + CPP sample on day 6. On day 8, the vitamin content decreased to 81 and 84%, respectively, indicating further degradation of D_3 and the approach of the system to a critical state in terms of the target vitamin content.

Table 3 – Effect of pasteurization on calcium-binding components and stability parameters of experimental milk samples

Sample	Parameter	Before pasteurization	After pasteurization	Recovery, %
Control	Total calcium, mg/100 g	120	119	—
	Soluble calcium, mg/100 g	38.0	36.0	—
	Soluble Ca^{2+}, % of total	31.7	30.3	—
	Viscosity, mPa·s (20 °C)	1.80	1.82	—
	Sediment after centrifugation (3000 rpm, 10 min), %	0.00	0.12	—
Milk + 0.15% calcium citrate + vitamin D_3	Total calcium, mg/100 g	155	154	—
	Soluble calcium, mg/100 g	50.0	47.0	—
	Soluble Ca^{2+}, % of total	32.3	30.5	—
	Viscosity, mPa·s (20 °C)	1.88	1.92	—
	Sediment after centrifugation (3000 rpm, 10 min), %	0.20	0.32	—
Milk + 0.15% calcium citrate + vitamin D_3 + 0.05% casein phosphopeptides	Total calcium, mg/100 g	155	154	—
	Soluble calcium, mg/100 g	53.0	50.0	—
	Soluble Ca^{2+}, % of total	34.2	32.5	—
	CPP, mg/100 g	50.0	47.0	94.0
	Viscosity, mPa·s (20 °C)	1.93	1.98	—
	Sediment after centrifugation (3000 rpm, 10 min), %	0.18	0.26	—

Note. The CPP values were obtained from a separate series of experiments in which CPP and vitamin D_3 were added before pasteurization in order to evaluate the effect of heat treatment.

The addition of CPP made it possible to reduce D_3 losses by an average of 3–4% throughout the entire observation period, as evidenced by the consistently higher retention values in the Ca + D_3 + CPP variant.

The obtained results (Table 4) indicate that, under storage conditions of 4 ± 1 °C, the vitamin D_3 content in enriched pasteurized milk remains at no less than 85% throughout the recommended storage period of 6 days. Up to this time point, pH, acidity, viscosity, and sediment formation also remain within technologically acceptable limits and sensory acceptability.

The eighth day is considered a control point at which a further decrease in vitamin D_3 content (to 81–84%) and an increase in the rate of its loss are observed, which limits the recommended shelf life of the product to 6 days.

Table 4 – Retention of vitamin D_3 during storage of the finished enriched milk at 4 ± 1 °C, % of the initial level

Storage time, days	Ca + D_3	Ca + D_3 + CPP
0	100	100
2	93	96
4	89	93
6	86	89
8	81	84

Quality characteristics of the finished product.

The finished enriched product (day 0 after production) was characterized by a homogeneous consistency without visible sediment, a clean milky taste and odor, pH of 6.61, titratable acidity of 19 °T, viscosity of 1.98 mPa·s, sediment after centrifugation of 0.26%, and a sensory score of 4.8 points. The obtained parameters indicate high colloidal stability of the product and the absence of a negative effect of the bioactive complex on its organoleptic properties.

Microbiological parameters during storage. As shown by the results in Table 5, TVC increased within the range characteristic of pasteurized milk, while coliform bacteria were not detected throughout the entire period; on day 8, TVC increased substantially compared with day 6, which further supported the choice of a 6-day recommended shelf life.

Table 5 – Microbiological parameters during storage of finished pasteurized milk (0.15% calcium citrate + D_3 2.5 μg/100 g + CPP 0.05%) at 4 ± 1 °C

Day	TVC, CFU/cm³	Coliform bacteria, in 0.1 cm³
1	1.0×10^3	not detected
3	1.0×10^4	not detected
6	3.0×10^4	not detected
8	9.0×10^4	not detected

Product condition at the end of the storage period. According to Table 6, pH decreased to 6.59; viscosity and sediment increased slightly; and vitamin D_3 content decreased to 81–84% (81% for the Ca + D_3 variant and 84% for the Ca + D_3 + CPP variant).

These changes confirm that the optimal storage period is 6 days.

Table 6 – Changes in the physicochemical and organoleptic parameters of enriched pasteurized milk at the end of storage (6 and 8 days)

Parameter	Day 6	Day 8
pH	6.60	6.59
Titratable acidity, °T	20.0	21.0
Soluble Ca^{2+}, % of total	32.5	32.0
Viscosity (20 °C), mPa·s	1.99	2.14
Vitamin D_3 retention, %	86–89	81–84
Sediment after centrifugation, %	0.28	0.30
Sensory score, points	4.80	4.70

Thus, the application of the bioactive complex consisting of 0.15% calcium citrate, D_3 at 2.5 μg/100 g, and CPP at 0.05%, in combination with the optimal addition scheme (CPP after pasteurization), promotes an increase in the proportion of soluble calcium, a reduction in sediment formation, and stabilization of the protein–mineral system, while also ensuring adequate vitamin D_3 retention and acceptable rheological, organoleptic, and microbiological characteristics of the product during 6 days of storage.

The technological flowchart for the production of the finished product includes the following main stages (Fig. 1):

Milk reception and preparation. Raw cow's milk is delivered to the processing facility, where organoleptic evaluation, determination of acidity, density, and fat content, as well as sampling for microbiological control, are carried out. Admission to further processing is allowed provided that the milk complies with the requirements of the current DSTU standards. The milk is purified using milk clarifier separators to remove mechanical impurities and part of the somatic cells, which increases microbiological stability and reduces the risk of sediment formation at subsequent stages.

Standardization of fat content to 2.5%. Milk is standardized by mixing whole milk and skimmed milk until a fat content of 2.5% is achieved. The selected fat level meets the requirements for pasteurized drinking milk and makes it possible to combine a standard consumer profile of the product with functional enrichment with calcium and vitamin D_3, without overloading the system with an excessive fat phase.

Addition of calcium citrate. Calcium citrate was introduced into standardized milk as a dry powder under intensive stirring or as a pre-dispersed aqueous suspension, ensuring a final calcium citrate concentration of 0.15%. The use of calcium citrate is justified by its technologically acceptable solubility, its

ability to maintain the optimal calcium–casein equilibrium, and its minimal effect on pH. The addition of calcium before homogenization ensures a uniform distribution of Ca^{2+} ions in the protein–fat system of milk.

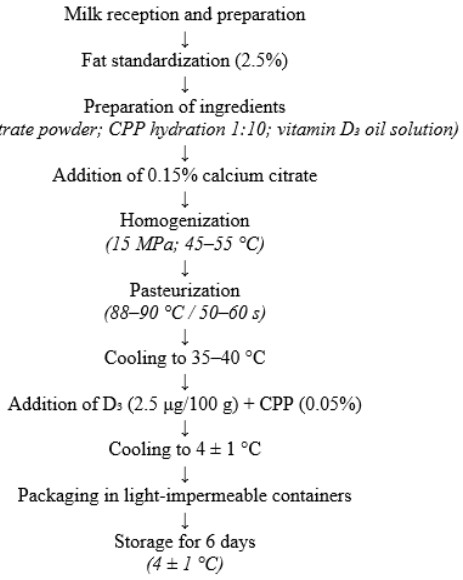

Fig. 1. Technological flowchart for the production of the finished product.

Heating to the homogenization temperature (45–55 °C). Before homogenization, milk with added calcium citrate is heated to 45–55 °C. This temperature range represents a compromise: at these temperatures, the fat acquires sufficient plasticity for effective disruption of fat globules in the homogenizer valve, while excessive heating of the calcium-enriched casein system is avoided, since at 60–65 °C it could intensify protein aggregation and increase the risk of coagulation even before pasteurization. Thus, the homogenization temperature, lower than that used in standard technology, was specifically selected with account for the presence of calcium citrate in the system.

Homogenization at a pressure of 15 MPa. Homogenization is carried out in a single-stage homogenizer at a pressure of 15 MPa and a temperature of 45–55 °C. The purpose of this operation is to reduce the size of fat globules, improve product homogeneity, and prevent separation of the fat phase during storage. For milk with a relatively low fat content (2.5%), this pressure is sufficient to ensure a stable emulsion without excessive mechanical impact on the calcium-sensitive protein phase.

Pasteurization (88–90 °C / 50–60 s). After homogenization, the milk is directed to a plate pasteurization-cooling unit, where pasteurization is carried out at 88–90 °C for 50–60 s. This elevated pasteurization regime was selected taking into account the need to ensure the appropriate microbiological condition of the enriched product and the stability of its parameters within the recommended storage period of 6 days.

During pasteurization, care is taken to prevent protein coagulation and the formation of visible sediment; the experimental data obtained (Table 3) confirm the preservation of colloidal stability under the selected regime.

Cooling to 35–40 °C and addition of CPP and vitamin D_3. Immediately after pasteurization, the milk is partially cooled in the same pasteurization-cooling unit to 35–40 °C. This temperature range is specifically selected because it minimizes thermal degradation of vitamin D_3, preserves the structural integrity of casein phosphopeptides, and keeps the system sufficiently mobile from a technological point of view (i.e., with low enough viscosity for effective mixing).

Into the milk stream at 35–40 °C, the following components are added: vitamin D_3 in the form of an oil solution in an amount ensuring 2.5 µg/100 g of product, and pre-hydrated casein phosphopeptides (1:10 suspension) added to reach a CPP content of 0.05%.

The components are introduced gradually into the milk stream under intensive mixing for at least 5 min, which ensures uniform distribution of the pre-hydrated CPP suspension and the vitamin D_3 oil solution and reduces the risk of locally supersaturated zones and subsequent sedimentation.

Final cooling to 4 ± 1 °C. After the addition of vitamin D_3 and CPP, the product is further cooled to 4 ± 1 °C. This temperature corresponds to the standard storage conditions for pasteurized milk, slows down oxidative processes, vitamin D_3 degradation, and changes in the protein–mineral equilibrium, and ensures the preservation of the structural and sensory properties of the product throughout the entire storage period.

Packaging in light-impermeable containers. The cooled enriched milk is supplied to filling and sealing equipment, where it is packaged into light-impermeable polymer containers. The use of opaque packaging is important for two reasons: it protects vitamin D_3 from photodegradation and reduces the risk of oxidation of the fat phase under light exposure.

Packaging is carried out in as closed a system as possible in order to minimize secondary microbial contamination.

Storage and quality control. The finished product is stored at 4 ± 1 °C for up to 6 days. During storage, the following are monitored: physicochemical parameters (pH, acidity, viscosity, sediment, soluble Ca^{2+}, and vitamin D_3 content), organoleptic characteristics (taste, odor, consistency, presence/absence of sediment), and microbiological indicators (TVC and coliform bacteria).

The obtained results (Tables 4–6) indicate that it is up to day 6 of storage that the optimal combination of microbiological safety, vitamin D_3 stability, a high proportion of soluble calcium, and sensory acceptability is ensured. Day 8 is considered a control point

demonstrating the limiting changes and defining the recommended shelf life.

Conclusion

1.It was established that the technologically appropriate concentration of calcium citrate is 0.15%: it does not disturb colloidal stability and does not cause substantial sediment formation; in the CPP-containing sample after pasteurization, sediment amounted to 0.26%, indicating the preservation of colloidal stability.

2. It was shown that vitamin D_3 is retained at a level of no less than 85% during 6 days of storage at 4 ± 1 °C, indicating adequate preservation of the vitamin throughout the recommended shelf life.

3. It was established that the addition of casein phosphopeptides at 0.05% contributes to an increase in the proportion of soluble calcium and a reduction in sediment formation without impairing the sensory properties of the product.

4. It was substantiated that the optimal addition scheme is as follows: calcium citrate before homogenization, and CPP and vitamin D_3 after pasteurization at 35–40 °C, which helps reduce losses of bioactive components and supports the stability of the enriched milk.

5. It was established that the introduction of the Ca + D_3 + CPP complex does not exert a negative effect on pH, viscosity, sediment formation, or sensory properties, while ensuring product stability throughout the recommended storage period of 6 days.

6. A technological flowchart for the production of enriched pasteurized milk was developed, ensuring micronutrient stability, microbiological safety, and high quality of the finished product.

The obtained results may be used in further studies on process scale-up, variation of concentrations, packaging types, and expansion of the range of functional dairy products.

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References:

1. Alnafisah R.Y., Aljefree N., Shatwan I.M. The impact and efficacy of vitamin D fortification: a review. Nutrients. 2024; 16(24): 4322. DOI: 10.3390/nu16244322.
2. Pellegrino L., Hogenboom J.A., De Noni I. Vitamin D fortification of consumption cow's milk: technological challenges and perspectives. Molecules. 2021; 26(17): 5289. DOI: 10.3390/molecules26175289.
3. Lavelli V., Guerrini L., Vannini M. Vitamin D incorporation in foods: formulation strategies, stability, and bioaccessibility as affected by the food matrix. Foods. 2021; 10(9): 1989. DOI: 10.3390/foods10091989.
4. Symakhina H.O., Naumenko N.V. Nutrition as the main factor in maintaining population health. Problemy starinnia i dovholittia. 2016; 25(2): 204–214 [in Ukrainian].
5. Holick M.F. Vitamin D deficiency. N Engl J Med. 2007; 357(3): 266–281. DOI: 10.1056/NEJMra070553.
6. Uwitonze A.M., Razzaque M.S. Role of magnesium in vitamin D activation and function. J Am Osteopath Assoc. 2018; 118(3): 181–189. DOI: 10.7556/jaoa.2018.037.
7. Henriksen C., Aaseth J.O. Magnesium: a scoping review for Nordic Nutrition Recommendations 2023. Food Nutr Res. 2023; 67: 10314. DOI: 10.29219/fnr.v67.10314.
8. Pawlos M., Znamirowska-Piotrowska A., Kowalczyk M., Zaguła G., Szajnar K. Possibility of Using Different Calcium Compounds for the Manufacture of Fresh Acid Rennet Cheese from Goat's Milk. Foods. 2023; 12(19): 3703. DOI: 10.3390/foods12193703.
9. Korhonen H., Pihlanto A. Bioactive peptides: production and functionality. Int Dairy J. 2006; 16(9): 945–960. DOI: 10.1016/j.idairyj.2005.10.012.
10. Meisel H., FitzGerald R.J. Biofunctional peptides from milk proteins: mineral binding and cytomodulatory effects. Curr Pharm Des. 2003; 9(16): 1289–1295. DOI: 10.2174/1381612033454847.
11. Zahedirad M., Jafari S.M., Assadpour E., et al. Fortification aspects of vitamin D in dairy products: A review. Int Dairy J. 2019; 94: 53–64. DOI: 10.1016/j.idairyj.2019.01.013.
12. Jafari T., Askari G., Mirlohi M., Javanmard S.H., Faghihimani E., Fallah A.A. Stability of Vitamin D_3 in fortified yoghurt and yoghurt drink (Doogh). Advanced Biomedical Research. 2016; 5: 52. DOI: 10.4103/2277-9175.178796.
13. Brothersen C., McMahon D.J., Legako J., Martini S. Comparison of milk oxidation by exposure to LED and fluorescent light. J Dairy Sci. 2016; 99(4): 2537–2544. DOI: 10.3168/jds.2015-9849.
14. Vieira E.F., Soares J., Pintado M. Strategies for improving stability and bioaccessibility of vitamin D in beverages. Foods. 2022; 11(2): 278. DOI: 10.3390/foods11020278.
15. Liu G., Guo B., Sun S., Luo M., Liu F., Miao J., Tang J., Huang Y., Cao Y., Song M. A comprehensive review on preparation, structure-activities relationship, and calcium bioavailability of casein phosphopeptides. Crit Rev Food Sci Nutr. 2024; 64(4): 996–1014. DOI: 10.1080/10408398.2022.2111546.
16. Tsuchita H., Suzuki T., Kuwata T. The effect of casein phosphopeptides on calcium absorption from calcium-fortified milk in growing rats. Br J Nutr. 2001; 85(1): 5–10. DOI: 10.1079/BJN2000206.
17. Pei J., Gao X., Pan D., Hua Y., He J., Liu Z., Dang Y. Advances in the stability challenges of bioactive peptides and improvement strategies. Curr Res Food Sci. 2022; 5: 2162–2170. DOI: 10.1016/j.crfs.2022.10.031.
18. Liao W., Sun G., Xu D., Wang Y., Lu Y., Sun J., Xia H., Wang S. The blood-pressure-lowering effect of food-protein-derived peptides: a meta-analysis of recent clinical trials. Foods. 2021; 10(10): 2316. DOI: 10.3390/foods10102316.
19. Costello R.B., Rosanoff A., Dai Q., Saldanha L.G., Potischman N.A. Perspective: Characterization of dietary supplements containing calcium and magnesium and their respective ratio—Is a rising ratio a cause for concern? Adv Nutr. 2021; 12(2): 291–297. DOI: 10.1093/advances/nmaa160.
20. Zareie M., Hosseini S.M., Nematollahi A. Influence of storage conditions on the stability of vitamin D in dairy beverages. *J Food Qual*. 2021; 2021: 9937452. DOI: 10.1155/2021/9937452.
21. Sood S, Methven L, Cheng Q. Role of taste receptors in salty taste perception of minerals and amino acids and developments in salt reduction strategies: A review. Crit Rev Food Sci Nutr. 2025;65(18):3444-3458. doi: 10.1080/10408398.2024.2365962. Epub 2024 Jun 22. PMID: 38907620.

ТЕХНОЛОГІЧНІ АСПЕКТИ ВИРОБНИЦТВА ПАСТЕРИЗОВАНОГО МОЛОКА З БІОАКТИВНИМ МІНЕРАЛЬНО-ПЕПТИДНИМ КОМПЛЕКСОМ

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Анотація. Дефіцит кальцію та вітаміну D в Україні й Європі пов'язаний із ризиком порушень кістково-м'язової системи. Пастеризоване молоко є зручною матрицею для збагачення, однак поєднання Ca з вітаміном D_3 і молочними пептидами потребує перевірених технологічних рішень, що зберігають стабільність і сенсорні показники продукту протягом 6 діб. Мета – обґрунтувати технологію пастеризованого молока, збагаченого цитратом кальцію, вітаміном D_3 і казеїновими фосфопептидами (КФП), із забезпеченням однорідності, колоїдної стабільності та прийнятних органолептичних показників. Базою було нормалізоване молоко 2,5 % жиру. Дослідні варіанти: (1) цитрат кальцію 0,15 % + вітамін D_3 2,5 мкг/100 г; (2) те саме + КФП 0,05 %. Вивчали pH, титровану кислотність, в'язкість, частку розчинного Ca, осад після центрифугування, збереження D_3 (6 діб, 4 ± 1 °C) та сенсорні показники (п'ятибальна шкала). Пастеризація – 88–90 °C/50–60 с; гомогенізація –15 МПа. За технологічно доцільної концентрації 0,15 % Ca-цитрату фізико-хімічні показники зразків залишалися в межах технологічно прийнятних значень; для готового продукту pH становив 6,61, титрована кислотність – 19 °T. У фінальних зразках осад не перевищував 0,5 %. У присутності 0,05 % КФП частка розчинного кальцію (при 0,15 % Ca-цитрату) зростала з 31,7 % (у зразку без КФП) до 34,2 % (у зразку з КФП), що супроводжувалося зменшенням осаду (0,26 % проти 0,32 %). Пастеризація не впливала на загальний Ca (≤1 %), але зменшувала розчинний Ca на 5–6 %; КФП забезпечували вищий абсолютний вміст розчинного Ca після пастеризації. При дозі 2,5 мкг/100 г збереження вітаміну D_3 через 6 діб становило 86 % у зразку Ca + D_3 і 89 % у зразку Ca + D_3 + КФП у світлонепроникній тарі; присутність КФП зменшувала втрати вітаміну на 3–4 % упродовж періоду спостереження. Сенсорні оцінки залишалися високими: 4,7 (Ca+D_3) та 4,8 (Ca+D_3+КФП) проти 4,9 у контролі. В'язкість готового продукту становила 1,98 мПа·с і залишалася близькою до цього рівня на 6-ту добу зберігання. Комбінація 0,15 % цитрату кальцію, 2,5 мкг/100 г вітаміну D_3 та 0,05 % КФП забезпечує стабільність і однорідність пастеризованого молока без істотного впливу на сенсорику, підвищує частку розчинного Ca та забезпечує належне збереження D_3 протягом 6 діб. Технологічно доцільним є внесення КФП після пастеризації (35–40 °C), а вітаміну D_3 — переважно після пастеризації або, за необхідності, перед гомогенізацією з урахуванням можливих термовтрат.

Ключові слова: пастеризоване молоко; цитрат кальцію; вітамін D_3; казеїнові фосфопептиди; розчинність кальцію; колоїдна стабільність; сенсорні властивості.

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