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Transforming ordinary yogurt into functional yogurt using low doses of ethanolic extract of *Origanum vulgare* L.

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ABSTRACT

Background: Medicinal plants are widely known for their antimicrobial properties. However, the concentrations required to enhance the shelf life or enrich food products are often relatively high. When applied during fermentation, these levels may inhibit the growth of starter cultures, thereby negatively affecting the final product's quality. As a result, exploring the influence of low doses of plant extracts on fermentation processes holds both scientific and practical significance.

Objective: This study aimed to investigate the dose-dependent effects of *Origanum vulgare* ethanolic extract (OVEE) on the growth of lactic acid bacteria (LAB) starters, their proteolytic activity, the fermentation rate of milk, and the resulting yogurt's functional and sensory characteristics.

Methods: The dose-response relationship was evaluated by measuring the optical density (OD600) of LAB cultures incubated for 6 hours in the presence of serial two-fold dilutions of 50 mg/mL OVEE. Proteolytic activity was assessed using the disk diffusion method on milk agar plates. For yogurt production, OVEE was added at various concentrations to milk pasteurized at 85°C for 15 minutes, then cooled to 45°C. The milk was inoculated with 3% LAB starter culture and fermented at 37°C. After coagulation, the yogurt samples were stored at 5°C for ripening. Analyses were conducted on days 1, 14, and 28 of refrigerated storage. Sensory evaluation was performed by a trained panel of 12 participants using a 10-point hedonic scale.

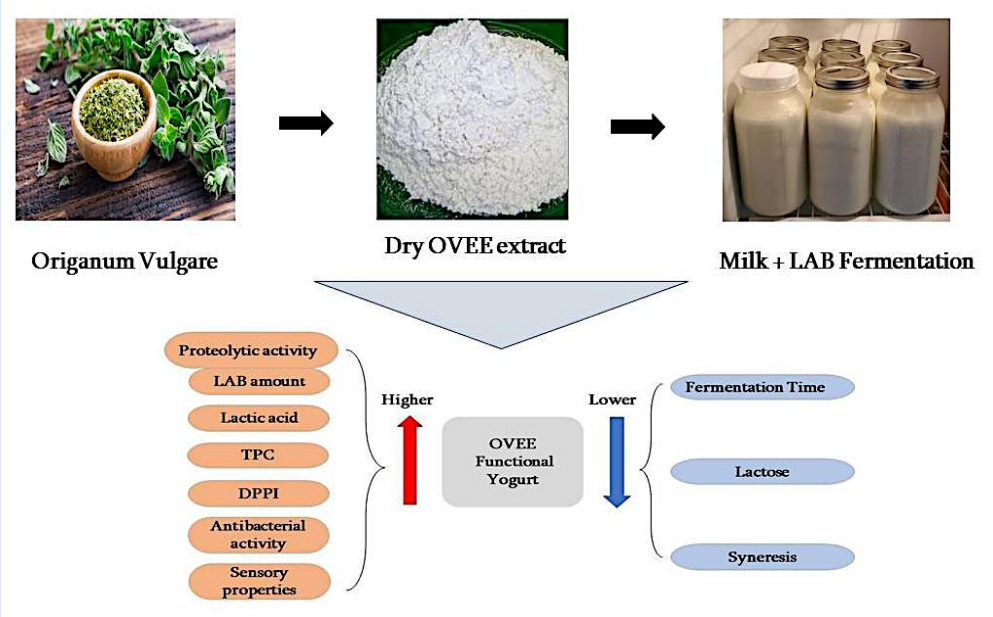
Results: Lactic acid bacteria, unlike pathogenic microorganisms, are highly resistant to medicinal plant extracts. OVEE exhibited only mild inhibitory activity against yogurt LAB strains (MIC > 100 mg/mL). Moreover, certain concentrations of OVEE in the range of 0.1% to 1.0% stimulate the growth of LAB, with the most effective concentration being 0.3%. The addition of OVEE significantly enhanced the proliferation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk, increased the total proteolytic activity, accelerated acidification and clot formation, and enhanced the antioxidant and antimicrobial properties of yogurt. Additionally, OVEE supplementation led to higher lactic acid production and a corresponding reduction in lactose content. It also improved cold storage tolerance, reduced syneresis, and contributed to a more uniform yogurt texture throughout the 28-day cold storage period. Overall, OVEE-treated samples received higher sensory scores in most evaluated attributes.

Conclusion: Specific concentrations of OVEE are sufficient to stimulate microbial activity, enhance proteolytic processes, and promote the release of bioactive peptides from milk proteins, including antioxidants. The resulting yogurts exhibit deeper fermentation, reduced lactose levels, improved digestibility, and a broader range of health-promoting bioactive compounds. Therefore, carefully selected doses of plant extracts can play an active role in fermentation and elevate conventional dairy products into functional foods.

Novelty: The main novelty of this work lies in demonstrating that specific doses of *Origanum vulgare* extract can enhance fermentation and enrich yogurt with bioactive compounds, primarily produced by lactic acid bacteria from milk.

Keywords: *Origanum vulgare*, low dose ethanolic extract, lactic acid bacteria (LAB), microbial growth enhancement, functional yogurt, proteolytic activity, antioxidant activity of yogurt, bioactive compounds, sensory evaluation of yogurt.

Graphical Abstract: Functional yogurt created using ethanol extract of oregano



INTRODUCTION

In recent years, the demand for functional foods has grown considerably, driven by increasing consumer awareness of the link between nutrition and overall health. Fermented dairy products, particularly yogurt, are among the most widely consumed functional foods, primarily due to their probiotic content and associated health benefits. Lactic acid bacteria (LAB), essential in yogurt fermentation, contribute not only to acidification and texture development but also to the generation and release of bioactive peptides from milk proteins. These compounds may exhibit antioxidant, antimicrobial, and immunomodulatory properties [1-5].

Yogurt stands out among dairy products as an especially suitable matrix for functional food development, owing to its high digestibility, rich nutrient content, and probiotic nature. In recent years, incorporating medicinal herbs and their extracts into fermented dairy products has emerged as a natural strategy to boost their nutritional and functional properties. Medicinal plants are well known for their abundance of biologically active constituents, including flavonoids, polyphenols, phenolic acids, terpenoids, sulfides, carotenoids, coumarins, lignans, saponins, curcumins, phthalides, and plant sterols. These compounds are associated with a wide range of biological activities, such as antimicrobial, antioxidant, anti-inflammatory, anti-allergic, and hypotensive effects [6-9].

According to the FFC definition, functional foods are "natural or processed foods containing bioactive compounds that, in defined, effective, non-toxic amounts, provide clinically proven and documented health benefits using defined biomarkers to support optimal health and reduce the risk of chronic/viral diseases and control their symptoms" [10]. Fortification of yogurt with bioactive compounds derived from plant extracts offers a promising avenue for enhancing its health-promoting properties. While the individual effects of probiotics and medicinal herbs have been extensively documented, their combined impact particularly the interactions between plant-derived compounds and LAB remains insufficiently explored. Depending on concentration, plant extracts may either stimulate or inhibit the growth and metabolic activity of LAB [11- 12]. This dual role highlights the need for systematic evaluation of concentration-dependent effects on starter culture performance. Currently, limited research is available that specifically examines how varying doses of specific plant extracts influence LAB growth and milk fermentation processes [10-11, 13].

Origanum vulgare (commonly known as oregano), a widely used culinary and medicinal herb, is particularly rich in phenolic compounds such as carvacrol, thymol, and rosmarinic acid, along with other bioactive phytochemicals. These constituents are known for their anti-inflammatory, antimicrobial, and immunomodulatory activities, making oregano extract a compelling candidate for enhancing the functional value of food products. Incorporating such extracts into yogurt formulations may enhance nutritional quality, extend product shelf life, and provide added health benefits to consumers [10, 14-16]. The present study aims to explore the potential of *Origanum vulgare* ethanolic extract to enhance the intrinsic functional properties of yogurt. The goal is to achieve this enhancement without negatively affecting the physicochemical characteristics or sensory quality of the product, aligning with modern consumer preferences for clean-label, health-promoting foods.

MATERIALS AND METHODS

Microorganisms and Culture Conditions: All bacterial strains used in this study were obtained from the American Type Culture Collection (ATCC), a globally recognized resource for standardized microbial strains recommended for scientific research. The strains included *Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC

11842 and *Streptococcus thermophilus* ATCC 19258. The cultures were revived from lyophilized stocks following ATCC protocols.

Lactobacillus species were cultivated in LAPTg medium (comprising 1.5% peptone, 1% tryptone, 1% yeast extract, 1% glucose, and 0.1% Tween 80, pH 6.5) in either broth or agar form. *Streptococcus thermophilus* was grown in M17 broth. All cultures were incubated at 37°C for 24 hours under microaerophilic conditions. Additional media used included Nutrient Broth (NB, Condalab, Spain) and MRS (Hi media, India). Stock cultures were maintained on their respective solid media at 4°C and subcultured biweekly to maintain viability.

To evaluate the antimicrobial activity of the ethanolic extract, the specific test strain *Escherichia coli* ATCC 8739 was selected.

Preparation of *Origanum vulgare* Ethanolic Extract (OVEE): The aerial parts of *Origanum vulgare* were harvested from the Tavush region in Armenia and authenticated by a qualified botanist. For extraction, 150 g of dried plant material underwent cold maceration in 50% ethanol. Each extraction cycle lasted 72 hours and was repeated three times on the remaining plant material. The combined extracts were filtered through Whatman No. 1 filter paper. The solvent was subsequently evaporated under reduced pressure using vacuum drying at 50°C to obtain the crude ethanolic extract.

Determination of LAB growth rate depending on the extract dose: To evaluate the dose-dependent effects of the extract on lactic acid bacteria, a modified version of the two-fold serial dilution method (typically used for determining MICs) was employed. This adaptation enabled monitoring of bacterial growth during the logarithmic phase.

Initially, 200 mg of dried OVEE was dissolved in 2 mL of nutrient broth and sterilized using Millipore membrane filters (0.45 µm pore size; Schleicher & Schuell, Microscience, Dassel, Germany). Ten sterile test tubes were each filled with 2 mL of LAPTg broth. The first tube received 2 mL of the 1.0% OVEE solution, which was mixed thoroughly. From this, 2 mL was transferred to the second tube, continuing the serial dilution through the tenth tube. To maintain a consistent volume, 2 mL was discarded from the last tube.

Each tube was then inoculated with 0.1 mL of a mixed suspension of *L. delbrueckii* subsp. *Bulgaricus* and *S. thermophilus* (final concentration ~10^7 CFU/mL). The cultures were incubated under static conditions at 37°C for 6 hours. Optical density (OD) at 600 nm was measured using a BNUV-S650 spectrophotometer. The 6-hour time point was selected to capture the exponential (log) phase, corresponding to approximately 6–7 bacterial generations. This experimental design allows for the simultaneous evaluation of both inhibitory and stimulatory effects of plant extracts on LAB growth.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Yogurt Samples against *E. coli* ATCC 8739: Yogurt samples (100 g) were homogenized at 3000 rpm for 10 seconds to achieve a uniform liquid consistency. The whey was then separated by centrifugation at 3000 × *g*, and the resulting supernatant was collected and stored at 4 °C until further analysis.

To determine the minimum inhibitory concentration (MIC), the broth microdilution method with two-fold serial dilutions was employed. Overnight cultures of the test microorganisms were adjusted to a final concentration of approximately 10^5 CFU/mL and inoculated into test tubes containing serial dilutions of the yogurt extract in LAPTg broth. The tubes were incubated at 37 °C for 18 hours under static conditions. MIC was recorded as the lowest concentration of the yogurt extract at which no visible bacterial growth was observed.

The minimum bactericidal concentration (MBC) was determined by subculturing 10 µL from each clear (growth-free) MIC test tube onto fresh Mueller-Hinton agar plates. Plates were incubated for 24 hours at 37 °C. The MBC was defined as the lowest concentration at which no microbial colonies formed, indicating complete bactericidal activity.

Preparation of Fermented Milk Supplemented with OVEE: Reconstituted skim milk (13% w/v) was fortified with *Origanum vulgare* ethanolic extract (OVEE) at concentrations of 0.3% (w/v). The mixture was pasteurized at 85 °C for 15 minutes, and then cooled to 45 °C. Subsequently, it was inoculated with 2% (v/v) of each activated lactic acid bacteria (LAB) culture and incubated at 37 °C until curd formation. After coagulation, the fermented milk samples were stored at 5 °C and analyzed on days 1, 14, and 28.

Determination of pH and Lactic Acid Content: The pH values of the fermented milk samples were measured using a digital pH meter (Checker, HANNA Instruments Inc., USA). The lactic acid content was estimated by titratable acidity using the Thorner method, where one Thorner degree (°Th) corresponds to 9 mg of lactic acid per 100 mL of sample. The titration results were then converted to lactic acid concentration (mg/100 mL).

Enumeration of Viable LAB Counts in Yogurt: To assess bacterial viability, 10 g of each yogurt sample was homogenized and serially diluted in sterile Ringer's solution. Viable counts of *Streptococcus thermophilus* were determined by pour-plating on M17 agar (pH 6.8) followed by aerobic incubation at 37 °C for 48 hours. For *Lactobacillus bulgaricus*, MRS agar (pH 5.4) was used, with anaerobic incubation at 37 °C for 72 hours. Colony-forming units (CFU) were counted on plates containing 30–300 colonies, and the results were expressed as the logarithm (log_{10}) of CFU per gram of yogurt (log CFU/g).

Preparation of Liquid Contents of Yogurt (LCY): The liquid fraction of yogurt was prepared following the method of Shori et al. [17], with minor modifications. A 100 mg sample of yogurt was homogenized at 3000 rpm for 20 s. The pH of the mixture was adjusted to 4.0 using 0.1 M hydrochloric acid (HCl), followed by incubation in a water bath at 45 °C for 10 minutes. The samples were then centrifuged at 5000 rpm for 10 minutes at 4 °C. The supernatant was collected and neutralized to pH 7.0 using 0.1 M sodium hydroxide (NaOH). A second centrifugation was performed under the same conditions, and the resulting clear supernatant was collected for further analysis, including DPPH radical scavenging activity, total phenolic content (TPC), and total flavonoid content (TFC).

DPPH Free Radical Scavenging Activity: The antioxidant capacity of the liquid content of yogurt (LCY) was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Briefly, 2.5 mL of LCY was mixed with 3 mL of ethanol and 0.5 mL of a 55 µM DPPH solution. The mixture was vigorously shaken and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm with a spectrophotometer. A blank containing only DPPH dissolved in methanol was used as a reference. Ascorbic acid served as the positive control antioxidant.

The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH radicals using the formula:

$$RSA\ (\%) = [1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ blank}] \times 100$$

Determination of Total Phenolic Content (TPC): The total phenolic content in OVEE and LCY was measured using a modified version of the Singleton and Rossi method [18]. In this procedure, 0.5 mL of the extract or sample was transferred into test tubes, followed by the addition of

2.5 mL of 10% Folin–Ciocalteu reagent and 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution. The mixtures were vigorously vortexed and then left to react at room temperature for 30 minutes. Absorbance was measured at 765 nm using a spectrophotometer. Total phenolic content was calculated based on a gallic acid standard curve and expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

Determination of Total Flavonoid Content (TFC): The total flavonoid content was measured using the aluminum chloride colorimetric method according to Timothy [19]. Briefly, 0.2 mL of 10% aluminum chloride ($AlCl_3$) solution was mixed with 0.2 mL of the extract or LCY. Subsequently, 0.2 mL of 1 M potassium acetate (CH_3COOK) and 1.12 mL of distilled water were added. The mixture was thoroughly homogenized and incubated at room temperature for 30 minutes. Absorbance was measured at 415 nm against a reagent blank. Quercetin standards ranging from 0 to 1 mg/mL were used to construct a calibration curve, and the flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry matter (mg QE/g DM).

Evaluation of Proteolytic Activity: Proteolytic activity of lactic acid bacteria (LAB) strains was evaluated using a modified agar diffusion assay. Overnight LAB cultures were centrifuged at 6000 rpm for 10 minutes at 4°C to obtain cell-free supernatants. Sterile paper discs (6 mm diameter) were soaked in the supernatants and placed onto Eickman agar plates prepared with 10% (w/v) reconstituted skim milk, serving as the protein substrate.

The plates were incubated aerobically at 37°C for 48 hours. Proteolytic activity was indicated by clear zones (halos) surrounding the discs, resulting from casein hydrolysis by extracellular proteases secreted by the LAB. The diameters of these zones were measured in millimeters using a digital caliper. Larger clear zones corresponded to higher proteolytic activity.

This assay, based on the degradation of casein, was performed in triplicate, and mean values were used for further analysis. The method is adapted from Morandi with modifications [20].

Dynamic Viscosity Measurement: The dynamic viscosity of yogurt samples was measured using a Hoppler ball drop viscometer, and results were expressed in mPa·s. The Hoppler viscometer functions based on the principle of a falling ball, allowing for quick and reliable determination of both kinematic and dynamic viscosity. Its suitability for Newtonian and non-Newtonian fluids makes it ideal for assessing the rheological characteristics of fermented dairy products such as yogurt.

Evaluation of Syneresis: Syneresis, or the separation of whey from fermented milk samples, was evaluated following the method outlined by Panesar et al. [21]. In brief, 20 g of each sample was centrifuged at 500 rpm for 5 minutes. The volume or weight of the expelled whey (supernatant) was then recorded. The extent of syneresis was calculated using the formula:

$$Syneresis\ (\%) = \frac{Weight\ of\ supernatant}{Weight\ of\ sample} \times 100$$

Sensory Analysis: Sensory evaluation was performed by a trained panel consisting of 12 individuals from the Laboratory of Lactic Acid Bacteria at the S&P Center of "Armbiotechnology." Prior to testing, panelists were briefed on the evaluation procedures. The order of sample presentation was randomized, and palate cleansers (water) were provided between samples to prevent carryover effects.

Panelists rated their preferences for seven specific attributes, organized into three categories:

Appearance: color and syneresis, Flavor: aroma and balance of acidity versus sweetness, Texture: firmness, creaminess, and homogeneity.

A 10-point hedonic scale was employed, where 1 represented "extreme dislike" and 10 represented "extreme liking," allowing panelists to express their degree of preference for each attribute.

Statistical Analysis: All experimental data are presented as mean values ± standard deviation (SD). Group differences were analyzed using Duncan's multiple range test. Statistical analyses were conducted using IBM SPSS Statistics software, version 22 (IBM Corp., USA). A p-value below 0.05 was considered indicative of statistical significance.

RESULTS AND DISCUSSION

The impact of OVEE on the growth rate and biomass accumulation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*: The impact of varying concentrations of OVEE (ranging from 0.50 to 0.045 mg/mL) on the growth rate and biomass accumulation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* during the logarithmic growth phase was investigated using a two-fold serial dilution method in LAPTg medium (see Fig. 1). This approach enables the simultaneous detection of both stimulatory and inhibitory effects of the plant extracts on bacterial growth.

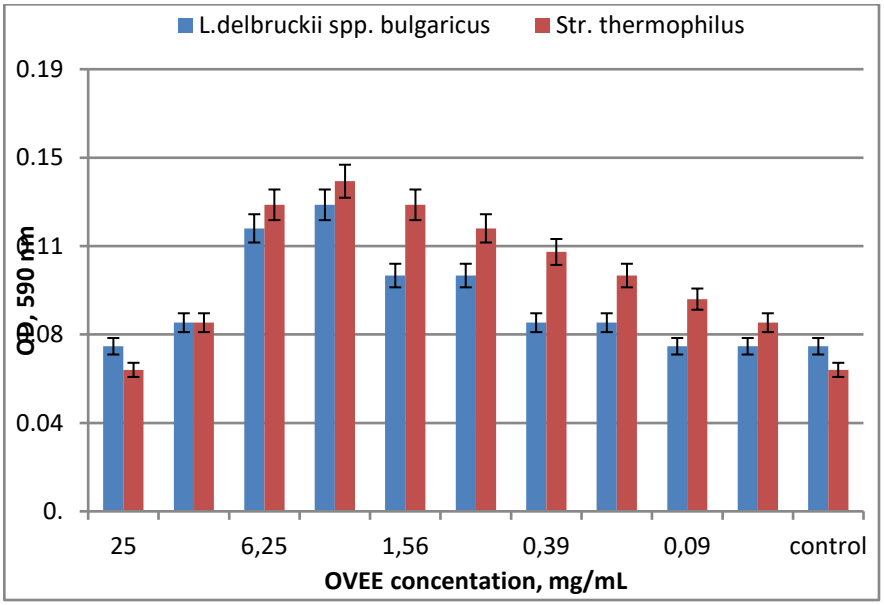


Figure 1. The effect of different concentrations of OVEE on the growth of *L. delbrueckii*, spp. *bulgaricus*, *Streptococcus thermophilus* in LAPTg medium.

As illustrated in Fig. 1, the ethanolic extract of *Origanum vulgare* exerts concentration-dependent effects on bacterial growth. Initially, the inhibitory impact of OVEE decreases at concentrations down to 25 mg/mL, followed by a gradual increase in stimulatory effect, which peaks at 3.125 mg/mL. Beyond this concentration, the stimulatory effect diminishes proportionally with increasing OVEE concentration. Concentrations between 0.045% and 0.39% showed no significant influence on the growth rate or biomass accumulation of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The most pronounced stimulation was observed at concentrations ranging from 0.156% to 6.25% ($p < 0.05$). Notably, supplementation with 3.125 mg/mL OVEE enhanced the biomass production of these starter cultures by two to three times.

The extent of bacterial growth stimulation appears to depend on the balance between growth-promoting and inhibitory compounds present in the plant extract. It is well-documented that lactic acid bacteria (LAB) possess

intrinsic resistance to certain antibiotics [22, 23]. In this study, *S. thermophilus* and *L. bulgaricus* exhibited resistance to OVEE, with minimum inhibitory concentrations (MIC) exceeding 100 mg/mL, whereas *Escherichia coli* showed susceptibility at MIC values ≤ 50 mg/mL. This aligns with previous findings indicating that the effects of plant extracts on microorganisms vary depending on both the microbial species and the extract concentration [24-26].

Polyphenolic compounds are known to play a pivotal role in the biological activity of crude plant extracts [15, 27]. Our analysis revealed that the alcoholic extract of OVEE contained high levels of phenolics and flavonoids, measured at 83.5 ± 2.8 mg GAE/g and 37.4 ± 3.2 mg CE/g, respectively. These results are consistent with those reported by Piekarska-Radzik et al. [24], who demonstrated that polyphenols from pomace extracts of *Rosa rugosa* Thunb. can stimulate the growth of *Lactobacillus* species in a concentration-dependent manner. Accordingly, the presence of phenolic compounds at approximately 3.0 mg/mL in cultures of *L. bulgaricus* and *S. thermophilus* is a key factor driving biomass increase. Similarly, Piekarska-Radzik [24] found that polyphenols in a water-ethanol extract enhanced the biomass of *Lactobacillus acidophilus* ŁOCK 0928 by nearly 15% compared to control cultures.

Influence of OVEE on the Microbiological Properties of Yogurt: The growth kinetics of *S. thermophilus* and *L. bulgaricus* in yogurt during fermentation are illustrated in Fig. 2. Yogurt supplemented with *Origanum vulgare* ethanolic extract (OVEE) exhibited a faster increase in microbial populations compared to the control (plain) yogurt. The viability of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus* in the experimental yogurt increased significantly, by approximately 3.8 log CFU/mL and 2.1 log CFU/mL, respectively, from the start of fermentation. By the end of the fermentation process, viable counts in all yogurt samples remained high, exceeding 9.4 log CFU/mL for *S. thermophilus* and 7.3 log CFU/mL for *L. bulgaricus*. These findings suggest that OVEE can effectively stimulate the growth of starter cultures, indicating its potential application in yogurt production to shorten fermentation time.

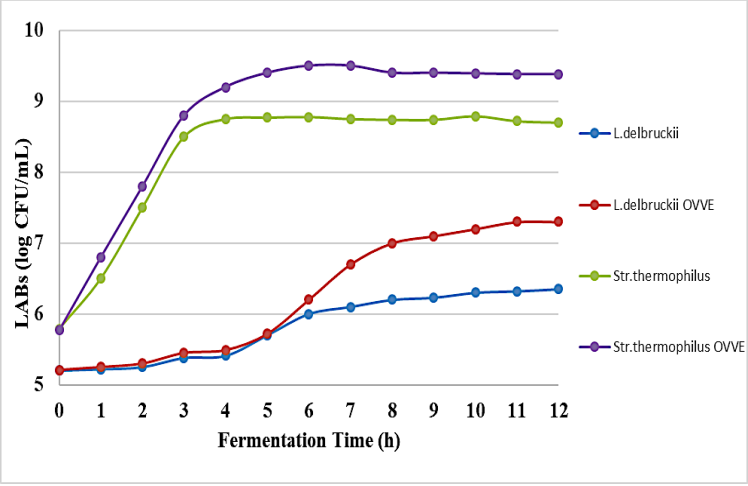


Figure 2. Growth of *S. thermophilus* and *L. bulgaricus* in milk supplemented with plant extracts during fermentation. Mean ± SEM (n = 3).

Effect of OVEE on the Physicochemical, Microbiological, Sensory, Antioxidant, and Antimicrobial Properties of Yogurts during Refrigerated Storage: For successful growth and fermentation of milk, LAB starters must have high exoprotease activity. The relative proteolytic activity of OVEE and plain yogurts is presented in Fig. 3.

Figure 3. Effect of ethanol extract of oregano on proteolytic activity of yogurt. The left zone of milk hydrolysis is formed by OVEE yogurt, and the right one by plain yogurt.

As illustrated in Fig. 3, the addition of 0.3% OVEE significantly increased the proteolytic activity of yogurt by approximately threefold ($p < 0.05$). This enhancement is particularly important because elevated proteolytic activity promotes the release of bioactive peptides from milk proteins [14, 28]. Similarly, Shori and Baba [29] reported that cinnamon supplementation in cow, goat, and camel milk yogurts stimulated both the growth of lactic acid bacteria and proteolytic activity during fermentation.

The impact of OVEE at the optimal concentration of 0.3% on various parameters of yogurt—including the viability of lactic acid bacteria (LAB) starters, apparent viscosity, syneresis, lactic acid synthesis, pH, antioxidant capacity, and antimicrobial activity—was evaluated during refrigerated storage at 1, 14, and 28 days (see Table 1).

Table 1. Comparative analysis of LAB viability, apparent viscosity, syneresis, titratable acidity, pH, antioxidant, and antimicrobial properties of plain yogurt and yogurt supplemented with 0.3% OVEE during refrigerated storage.

Properties	**Refrigerator storage, days**					
	1		**14**		**28**	
	Control	**+ OVEE**	**Control**	**+ OVEE**	**Control**	**+ OVEE**
***S. thermophilus*, log CFU/mL**	8.78	9.4	9.1	9.4	9.0	9.4
***L. bulgaricus*, log CFU/mL**	6.35	7.73	6.68	7.9	6.63	7.7±
pH	4.2	3.65	3.75	3.60	3.3	3.4
Lactic acid, g/L	17.4	18.2	17.9	18.8	18.4	19.2
Viscosity, mPa·sec	44.2	62.9	47.58	60.28	48.57	60.1
Syneresis, %	2	< 1	7.8	5.3	14.3	9.5
Antioxidant activity, %	28.6	94.5	27.5	93.2	27.7	93.3
MIC/MDC for *E. coli* ATCC 8739, µg/mL	500/1000	250/500	250/500	125/250	250/500	125/250

The data is represented as mean ± SD (standard deviation) ($n = 3$) and p-values indicating statistical significance ($p < 0.05$)

On the first day, the acidity (pH) and lactic acid content of the OVEE-supplemented yogurt were higher than those of the plain yogurt, likely due to the enhanced

growth of starter cultures stimulated by the plant extract (Table 1). Elevated lactic acid levels indicate a corresponding reduction in lactose content in the OVEE yogurt.

The viable count of *S. thermophilus* in OVEE yogurt increased up to day 14 of storage before declining slightly, but it never dropped below the initial level. Interestingly, the increase in *S. thermophilus* counts was greater in plain yogurt compared to the OVEE-supplemented samples, which may be attributed to the higher sensitivity of *S. thermophilus* to lactic acid accumulation; plain yogurt had lower lactic acid levels. This observation aligns with Getsemani LópezGea [30], who reported that at pH values between 4.3 and 4.5, which most yogurts reach after 3 to 4 weeks of cold storage, the growth and metabolism of *S. thermophilus* are typically inhibited. By the end of refrigerated storage, *S. thermophilus* counts in both plain and OVEE yogurts were similar, approximately 8.77 log CFU/mL.

In contrast, yogurts containing OVEE showed significantly higher counts of *L. bulgaricus* than plain yogurt throughout the storage period. Both plain and OVEE-supplemented yogurts exhibited a significant increase *in L. bulgaricus* counts from day 0 to day 14. Notably, the lactic acid content was higher in the OVEE-supplemented yogurt, consistent with the sharp initial increase in *L. bulgaricus* counts during storage. This enhanced viability of starter cultures in the experimental yogurts may be due to a lower lactic acid production rate during cold storage caused by OVEE [17, 29, 31-35]. The observed slowdown in yogurt acidification is likely the result of the plant extract hindering cell-substrate interactions. Therefore, OVEE ensured that both starters maintained sufficiently high concentrations throughout the entire refrigerated storage period.

The pH of both yogurt samples decreased gradually during storage (Table 1). Yogurt with added OVEE consistently showed a slightly lower pH (~ 0.05 units) than plain yogurt throughout the entire refrigerated storage period, as OVEE promoted lactic acid production by the starter cultures.

Apparent viscosity and syneresis are critical quality attributes that affect consumer acceptance of fermented milk products [20-26, 35]. Notably, the viscosity of OVEE-supplemented yogurt is significantly higher even after 28 days, with an average value of 60.1 mPa·s ($p \leq 0.05$). The increase in viscosity is attributed to interactions between whey proteins and casein micelles, which correlate with pH changes. Additionally, protein agglomeration at low temperatures may contribute to this effect [8, 13, 24]. Viscosity fluctuations are further influenced by pH-dependent factors such as particle size and protein deposition. These variations relate to changes in casein micelle volume during storage, possibly due to micelle-binding factors. During the first 14 days of storage, the apparent viscosity of both control and OVEE-enriched yogurt increased, although these changes were not statistically significant ($p > 0.05$). In contrast, the decrease in viscosity observed in plain yogurt during refrigerated storage may be due to a significant reduction in starter culture viability and subsequent release of catabolic enzymes during cell lysis.

Syneresis is commonly defined as the spontaneous release of water from a gel caused by gel contraction [11, 37-39]. Storage time generally has a significant impact on syneresis; during this period, bacterial counts decrease, acidity increases, syneresis occurs, and often off-flavors develop. As shown in Table 1, the addition of OVEE significantly reduced syneresis formation ($p < 0.05$). On day 14 of refrigerated storage, syneresis in the control yogurt was 7.8%, whereas in the OVEE yogurt it was only 5.3%. This significant difference ($p < 0.05$) persisted throughout the entire storage period despite variability. By day 28, syneresis values increased to 14.3% in the control and 9.5% in the OVEE-supplemented yogurt. These results are consistent with other studies

demonstrating that yogurts containing herbal extracts exhibit lower syneresis [5, 13-16, 31- 34, 40]. The reduction in syneresis is attributed to the positive effects of the extracts on the yogurt's rheological properties. Additionally, the increased yield of bioactive compounds stimulated by plant extracts may contribute to reduced syneresis [41].

DPPH Free Radical Scavenging Activity of OVEE Yogurt: The DPPH scavenging assay is a widely used method to assess the antioxidant capacity of fermented foods and herbal extracts. The impact of OVEE on the antioxidant activity of yogurt is presented in Table 1. As shown, yogurt supplemented with OVEE displayed significantly higher antioxidant activity compared to plain yogurt ($p < 0.05$). The addition of 0.3% OVEE enhanced antioxidant activity by 92.7% ($p < 0.05$). A positive correlation was observed between antioxidant activity and the abundance of starter cultures as well as proteolytic activity in the yogurt. Despite the slow ongoing growth of starters, the antioxidant activity of OVEE yogurt remained stable over 28 days of cold storage, whereas plain yogurt showed a marked decline. The supplemented yogurt exhibited antioxidant activity approximately 3.2 times greater (97.0 ± 1.7%) for DPPH than plain yogurt and maintained this advantage throughout the storage period.

According to Sari et al. [43], the enhanced antioxidant activity in yogurts fortified with plant extract is primarily due to the phytochemical content of the extracts and microbial metabolic activity [42-43]. Continued microbial growth during storage likely increases protease production, which in turn promotes the release of antioxidant peptides from caseins [14, 17, 21]. Although phenolic compounds in OVEE possess DPPH scavenging activity, their direct contribution to the antioxidant capacity of yogurt is minimal due to the low concentration used (0.3%). However, the metabolic activity of starter cultures may generate small amounts of phenolic compounds exhibiting antioxidant properties in the yogurt.

Supplementation of milk prior to fermentation with OVEE increased total phenolic content (TPC) up to 9.0 mg GAE/g. Herbal yogurts fortified with extracts from peppermint, dill, and basil have similarly shown elevated antioxidant activity linked to increased proteolytic activity. Conversely, previous studies have attributed fluctuations in microbial activity during storage to the degradation of phenolic compounds and/or enhanced milk protein–polyphenol interactions [39-41]. Furthermore, plant extracts can improve the antioxidant properties of yogurt, thereby enhancing its overall quality and stability [15]. When combined with LAB, oregano extracts may stimulate microbial metabolism, leading to the increased release of health-promoting substances from casein molecules.

Antibacterial Activity: Antibacterial activity is a key characteristic of probiotic bacteria and functional foods. Lactic acid bacteria produce various antimicrobial compounds, including bacteriocins, lactic acid, hydrogen peroxide, and diacetyl. These substances enable LAB to inhibit the growth of pathogenic bacteria, thereby reducing the risk of foodborne infections. As anticipated, the addition of OVEE during fermentation significantly enhanced the antibacterial activity of the yogurt, likely due to the increased number of viable LAB starter cultures (Table 1). The minimum inhibitory concentration (MIC) (<500 µg/mL) and minimum bactericidal concentration (MBC) (<250 µg/mL) for OVEE-supplemented yogurt were more than twice as low compared to plain yogurt. Moreover, the antibacterial activity of OVEE yogurt increased approximately twofold. Similar to antioxidant compounds, the increase in the number of microbial cells leads to increased production of secondary antimicrobial metabolites. The resulting functional herbal yogurt mainly contains bioactive components created by lactic acid bacteria from milk molecules.

Sensory Evaluation of Yogurt Supplemented with OVEE: The incorporation of herbal extracts into dairy products is known to enhance desirable organoleptic properties and flavor profiles. The sensory characteristics of yogurt supplemented with 0.3% OVEE were assessed, focusing on appearance, odor, color, aroma, acidity, and overall acceptability. The hedonic scores for both the experimental and plain yogurts are illustrated in Figure 4.

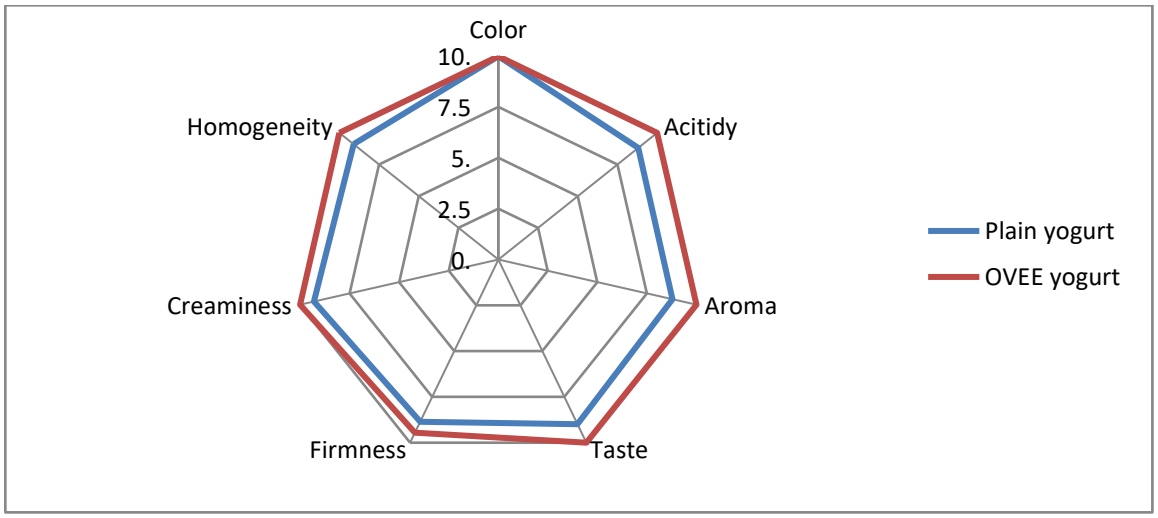


Figure 4. Comparative evaluation of sensory characteristics of yogurts fermented with and without the addition of OVEE. Mean ± standard deviation**.**

The results illustrated in Figure 4 demonstrate that OVEE significantly enhances multiple sensory attributes of the yogurt, including appearance, odor, color, aroma, pleasant sourness, and overall acceptability. Yogurt enriched with OVEE exhibited a smoother and more delicate texture, which was preferred by the sensory panel. Across nearly all evaluated parameters, the OVEE-supplemented yogurt received higher scores compared to the plain yogurt. Comparable improvements in flavor and texture have also been reported in yogurts fortified with extracts of *Rosmarinus officinalis* and Lemon balm, alongside positive effects on microbiological and physicochemical characteristics [26, 45, 46]. The high-quality functional yogurt developed in this study, with enhanced organoleptic qualities, is recommended for regular consumption as well as for supporting the prevention and management of various gastrointestinal conditions.

This research highlighted the synergistic effect of *Origanum vulgare* ethanolic extract on lactic acid bacteria activity within fermented dairy products. The activation of LAB by the oregano extract improved the recovery and bioavailability of health-promoting compounds during fermentation. Understanding this plant-microbe interaction paves the way for the development of novel functional dairy foods and expands opportunities to leverage such synergy for human health benefits.

Scientific Innovations: Unlike enrichment with large amounts of fruit or plant extracts, which often alters the organoleptic characteristics of the product and may inhibit fermentation, the use of low doses of plant extracts activates the intrinsic functional potential of fermented dairy products. This is achieved through the active involvement of high-performing lactic acid bacteria starter cultures in the fermentation process, resulting in enhanced functional properties with minimal changes to the original sensory profile.

Practical Implications: Incorporating low doses of herbal extracts preserves the profitability of yogurt production

while providing additional benefits such as accelerated fermentation and extended shelf life.

CONCLUSIONS

Specific concentrations of OVEE effectively stimulate microbial growth, boost overall proteolytic activity, and enhance the release of bioactive peptides from milk proteins, including antioxidants. The resulting dairy products exhibit more thorough fermentation, reduced lactose levels, improved digestibility, and a diverse range of bioactive peptides with potential health benefits. Therefore, carefully dosed plant extracts, by actively engaging in the fermentation process, can convert conventional dairy products into functional foods.

List of Abbreviations: LAB, Lactic acid bacteria; DPPP, 2,2-diphenyl-1-picrylhydrazyl; GAE, Gallic acid equivalent; QE, Quercetin equivalent; ATCC, American Type Culture Collection; OVEE, *Oreganum vulgare* ethanolic extract.

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Author contributions L.V. D: Investigation, Methodology; Visualisation; Writing - review and editing; Software; Project administration; A.H.B: Investigation; Formal analysis; Supervision; I.E.M: Investigation; Formal analysis; N.G.M: Investigation; Conceptualization; Data curation; Validation Investigation; M.G.Kh: Investigation; Visualization; M.H.H: Investigation; Visualization. H.G.H: Methodology; Visualisation, Writing - original draft; Formal analysis.

REFERENCES

1. Danielyan LV, Hovhannisyan HG, Baghdasaryan LG, Barseghyan AH. New generation of functional yogurts fermented with probiotic lactic acid bacteria isolated from human milk, *Funct. food health dis.*2024; V. 14(4): 232–247. Adolfsson O, Meydani SN, Russell RM (2004) Yogurt and gut function. AJCN 80(2): 245-256. DOI: https://doi.org/10.1093/ajcn/80.2.245.
2. Gjorgievski N, Tomovska J, Dimitrovska G., Makarijoski B., Shariati MA. Determination of the Antioxidant Activity in Yogurt. Journal of Hygienic Engineering and Design 2020; p.88-92. DOI: https://doi.org/10.17265/2161-6213/2016.11-12.006.
3. Papadaki E, Roussis IG. Assessment of Antioxidant and Scavenging Activities of Various Yogurts Using Different Sample Preparation Procedures. Applied Sciences 2022;12(18), 9283. DOI: https://doi.org/10.3390/app12189283.
4. Proestos C, The Benefits of Plant Extracts for Human Health. Foods 2020; 9(11), 1653. DOI: https://doi.org/10.3390/foods9111653.
5. Patrick M, Peter C, Mary M. The potential health benefits of a novel synbiotic yogurt fortified with purple-leaf tea in modulation of gut microbiota, *Bioact. compd. health dis.*2024; 7(4):170-184. DOI: https://doi.org/10.31989/bchd.v7i3.1323.
6. Roasa J, Villa R D. Mine Y, Tsao R. Phenolics of cereal, pulse and oilseed processing by-products and potential effects of solid-state fermentation on their bioaccessibility, bioavailability and health benefits: A review. Trends Food Sci. Technol. 2021; 116, 954–974. DOI: https://doi.org/10.1016/j.tifs.2021.08.027.
7. Khan SA, Lai T, Zhang R, Wei Z, Xiao J, Deng Y, Zhang M. Phenolic profile, free amino acids composition and antioxidant potential of dried longan fermented by lactic acid bacteria. J. Food Sci. Technol. 2018, 55, 4782–4791. DOI: https://doi.org/10.1007/s13197-018-3411-8.
8. Liu L Zhang C, Zhang H, Qu G, Chun L, Liu L. Biotransformation of Polyphenols in Apple Pomace Fermented by β-Glucosidase-Producing *Lactobacillus rhamnosus* L08. Foods 2021, 10, 1343. DOI: https://doi.org/10.3390/foods10061343.
9. Marhamatizadeh MH, Rafat MM. The Effect of Medicinal Plants on Probiotic Bacteria:A Review.Int.J.Nutr.Sci.2024;9(3):163-173. DOI: https://doi.org/10.30476/ijns.2024.100366.1276.
10. Martirosyan D, Lampert T , Ekblad M Classification and regulation of functional food proposed by the Functional Food Center. *Funct. food sci.* 2022; 2(2): 25–46. DOI: https://doi.org/10.31989/ffs.v2i2.890.
11. Joung JY, Lee JY, Ha YS, Shin YK, Kim SH Oh NS. Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts.

Korean Journal Food Science Animal Resource 2016; 36:1;90–99.
DOI: https://doi.org/10.5851/kosfa.2016.36.1.90.

12. Nurzy´nska-Wierdak RN and Walasek-Janusz M, Chemical Composition, Biological Activity, and Potential Uses of Oregano (*Origanum vulgare* L.) and Oregano Essential Oil. Pharmaceuticals 2025; 18(2), 267;
DOI: https://doi.org/10.3390/ph18020267.
13. Kiani H, Mousavi ME, Razavi H, Morris ER. Effect of gellan, alone and in combination with high-methoxy pectin, on the structure and stability of doogh, a yogurt-based Iranian drink. Food Hydrocolloids. 2010; V.24 Issue 8. 744-754.
DOI: https://doi.org/10.1016/j.foodhyd.2010.03.016.
14. Hovhannisyan HG, Pashayan MM, Barseghyan AH, Grigoryan GG, Gaboyan EH, Danielyan LV Health Promoting Potentials of Armenian Functional Sour Milk "NARINE" and its Starter *Lactobacillus helveticus* MDC 9602. The New Armenian Medical Journal 2023; 17: 3 66-73.
DOI: https://doi.org/10.56936/18290825-2023.17.3-66.
15. Mueed A, Shibli S, Al-Quwaie DA, Ashkan MF, Alharbi M, Alanazi H, et al. Extraction, characterization of polyphenols from certain medicinal plants and evaluation of their antioxidant, antitumor, antidiabetic, antimicrobial properties, and potential use in human nutrition. Front Nutr 2023; 10: 1125106.
DOI: https://doi.org/10.3389/fnut.2023.1125106.
16. Dordevi´c SM, Stanisavljevi´c DM, Milenkovi´c MT, Karabegovi´c IT, Lazi´c ML, Nikolova MT, et al. DT Formulation of refreshing nonalcoholic beverage with extracts of medicinal plants. Progress in Nutrition 2019; 21:3, 620–630.
DOI: https://doi.org/10.23751/pn.v21i3.7700.
17. Shori AB, Aljohani GS, Al-zahrani AJ, Al-sulbi OS, Baba AS. Viability of probiotics and antioxidant activity of cashew milk-based yogurt fermented with selected strains of probiotic *Lactobacillus* spp. LWT-Food Sci Technol 2022;153:112482.
DOI: https://doi.org/10.1016/j.lwt.2021.112482.
18. Singleton V and Rossi J. Colorimetry of Total Phenolic Compound with Phoshomolybdic-Phospotungstic Acid reagents. American Journal of Enology and Viticulture. 1965; V. 16, 144–158
19. Timothy CN, Priya VV, Gayathri GR, Phytochemical analysis and total phenolic content of *Origanum vulgare* (oregano). Drug Invent. Today 2018; 10(10):1903-1905.
20. Morandi S, Pica V, Masotti F, Cattaneo S, Brasca M, Noni ID, and Silvetti T. Proteolytic Traits of Psychrotrophic Bacteria Potentially Causative of Sterilized Milk Instability: Genotypic, Phenotypic and Peptidomic Insight. Foods 2021, 10(5), 934; DOI: https://doi.org/10.3390/foods10050934.
21. Panesar PS, Shinade C. Effect of storage on syneresis, pH, *Lactobacillus acidoohilus* count *Bifidobacterium bifidum* count of *Aloe vera* fortified probiotic yogurt. Current Research in Dairy Sciences 2012; 4: 1.17-23.
DOI: https://scialert.net/abstract/?doi=crds.2012.17.23.
22. Wang Y, Dong J, Wang J, Chi W, Zhou W, Tian Q, et al. Assessing the drug resistance profiles of oral probiotic lozenges. J Oral Microbiol. 2022;14(1):2019992.
DOI: https://doi.org/10.1080/20002297.2021.2019992.
23. Duskova M, Mor´avkov´a M, Mr´azek J, Florianov´a M, Vorlov´a L, Karpí˘skov´a. R: Assessment of antibiotic resistance in starter and non-starter lactobacilli of food origin. Acta Vet Brno. 2020;89(4):401–11.
DOI: https://doi.org/10.2754/avb202089040401.
24. Piekarska-Radzik L, Klewicka E. Mutual infuence of polyphenols and *Lactobacillus* spp. bacteria in food: a review. European Food Research and Technology 2021; 247:9–24.
DOI: https://doi.org/10.1007/s00217-020-03603-y.
25. Jakopovi´c LK, Repaji´c M, Samarin R, Božani´c RI, Blaži´c M, Baruk˘ci´c J. Fortification of Cow Milk with Moringaoleifera Extract: Influence on Physicochemical Characteristics, Antioxidant Capacity and Mineral Content of Yoghurt. Fermentation 2022: 8: 545.
DOI: https://doi.org/10.3390/fermentation8100545.
26. Hovhannisyan HG, Danielyan LV, Baghdasaryan LG, Melkumyan IE, Barseghyan AH. The influence of the alcoholic extract of lemon balm on the growth of *Lactobacillus helveticus* MDC 9602 and the technological, physicochemical, organoleptic, and antioxidant properties of fermented milk. J Food Sci Technol 2024; 62, p.1503–1512, DOI: https://doi.org/10.1007/s13197-024-06121-x.
27. Papastavropoulou K, Oz E, Oz F, Proestos C. Polyphenols from plants: Phytochemical characterization, antioxidant capacity, and antimicrobial activity of some plants from different sites of Greece. Separations 2022; 9, 186.
DOI: https://doi.org/10.3390/separations9080186.
28. Kieliszek M, Pobiega K, Piwowarek K, Ko AM. Characteristics of the Proteolytic Enzymes Produced by Lactic Acid Bacteria Molecules 2021; 26, 1858.
DOI: https://doi.org/10.3390/molecules2607185.
29. Shori AB, Baba AS, Cinnamomum verum improved the functional properties of bioyogurts made from camel and cow milks. Journal of the Saudi Society of Agricultural Sciences. 2011; V.10, 2, p.101-107.
DOI: https://doi.org/10.1016/j.jssas.2011.04.005.

30. Getsemani LópezGea, Víctor ManueToledoLópez , Maira Rubi SeguraCampos, Moo Manuel HuchinVíctor, VargasVargas María de Lourdes1 and MaderaSantana Tomás Jesús. Evaluation of the addition of *Moringa oleifera* L. and *Salvia hispanica* L. on the antioxidant activity and viability of lactic acid bacteria in yogurt during refrigerated storage, Dairy Science and Management 2025; 2:4
DOI: https://doi.org/10.1186/s44363-025-00005-x.
31. Nixon JE, Guzel-Seydim ZB, Seydim AC, Jiang X, Bridges WC Jr, Greene AK. Black seed oil impact on authentic kefir microbiota, *Funct. food sci.* 2023; 3(10): 217-227. DOI: https://doi.org/10.31989/ffs.v3i10.1210.
32. Sıçrama H. Turmeric-Enriched Yogurt: Increased Antioxidant and Phenolic Content. Fermentation 2025; 11(3), 127.
DOI: https://doi.org/10.3390/fermentation11030127.
33. Bautista-Hernández I, Gómez-García R, Guillermo CG, Martínez-Ávila, Aguilar CN, Torres-León C, et al. Solid-State Fermentation for Phenolic Compounds Recovery from Mexican Oregano (Lippia graveolens Kunth) Residual Leaves Applying a Lactic Acid Bacteria (*Leuconostoc mesenteroides*), Agriculture 2024; 14, 1342.
DOI: https://doi.org/10.3390/agriculture14081342.
34. Valencia-Avilé E, García-Pérez ME at all. Chemical composition, physicochemical evaluation and sensory analysis of yogurt added with extract of polyphenolic compounds from Quercuscrassi folia oak bark. *Funct. food health dis.*. 2022; 12(9): 502-517.
DOI: https://doi.org/10.31989/ffhd.v12i9.951.
35. Hovhannisyan H, Danielyan L, Chichoyan N, Pashayan M, Baghdasaryan L, Melkumyan I, et al. Improvement of Functional and Sensory Properties of Fermented Dairy Drink NARINE using Raw Apricot Gum, *Funct. food health dis.*, 2024; 14(8):600-614.
DOI: https://doi.org/10.31989/ffhd.v14i8.1398.
36. Ranjbary AG, Rostampour M, Goraghani JS, Varzandian S, Ranjbary Z G. The Effects [[of *Origanum vulgare* on the growth of *Lactobacillus casei* and *Lactobacillus paracasei* in probiotic milk, Int. J. Adv. Biol. Biomed. 2014; 2(3):591-596.
37. Beermann C, Hartung J. Physiological properties of milk ingredients released by fermentation. Food Funct. 2013; 4:185–199. DOI: https://doi.org/10.1039/C2FO30153A.
38. Benezech T, Maingonnat JF. Characterization of the rheological properties of yoghurt—A review. J. Food Eng. 1994; 21:447–472.
DOI: https://doi.org/10.1016/0260-8774(94)90066-3.
39. Buitimea-Cantúa NE, Gutiérrez-Uribe JA, Serna-Saldívar SO. Phenolic–Protein Interactions: Effects on Food Properties and Health Benefits, Journal of Medicinal Food, 2018; Vol. 21, N 2. DOI: https://doi.org/10.1089/jmf.2017.0057.
40. De Montijo-Prieto S, Razola-Díaz M.d.C, Barbieri F, Tabanelli G, Gardini F, Jiménez-Valera M, et al. Impact of Lactic Acid Bacteria Fermentation on Phenolic Compounds and Antioxidant Activity of Avocado Leaf Extracts, Antioxidants 2023, 12, 298.
DOI: https://doi.org/10.3390/antiox12020298.
41. Güldane M, Effect of enrichment with spice extracts on yoghurt quality: Optimizing the extraction process and sensory properties. Journal of Food Measurement and Characterization, 2025; 19:4354–4367.
DOI: https://doi.org/10.1007/s11694-025-03257-y.
42. Kozłowska M, Scibisz I, Zar˛eba D, Ziarno M. Antioxidant properties and effect on lactic acid bacterial growth of spice extracts. CyTA - Journal of Food 2015; 13:573–577.
DOI: https://doi.org/10.1080/19476337.2015.1022228.
43. Marhamatizadeh MH, Nikbakht M, Rezazadeh S, Marhamati Z, Hosseini M, Yakarim M. Effect of Oregano on the Growth of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in Probiotic Dairy Products, World Applied Sciences Journal 2012; 18 (10): 1394-1399,
DOI: https://doi.org/10.5829/idosi.wasj.2012.18.10.63143.
44. Sari IP, Hidayati AR, Muliasari H. Perbandingan Aktivitas antioksidan infusa simplisia segar dan simplisia kering daun Buni (*Antidesma buniues* L. Spreng) dengan metode DPPH. Jurnal Sains dan Kesehatan 2023, 5(5), 605-614.
DOI: https://doi.org/10.25026/jsk.v5i5.1792.
45. Mathews IM, EastwooJd , Lamport DJ, Cozannet RL, Fanca-Berthon P, Williams CM, Clinical Efficacy and Tolerability of Lemon Balm (*Melissa officinalis* L.) in Psychological Well-Being: A Review, Nutrients, 2024; 16(20), 3545.
DOI: https://doi.org/10.3390/nu16203545.
46. Haider IA, Dey M, Alzubaidi A K, Alneamah SJA, Altemimi AB, Pratap-Singh A. Effect of Rosemary (Rosmarinus officinalis L.) Supplementation on Probiotic Yoghurt: Physicochemical Properties, Microbial Content, and Sensory Attributes. Foods 2021; 10:10 2393.
DOI: https://doi.org/10.3390/foods10102393.