Influence of functional additives on microbiological and physico-chemical characteristics of yogurt

Myroslav Khonkiv, Ivan Dmytrenko, Andrii Marynin, Galyna Polishchuk, Viktor Stabnikov

National University of Food Technologies, Kyiv, Ukraine

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Corresponding author:

Viktor Stabnikov
E-mail: vstabnikov1@gmail.com

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Abstract

Introduction. Enriching yogurt with functional ingredients is a relevant task; however, their incorporation may significantly affect the sensory and physicochemical properties of the product, necessitating additional study.

Materials and methods. The technological effectiveness of adding (a) the LBLC + SeNPs supplement (biomass of *Lactobacillus bulgaricus* 3511 and *Lactococcus cremoris* 1220 containing selenium nanoparticles synthesized by these strains) and (b) cardamom as a source of technologically active extractive compounds is studied.

Results and discussion. The LBLC+SeNPs supplement substantially affected the activity of the starter culture LAB in yogurt, demonstrating a characteristic U-shaped dose-response relationship within the concentration range from 2.25 to 13.75 µg Se/100 mL. At a selenium content of 6.88 µg/100 mL, activation of the starter culture LAB was observed, whereas exceeding this concentration led to inhibition of their activity. The values of active acidity and syneresis of the yogurt samples correlated with LAB activity throughout fermentation, indirectly confirming the interrelationship among these indicators. Within the tested selenium concentration range, the selenium-containing supplement significantly influenced the apparent viscosity and thixotropic behavior of yogurt, increasing the values of these parameters nearly twofold compared with the control; this effect is attributed to selenium's impact on LAB metabolism.

The extractive compounds of dried cardamom, added at an initial level of 0.1–0.5%, caused a slight suppression of LAB activity during fermentation. The effect of cardamom on the apparent viscosity of fermented milk was minor; however, it enhanced thixotropy, which is technologically important in the production of drinking yogurt. When applied at rational dosages, both functional additives positively contributed to the overall quality of the final product during 28 days of storage, including the prevention of excessive post-acidification. Further research should focus on combining the functional and technological properties of the selenium-enriched biopreparation and cardamom extractives to formulate a new type of nutritionally enhanced yogurt.

Conclusions. The incorporation of the studied functional additives into yogurt significantly improves the overall quality characteristics of the product.

Introduction

Selenium is an essential trace element required for maintaining human health (Dinh et al., 2018; Niu et al., 2024; Skrotska et al., 2025). It is a key component of numerous selenoproteins involved in immune function, thyroid hormone metabolism, antioxidant protection, reproduction, and cardiovascular health. Adequate selenium intake helps prevent various disorders, including cardiovascular diseases, infertility, muscle degeneration, cognitive decline, and increased susceptibility to infections (Brigelius-Flohé, 2018). Its well-known antioxidant role is linked to glutathione peroxidase, which protects cells from oxidative damage (Hariharan and Dharmaraj, 2020; Zoidis et al., 2018). Although selenium deficiency poses serious health risks, its safe intake range is narrow, making careful dietary control essential. Recommended daily intake for adults generally ranges from 30 to 75 µg/day, depending on national guidelines (IMFNB, 2000; Kieliszek and Blazejak, 2016; Stabnikova et al., 2022).

In the food industry, selenium-enriched microbial products are primarily developed for the production of functional foods. These selenium-fortified products not only meet the growing consumer demand for nutrition and wellness but also enhance the added value of food items. Common applications include the use of selenium-enriched yeast and lactic acid bacteria in the manufacture of bakery and dairy products, as well as in the development of selenium-rich functional beverages (Du et al., 2024; Luo et al., 2025; Stabnikova et al., 2008, 2022; Shu et al., 2020; Wang et al., 2025).

It is known that Se can affect the physicochemical characteristics of food systems. In particular, Du et al. (2024) established the mechanism by which selenium-enriched yeast affects dough rheology. The addition of sodium selenite to yeast cultures (to produce selenium-enriched yeast) has been found to inhibit the yeast's energy metabolism and the synthesis of glutathione, glycerol, and linoleic acid, ultimately leading to dough softening. It was shown that as the amount of selenium accumulated in yeast cells increases, their enzymatic activity and gas-forming capacity decline, resulting in slower fermentation and proofing of the dough (He et al., 2023; Stabnikova et al., 2008). Therefore, the impact of accumulated Se on the activity and viability of microorganisms employed in fermented food production requires further detailed investigation.

Selenium accumulated by yeasts and bacteria offers advantages when incorporated into fermented dairy products due to its low toxicity and high bioavailability (Kieliszek et al., 2023). It has been demonstrated that certain lactic acid bacteria (LAB), including *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Bifidobacterium longum*, are capable of biotransforming selenite (a toxic form) into bioavailable, less toxic selenium nanoparticles, and Se–amino acids (non-toxic forms) (Martínez et al., 2020). There is experience in enriching dairy products with selenium nanoparticles in the form of biomass of lactic acid bacteria that synthesized them during cultivation in media containing inorganic selenium (Stabnikov et al., 2025; Stabnikova et al., 2023).

Csapó et al. (2015) produced yogurt from milk obtained from cows fed selenium-enriched yeast (Selplex-2300). The milk, containing 53.0 ± 2.80 µg/kg of selenium, was pasteurized, cooled, and inoculated with a starter culture of *Lactobacillus delbrueckii* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* for fermentation. The resulting yogurt had a high selenium content of 58.5 ± 0.40 µg/kg.

A selenium-enriched yogurt was developed using the high–selenium-tolerant strain *Lactiplantibacillus plantarum* NML21, which is capable of converting inorganic selenium in the form of sodium selenite (Na_2SeO_3) into organic forms (Wang et al., 2025). The application of selenium-enriched *L. plantarum* NML21 in yogurt production made it possible to obtain a selenium-enriched functional product with high antioxidant activity and excellent physicochemical properties.

Guo et al. (2023) investigated quality changes of yogurt fermented with selenium-enriched lactic acid bacteria during 21 days of refrigerated storage. Compared with the control, selenium-enriched yogurt showed a slower increase in titratable acidity (approximately 10–15% lower by the end of storage), significantly reduced syneresis (by about 20–30%), and higher apparent viscosity and water-holding capacity throughout storage. In addition, viable LAB counts remained 0.5–1.0 $\log_{10}$ CFU/g higher than in the control at day 21, indicating improved microbial stability.

Osman et al. (2020) reported that during 10 days of storage, yogurt samples containing 0.2–0.6 mg/L of selenium exhibited a slight decrease in titratable acidity, an increase in viscosity, and a reduction in syneresis. A selenium concentration of 0.4 mg/L was identified as the threshold for producing yogurt without flavor or odor defects.

Thus, the available information on the effect of selenium on yogurt quality parameters is somewhat contradictory and requires clarification. The nature of this effect likely depends on the composition of the selected selenium preparation, its dosage and method of application, the type of lactic acid bacteria, and the duration and conditions of storage of the fortified yogurt, all of which should be considered in each specific case (Wang et al., 2021). Therefore, fortification of yogurt with selenium preparations requires detailed study to determine its specific impact on physicochemical, rheological, and microbiological parameters, including during storage.

To impart unique sensory properties to yogurts with Se and to offset potential flavor and aroma defects, natural flavor additives can be used. There is some experience in producing yogurts with natural flavor additives, including spices such as cinnamon, cardamom, thyme, and ginger. These additions can enhance organoleptic properties, provide antioxidant benefits, and generally maintain probiotic viability during storage.

Thus, probiotic yogurts have been developed with spice oleoresins (cardamom, cinnamon, and nutmeg) and probiotics (*Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis)*. The best sensory properties were obtained with cardamom oleoresin. The presence of spice oleoresins did not affect the probiotic populations in the yogurt during 4 weeks of storage (Vijayalakshmi et al., 2014). Yogurt samples with cardamom water extract at concentrations of 100–250 µg/L showed reduced pH, decreased peroxide value, and significantly improved sensory properties (Ismael et al., 2024). The suitability of combining yogurt with cardamom was also confirmed by Santos et al. (2025). Cardamom imparts a unique flavor to yogurt without negatively affecting the viability of lactic acid bacteria during storage. Furthermore, encapsulated cardamom oil is known to extend yogurt shelf life and stabilize its sensory and antibacterial properties for up to 21 days (Teymuri-Yeghaneh et al., 2025).

Therefore, it is worth investigating the potential use of cardamom in yogurt as a flavoring and aromatic ingredient, with a view to its possible combination with selenium. The aim of the present study was to investigate the effects of the LBLC+SeNPs supplement (lactic acid bacteria biomass with biosynthesized selenium nanoparticles) and cardamom as a flavoring additive on the physicochemical, rheological, and microbiological properties of yogurt.

Materials and methods

Materials

For yogurt production, the Iprovit starter culture, containing *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, was used.

Experimental yogurt samples enriched with selenium were prepared using a dietary supplement consisting of lactic acid bacteria biomass (*Lactobacillus bulgaricus* 3511 and *Lactococcus cremoris* 1220) together with selenium nanoparticles (LBLC+SeNPs) biosynthesized by these bacteria. The LBLC+SeNPs supplement was added to the yogurt as a powder suspension in 0.15 M NaCl, taking into account the Recommended Dietary Allowance (RDA) for selenium of 55 μg/day. The supplement was added at 0, 25, and 50% of the RDA, corresponding to selenium contents of 2.25, 6.88, and 13.75 μg/100 ml of yogurt, respectively.

To study the effect of natural flavor additives on yogurt properties, ground cardamom (origin: India; composition: proteins – 11%, fats – 7%, carbohydrates – 68%) was used as the additive.

Preparation of yogurt samples

Yogurt samples with a fat content of 1% were prepared from standardized milk: control sample without additives; sample 1 (2.25 μg Se/100 ml); sample 2 (6.88 μg Se/100 ml); sample 3 (13.75 μg Se/100 ml); sample 4 (0.1% cardamom); sample 5 (0.3% cardamom); sample 6 (0.5% cardamom).

Yogurt samples were prepared in 200 ml volumes. The dietary supplement with SeNPs was added to standardized milk before pasteurization, followed by stirring for 1 minute.

Dry cardamom was added to the standardized milk before pasteurization, during which, with vigorous stirring, the water-soluble extractive compounds of cardamom are extracted into the aqueous phase of the milk, eliminating the need for preliminary preparation of an aqueous cardamom extract (Ismael et al., 2024). Pasteurization was carried out at a temperature of 87±2 °C for 2–3 minutes. To separate the milk from the cardamom segments, the hot mixture was filtered through four-layer cheesecloth. The mixtures, cooled to a temperature of 40±1 °C, were inoculated with pure cultures of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (10^7 colony-forming units (CFU)/ml) and fermented until active acidity reached pH 4.8 or lower. Before fermentation, the dry lyophilized starter culture was activated for 2 hours at 40±1 °C in heat-treated milk. During fermentation, the active acidity of the yogurt samples was determined every hour.

The resulting yogurt samples were cooled to 4±2 °C. After 24 hours, the active acidity and degree of syneresis were determined. The viscosity and thixotropic capacity of the yogurt samples were determined after 14 days of storage, which is the average shelf life of the product. The concentration of lactic acid bacteria in the samples was determined after 1, 7, 14, and 28 days of storage.

Methods

Active acidity (pH) was measured potentiometrically using an ADWA AD1200 ATC laboratory pH/mV/ISE/temperature meter.

The number of lactic acid bacteria cells in yogurt samples was determined by serial tenfold dilutions. The yogurt sample was diluted with sterile saline (0.85% NaCl) and then plated on solid MRSA (De Man-Rogosa-Sharpe agar) medium (CondaLab, Spain). To create anaerobic conditions, the Petri dishes were overlaid with an additional layer of MRSA medium cooled to 45 °C. Colony counts and subsequent conversion to colony-forming units (CFU/ml yogurt) were performed after incubation at 37 °C for 48 hours.

Syneresis of milk protein yogurt curds was determined by centrifugation. After mixing, 25 mL of yogurt in a calibrated tube was centrifuged using a Sigma 2-6E laboratory centrifuge (Germany) for 20 min at 1,000 rpm and 20 °C. The volume of separated whey was measured and expressed as mL per 100 g of product (Polischuk et al., 2020).

The effective viscosity of yogurt samples was measured using a Kinexus Pro+ rotational rheometer (Malvern Instruments Ltd, United Kingdom). For the study, the upper geometry C25 DIN L0142 SS and lower geometry PC25 DIN C0350 AL were selected. Prior to measurement, yogurt samples were mixed for 30 s, poured into the measurement cylinder, the upper geometry was lowered, and the sample was equilibrated to 10 °C for 5 min. Effective viscosity was determined during a shear rate sweep in the forward direction (0.1–100 s^{-1}) and in the reverse direction (100–0.1 s^{-1}). Viscosity and thixotropic behavior of control and experimental yogurt samples were evaluated on the 14th day of storage, corresponding to the average shelf-life period when the product exhibits its most pronounced rheological characteristics.

The degree of structural recovery of yogurt samples, upon decreasing the shear rate to 0.1 s^{-1} at the end of rotational viscosity measurements, was determined using a calculation method, with the effective viscosity at the beginning of the measurement taken as 100% (Liang et al., 2022).

Statistical analysis

All assays were performed in triplicate. Means±standard deviation or mean values were reported. Statistical analysis of data was done using a one-way analysis of variance (ANOVA) and the differences between the means of samples were analyzed by Tukey's test at a significance level of 0.05.

Results and discussion

Activity and viability of lactic acid bacteria during yogurt fermentation and storage

Yogurt samples were fermented and stored at a temperature of 4±2°C for 2 hours. The cell concentration of lactic acid bacteria *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in fresh yogurt were determined (Figure 1).

Addition of Se-containing biopreparation had a significant effect on lactic acid bacteria cell counts in yogurt samples, exhibiting a characteristic U-shaped dose–response relationship. A similar dose–response relationship was reported by Yang et al. (2017). When the threshold selenium concentration is exceeded, bacterial growth may be suppressed due to the formation of reactive oxygen species and damage to cell membranes. In the present study, sample 1 contained a very low selenium dose and showed LAB counts comparable to those of the control, indicating no pronounced effect of selenium on the microbiological background of yogurt. In contrast, sample 2, with a selenium content of 6.88 µg/100 mL, demonstrated significant activation of *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, with cell numbers increasing 1.8-fold compared with the control (223×10^8 vs. 123×10^8 CFU/mL). This stimulation of LAB growth may be attributed to selenium involvement in the synthesis of antioxidant enzymes, thereby reducing oxidative stress in bacterial cells during fermentation (Zan et al., 2024). However, a further increase in selenium content in sample 3 resulted in a sharp decline in LAB counts, indicating inhibition of bacterial activity. Thus, determining the optimal dosage of the bioadditive for yogurt fortification is essential, as selenium concentration directly affects the development of starter culture lactic acid bacteria.

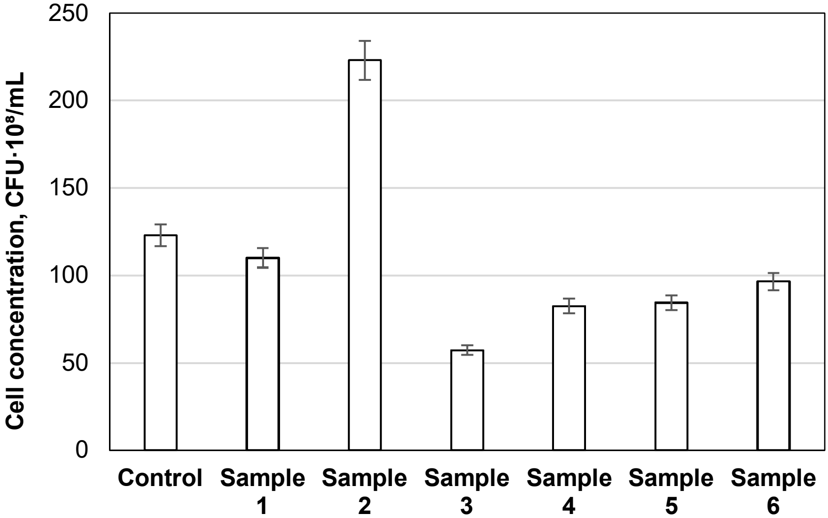


Figure 1. Lactic acid bacteria cell concentration in fresh yogurt

Regarding yogurt samples 4–6, a moderate inhibition of LAB activity by cardamom extractive compounds at the selected dosage was observed, as evidenced by a 1.3–1.5-fold reduction in bacterial cell counts compared to the control sample. Nevertheless, the overall LAB population in samples 4–6 remained sufficiently high, ranging from 82.5×10^8 to 96.5×10^8 CFU/mL, which is considered technologically acceptable for fermented dairy products.

Available literature data on the effects of cardamom extracts on lactic acid bacteria are inconsistent. In particular, Ismael et al. (2024) reported no inhibitory effect of aqueous cardamom extract on LAB at low concentrations and noted the formation of a product with high sensory quality and stable pH values. In contrast, the findings of Sobhy et al. (2023) demonstrated pronounced antibacterial activity of 1,8-cineole, the main component of cardamom essential oil, which may suppress not only pathogenic microorganisms but also other bacterial species, depending on the dosage and the composition of the growth medium. Therefore, further investigation of the effects of biologically active compounds of cardamom is required in each specific application, including their influence during product storage.

Additionally, the degree of syneresis and active acidity of the yogurt samples were studied (Table 1).

The values of active acidity of the yogurt samples generally correlate with the activity of lactic acid bacteria and indirectly confirm the relationship between these parameters. Regarding syneresis, the water-holding capacity is also associated with LAB activity, as these microorganisms are capable of producing viscous exopolysaccharides (EPS) (Brüls et al., 2024). The macromolecules of these biopolymers can significantly modify the microstructure of yogurt gels, in particular by binding water, reducing whey separation, and, under certain conditions, forming complex structured gels with milk proteins. However, this effect depends on the molecular structure and quantity of EPS produced (Jurášková, 2022). Therefore, the rheological characteristics of yogurt samples containing functional additives were also investigated in this research.

Table 1

Degree of syneresis and active acidity of yogurt samples

Yogurt samples	Degree of syneresis, mL/ 100 mL	Active acidity, pH
Control	30.3^{ab} ±1,7	4.68^{ab}±0.17
with LBLC + SeNPs		
Sample 1	19.0^{c}±1.1	4.72^{ab}±0.20
Sample 2	16.1^{d}±0.9	4.48^{b}±0.13
Sample 3	22.3^{bc}±0,9	4.78^{a}±0.15
with cardamom		
Sample 4	24.8^{b}±1.0	4.74^{ab}±0.20
Sample 5	28.5^{ab}±1.2	4.71^{ab}±0.21
Sample 6	31.2^{a}±1.5	4.70^{ab}±0.18

Note: Values are the means±standard deviation. Means within the same column with different superscripts are significantly different at $p \leq 0.05$.

Changes in the concentration of viabile LAB cells in yogurt samples during storage for up to 28 days is shown in Figure 2a, b, and their final amounts are shown in Figure 3.

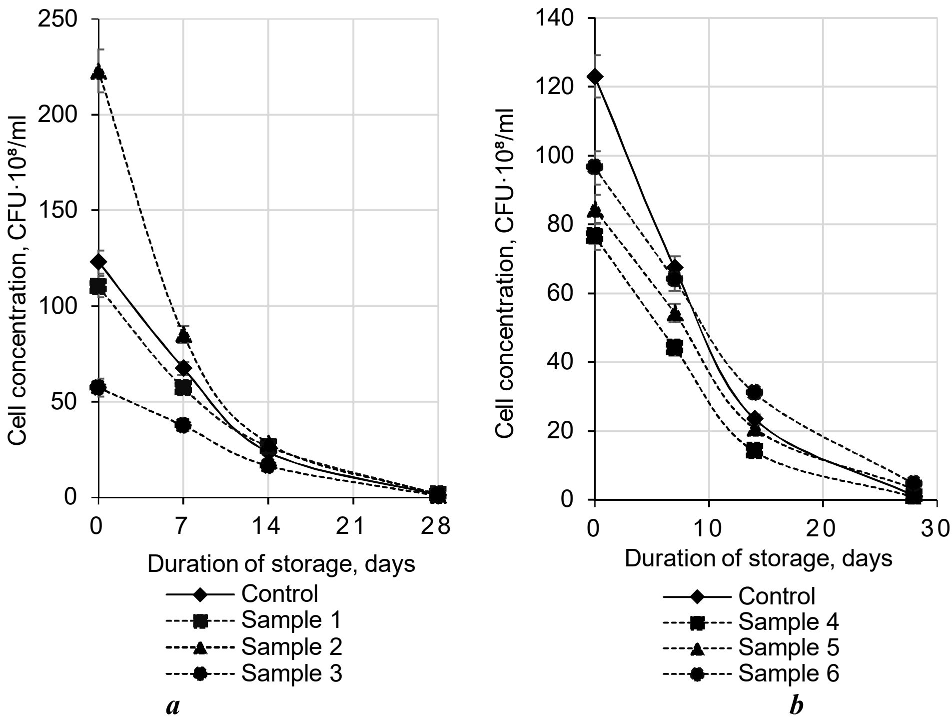


Figure 2. Changes in concentration of lactic acid bacteria cells during storage of yogurt samples with selenium (a) and cardamom (b)

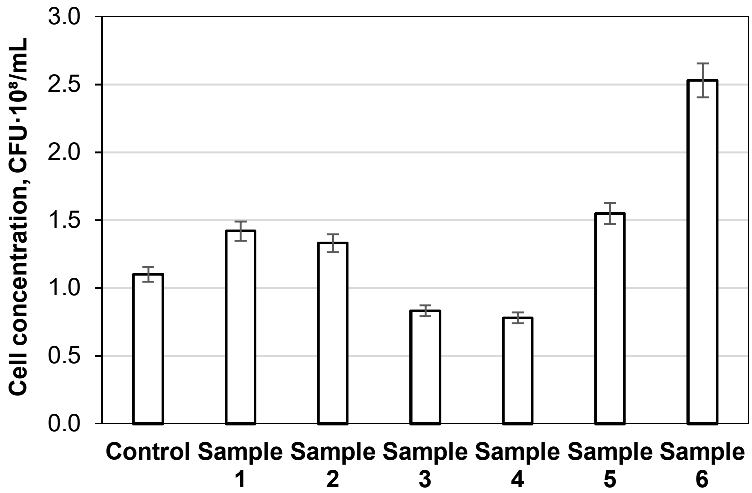


Figure 3. Lactic acid bacteria cell counts in yogurt samples on the 28th day of storage

According to Figures 2a and 3, the number of LAB cells in the yogurt samples containing the biopreparation LBLC + SeNPs gradually approaches that of the control, despite a substantial initial difference. This convergence is most pronounced between days 14 and 28, particularly in yogurt sample 1. Sample 2 exhibits the greatest change, whereas sample 3 shows the least variation in LAB counts in the yogurt during prolonged storage.

For the yogurt samples containing cardamom (samples 4–6), a gradual, nearly linear decrease in LAB cell counts is observed throughout the entire storage period (Figures 2b and 3).

According to international requirements (Codex Alimentarius, STAN 243-2003), yogurt must contain at least 10^7 CFU/g of viable microorganisms (in our case *L. bulgaricus* and *S. thermophilus*) at the time of consumption. All yogurt samples fully complied with these requirements throughout the selected storage period, despite the observed partial decline in LAB cell viability, especially between days 14 and 28.

However, different effects of functional additives on LAB cell concentrations in yogurt samples on day 28 (Figure 3) can be observed, particularly for samples 2 and 6. The maximum LAB count recorded in freshly prepared yogurt sample 2, supplemented with a selenium-containing preparation to achieve a Se concentration of 6.88 μg/100 mL, indicates stimulation of bacterial growth; however, after 7–14 days of storage, LAB counts approach those observed in the control sample.

At the same time, the freshly prepared Sample 6, containing the maximum level of cardamom (0.5%), exhibited the highest LAB content, indicating that compounds present in this spice support bacterial viability. This effect was similarly observed by Ismael et al. (2024).

The values of active acidity and syneresis of the yogurt samples generally correlate with the activity of lactic acid bacteria and indirectly confirm the relationship between these parameters. Functional additives exhibit different effects on the fermentation of the milk mixture and on the content of viable lactic acid bacteria cells in yogurt samples during fermentation. To activate LAB during fermentation and thus shorten the duration of the technological cycle, it is advisable to use a biopreparation LBLC + SeNPs providing a selenium content of 6.88 µg/100 mL. Cardamom at concentrations of 0.1–0.5% does not exert a significant effect on fermentation. The determined doses of functional additives are considered rational for preventing excessive post-acidification of yogurt containing cardamom.

Influence of functional additives on the rheological characteristics of yogurt

The rheological characteristics of yogurt depend on the composition of the milk base, particularly the content of total solids, protein, fat, and carbohydrates (Yu et al., 2016), as well as on the technological processing conditions applied to it (Ilić et al., 2024; Prócel et al., 2025; Sözeri Atik et al., 2024). A significant effect on yogurt viscosity and the structure of the protein gel is exerted by lactic acid bacteria, which are capable of producing viscous exopolysaccharides (EPS) (Brüls et al., 2024). The functional additives used in this study may indirectly influence EPS production through their specific effects on LAB activity or may suppress bacterial viability by day 14 of storage, which requires further investigation.

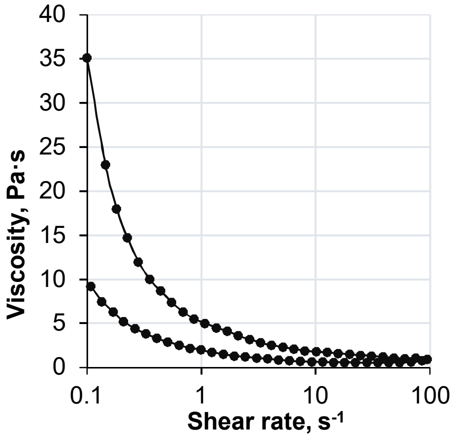
Figure 4 presents the changes in the effective viscosity of the control yogurt and samples containing the lowest and highest levels of the selenium-containing biopreparation (samples 1 and 3) and cardamom (samples 4 and 6) during forward measurement over a shear rate range from $\gamma = 0.1\ s^{-1}$ to $\gamma = 100\ s^{-1}$, as well as during backward measurement with decreasing shear, allowing assessment of the thixotropic behavior of each sample.

To gain a more detailed insight into the behavior of the studied food systems during rotational measurement, the most important values of their rheological characteristics were analyzed (Table 2).

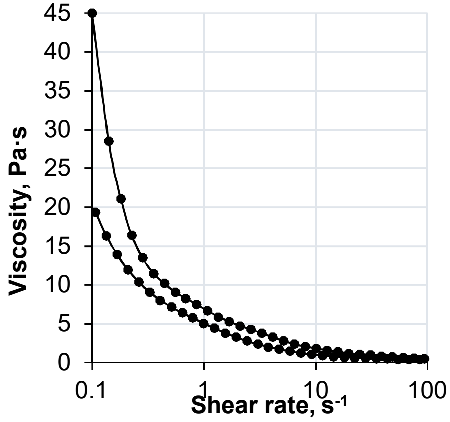
According to Figure 4 and Table 1, the most structured yogurt samples were 2 and 3, whose effective viscosity at the beginning of measurement (65.1172 and 68.2143 mPa·s, respectively) was almost twice that of the control sample (35.1276 mPa·s). This effect can be attributed to the influence of selenium on the metabolism of lactic acid bacteria. In particular, Krausova et al. (2020) found that selenium, especially when used to “selenize” LAB strains, enhances EPS production, which strengthens the protein gel structure. Changes in the viscosity characteristics of all yogurt samples containing the selenium biopreparation LBLC + SeNPs are linked to the effect of this element on LAB activity (Figs. 1 and 3).

The cardamom-containing samples (4–6) exhibited viscosities close to those of the control sample, indicating a minimal effect of this spice on lactic acid bacteria activity. The effect of cardamom on yogurt’s rheological characteristics depends on its form (powder, extract, or essential oil) (Ismael et al., 2024). Future studies should examine how different forms of cardamom influence the microbiological and viscosity properties of yogurt.

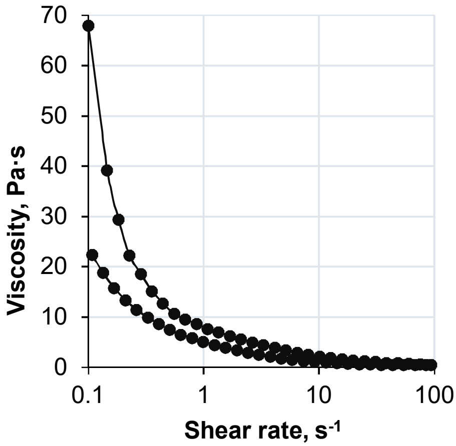
A comparative analysis of the thixotropic behavior of the yogurt samples was conducted using a criterion classifying food systems as exhibiting low or medium thixotropy. After disruption under high shear, yogurt gels typically recover up to 30% of their structure (Lee and Lucey, 2010). This value was therefore adopted as the threshold for assessing an adequate level of thixotropic behavior in the samples.



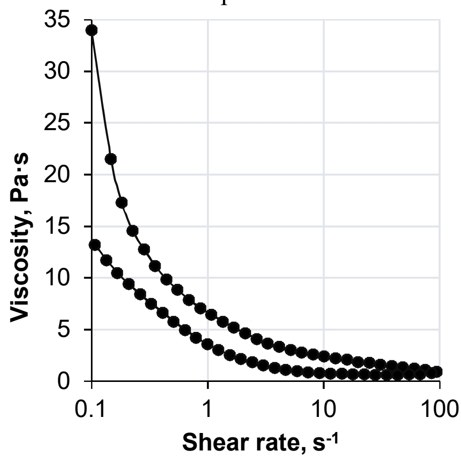
Control



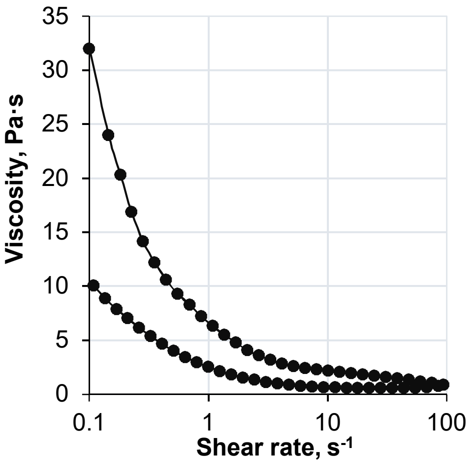
Sample 1



Sample 3



Sample 4



Sample 6

Figure 4. Change in effective viscosity of yogurt during rotational measurement

Table 2

Rheological characteristics of yogurt samples

Sample	Effective viscosity (mPa·s) under varying shear rate gradient			Degree of structure recovery, %
	γ = 0.1 s^{-1} (forward run)	γ = 100 s^{-1}	γ = 0.1 s^{-1} (backward run)	
Control	$35.1276^{c}\pm1.9312$	$0.5326^{c}\pm0.0173$	$9.2309^{e}\pm0.2543$	26.34
with LBLC + SeNPs				
Sample 1	$45.0034^{b}\pm2.0052$	$0.4513^{de}\pm0.01644$	$19.3601^{b}\pm1.0525$	43.02
Sample 2	$65.1172^{ab}\pm2.2107$	$0.5293^{cd}\pm0.0203$	$23.5216^{a}\pm1.5753$	36.12
Sample 3	$68.2143^{a}\pm1.9954$	$0.4780^{d}\pm0.0199$	$22.4233^{ab}\pm1.2410$	32.87
with cardamom				
Sample 4	$34.7599^{cd}\pm1.2954$	$0.6541^{a}\pm0.02621$	$13.2591^{c}\pm1.0385$	38.14
Sample 5	$33.4921^{cd}\pm1.5930$	$0.5966^{b}\pm0.0199$	$12.8738^{cd}\pm0.8941$	38.44
Sample 6	$32.7921^{cd}\pm1.9921$	$0.5628^{bc}\pm0.0203$	$10.0300^{d}\pm0.4603$	31.34

Note: Values are the means±standard deviation. Means within the same colunm with different superscripts are significantly different at $p \leq 0.05$.

A low degree of structure recovery (26.34%) was observed for the control sample. The high thixotropic behavior was demonstrated by samples 1, 2, 4, and 5, which contained moderate amounts of functional additives (no more than 2% for the selenium biopreparation and no more than 0.3% for cardamom). Exceeding these doses likely reduces the production of viscous exopolysaccharides by lactic acid bacteria, which, through numerous low-energy interactions, are capable of rapidly restoring the disrupted protein gel structure. In particular, Han et al. (2016) found that EPS macromolecules increase the relaxation time of structural bonds, thereby altering the thixotropy curve. It is also known that EPS reinforce the protein–casein gel by interacting with casein micelles or filling voids within the gel, which increases initial viscosity and provides better mechanical stability (Buldo et al., 2016). Thus, it can be concluded that the functional additives used in the yogurt positively influence the rheological characteristics of the final product, specifically by structuring the gel and enhancing its ability to spontaneously recover its structure after disruption, an important factor in the production of drinking yogurt obtained by tank fermentation.

The appearance of the yogurt samples after 1 day of storage at 4 ± 2 °C is shown in Figure 5.

Figure 5 visually demonstrates the significant impact of functional additives on the consistency of stirred yogurt. A thick, homogeneous texture was observed in sample 2, containing the selenium biopreparation LBLC + SeNPs at a Se concentration of 6.88 µg/100 mL, as well as in sample 5, with cardamom extract added at 0.3%.

Thus, based on a combination of physicochemical and microbiological indicators, the selected functional additives were shown to substantially improve yogurt quality. These findings are of practical significance and support further research on combining the functional-technological properties of LBLC + SeNPs and cardamom to develop a new type of fortified yogurt.

Control

Sample 1

Sample 2

Sample 3

Sample 4

Sample 5

Sample 6

Figure 5. Appearance of yogurt samples

Conclusions

The selenium-containing biopreparation LBLC + SeNPs affects lactic acid bacteria (LAB) activity in yogurt in a U-shaped dose-dependent manner, with the optimal effect at 6.88 µg/100 mL, while higher concentrations inhibit bacterial activity. Cardamom extracts slightly reduce LAB activity, including during storage. The addition of LBLC + SeNPs improved yogurt viscosity and thixotropic behavior, whereas cardamom mainly enhanced thixotropy. Using these functional additives at appropriate doses enhanced overall yogurt quality by maintaining LAB viability, improving gel recovery, and preventing excessive post-acidification.

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