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The Assessment the Quality of Probiotic Yogurt Using the *Rosmarinus Officinalis* Essential Oil

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ABSTRACT

Introduction: Antimicrobial compounds have recently attracted the attention of researchers across the world. Essential oils (EOs) could be used as potent herbal antimicrobial materials and natural antioxidants in the food industry. The present study aimed to assess the effects of *Rosmarinus officinalis* essential oil (REO) with variable phenol contents on the properties of probiotic yogurt containing *Bifidobacterium bifidum*.

Methods: Yogurt was prepared by adding *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as the starter and the *Bifidobacterium bifidum* as the probiotic strains. REO was also added at the three concentrations of 1%, 2%, and 3%. Titratable acidity, pH, syneresis, and rheological, microbial, and sensory properties were evaluated at three storage times (7, 14, and 21 days). The total phenol content was approximately 8.93±0.11 milligrams of gallic acid.

Results: The bacterial activity significantly declined in all the samples during 20 days of storage (P<0.05), and the addition of REO decreased the lowering rate of bacterial growth in the probiotic and starter strains over 21 days. Moreover, refrigerated storage decreased the pH and viscosity (P<0.05), which increasing increased titratable acidity and syneresis (P<0.05). The probiotic yogurt with REO exhibited thixotropic non-Newtonian behavior, and the samples containing 1% REO had the highest scores of flavor, odor, texture, and overall acceptability.

Conclusion: According to the results, high levels of phenolic compounds contributed to the biological, physicochemical, and rheological properties of probiotic yogurt, as well as its sensory scores.

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Introduction

Today, the consumption of functional foods has attracted attention owing to their health benefits (1). Functional foods also affect the food industry, especially fermented dairy products with nutritional values, such as proteins, vitamins, and minerals (2, 3). Such examples are yogurt, kefir, soy milk, whey drinks, and Kashk (drained yogurt) (4).

Numerous attempts have been made to fortify dairy products (5, 6). Probiotics are living microorganisms with beneficial health effects on the consumers (7).

Bifidobacteria are well-known strains (8), and *Bifidobacterium bifidum* is considered to be the most important species of this genus (9). *Bifidobacterium bifidum* improves the gastrointestinal tract function and immunity function against various diseases, such as influenza (10). The viability of probiotics in yogurt is often less than 6 log CFU/g during storage (11, 12). Changes in the final flavor of yogurt occur due to the presence of nonvolatile acids (e.g., pyruvic and lactic acid), volatile acids (e.g., butyric and acetic acid), and carbonyl compounds (e.g., acetaldehyde and diacetyl) (13). Compounds such as fruits, flavoring agents, and sweeteners may be added to yogurt to promote its flavor (14). These additives may affect microbial growth, texture, and flavor during fermentation and storage due to the presence of phenolic compounds and their interactions with proteins (15).

Essential oils (EOs) are oily aromatic liquids that are extracted from various plants (16, 17). The antimicrobial properties of EOs and spices could enhance the shelf life of foods (17) and inhibit the growth of the pathogens that could contaminate food, as well as the microorganisms that are responsible for food spoilage (18).

Reports have been published on the antibacterial effects of *Mentha spicata* and *Mentha aquatica* EOs against *Bifidobacterium animalis*, *Staphylococcus aureus*, *Lactobacillus reuteri*, and

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Clostridium perfringens in Kashk (19). Probiotic yogurt is produced with chamomile EOs at the concentrations of 0.2% and 0.4%. The probiotic types of yogurt with 0.4% EO constitute the largest population of probiotics (*B. lactis* BB-12) (20).

Rosmarinus officinalis (rosemary) is used in traditional medicine and food products for its antimicrobial properties (21). Several compounds have been isolated from rosemary, including flavonoids, diterpenes, steroids, and triterpenes. The major compounds of this plant with antimicrobial activity are α-pinene, bornylacetate, camphor, and 1,8-cineole (22).

The present study aimed to assess the effects of the *Rosmarinus officinalis* essential oil (REO) on the pH, microorganism survival, syneresis, rheological parameters, and sensory properties of probiotic yogurt during refrigerated storage.

Materials and Methods

Essential Oil Extraction

Rosmarinus officinalis (English: rosemary, Persian: Eklil-Kuhi/Rozmari) was obtained from a plant shop in Isfahan, Iran. The scientific name of the plant was confirmed by Isfahan University of Medical Sciences. The leaves of rosemary were air-dried in shade at the temperature of 25˚C, and the dried rosemary leaves were hydrodistilled for three hours with 500 milliliters of distilled water using a Clevenger apparatus. The extracted REO was collected and dried over anhydrous sodium sulfate and stored in sealed glass vials in a refrigerator at the temperature of 4˚C.

REO Analysis Using Gas Chromatography-Mass Spectrometry

The chemical composition of the REO was investigated using a gas chromatograph (Agilent Technologies, Wilmington, USA) with a mass spectrophotometer (Agilent Technologies, Wilmington, USA), and an HP-5Ms capillary column (30.0 m×0.25 mm×0.25 µm) was employed for separation. The initial temperature was 50ºC, which was maintained for two minutes and subsequently elevated to 200ºC at the rate of 3.5ºC/min. After a two-minute pause at the temperature of 200ºC, the temperature increased to 280ºC in steps of 7ºC/min.

Primary Culture Preparation

Bifidobacterium bifidum PTCC 1644 was obtained from the Iranian Research Organization for Science and Technology (IROST) culture collection. The lyophilized bacteria were transferred into a tube containing 10 milliliters of de Man-Rogosa-Sharpe (MRS) broth and incubated in an anaerobic jar at the temperature of 37°C for 24 hours. Afterwards, the bacteria were cultivated on the MRS-bile agar (Merck, Germany) and anaerobically incubated at the temperature of 37°C for 48-72 hours. The full-grown colonies were harvested to prepare a bacterial suspension with the turbidity of the McFarland standard.

Probiotic Yogurt Containing the REO

Fresh raw milk (2.5% fat) was obtained from a dairy farm (Isfahan, Iran) and heated to the temperature of 85°C for 20 minutes. Along with *Bifidobacterium bifidum*, yogurt starter cultures, *Lactobacillus delbrueckii*, and *Streptococcus thermophilus* (CHR Hansen, Denmark) were added to each container and cooled to the temperature of 44±1°C. Afterwards, various concentrations of REO (1%, 2%, and 3% [w/v]) were separately incorporated into the containers and thoroughly homogenized. At the next stage, the yogurt samples were incubated at the temperature of 43±1ºC, and incubation ended when the samples reached the pH of 4.6 by storage at the temperature of 4±1°C. The control samples were inoculated using both the starters and probiotics with no herbal additives. Each container was unsealed on days one, seven, 14, and 21 for the physiochemical analysis and microbial counts.

Chemical Analysis

Measurement of pH and Acidity

The pH values were gauged using a pH meter (Swiss, Metrohm 632) at the temperature of 4°C. The acidity test was performed based on lactic acid percentage using the titration method, along with 0.1 N sodium hydroxide and phenolphthalein (23).

Syneresis

At this stage, 10 grams of yogurt was poured into centrifuge tubes and weighed (Wg). The supernatant (whey) was removed after centrifugation at 350 G and the temperature of 10°C for 30 minutes (We). Following that, the tubes were weighed again, and the syneresis of the yogurt samples was calculated using the following formula (24):

$$\% \text{ Synereis} = (Wg - We) / Wg \times 100$$

where *We* is the weight of the whey released from the yogurt, and *Wg* represents the initial weight of the yogurt.

Rheological Properties

The apparent viscosity of the samples was measured using coaxial cylinders with the shear rate of 0-22 s^{-1} in up-down-up steps (Rheostress 300, Thermo Haake, Karlsruhe, Germany). The thixotropic index refers to the area between the upward and downward shear stress curves, which was determined in each of the yogurt samples. In addition, the Herschel-Bulkley model was utilized for the fitting of the data (25), as follows:

$$\sigma=\sigma 0+k\dot{\gamma}n$$

In the formula above, σ is the shear stress (Pa), $\sigma 0$ shows the yield stress (Pa), k is the consistency index (Pa.s^n), γ represents the shear rate (s^{-1}), and n is the flow index. The apparent viscosity (η) was also described as the ratio of shear stress (σ) to the shear rate (γ).

Microbial Analysis

The viability of *B. bifidum*, *L. bulgaricus*, and *S. thermophilus* was measured using one milliliter of the yogurt samples, which was diluted using nine milliliters of sterile peptone water (Merck, Germany). Following that, 0.1 milliliter of the dilutions was placed on the MRS-bile, MRS, and M17 agars. The first two plates were incubated at the temperature of 35-37°C for three days in anaerobic conditions, while the latter agar plate was subjected to incubation for two days at the temperature of 35-37ºC. The colonies were counted using a colony counter (26).

Sensory Analysis

The sensory evaluation was performed by 10 experienced panelists. The samples were served in 150-milliliter glass jars at the temperature of 20 °C after 21 days of storage (4°C). The organoleptic properties were scored based on a five-point hedonic scale within the range of 1-5 (1: Strongly Dislike, 2: Slightly Dislike, 3: Neither Like nor Dislike, 4: Slightly Like, 5: Strongly Like) (27).

Statistical Analysis

Data analysis was performed in SPSS version 20.0. The obtained data on the pH, titratable acidity, syneresis, rheological parameters, and cell counts were expressed as mean (M) and standard deviation (SD) in three replicates. The analysis of variance (ANOVA) and Duncan's multiple range test were used to assess the significant differences, and the P-value of less than 0.05 was considered significant in all the statistical analyses.

Results and Discussion

The Chemical Composition of REO

Table 1 shows the chemical composition of REO. In the present study, the REO comprised of 30 compounds, as well as α-pinene (15.11%), 1,8-cineole (14.17%), and camphor (10.17%). The mean total phenol content of the REO was 8.93±0.11 mg gallic acid/g (Figure 1). In a similar study, Bajlan et al. (2015) (28) used the leaves of rosemary, which were collected from the Zagros Mountains (Iran). The major compounds in the mentioned study included 1,8-cineole (18.29%), camphor (11.3%), and α-pinene (17.43%) (48), which is in line with our findings. On the other hand, Jaimand et al. (29) prepared REO using the microwave-assisted water distillation method, and the major compounds were camphene (28.22%), γ-terpinene (13.66%), and β-pinene (8.42%). In the mentioned study, the plant leaves were collected in 2017 from Tehran (Iran) (47). In another research, Jalali-Heravi et al. (30) described the REO collected in 2006 from the north of Tehran with five main constituents, including 1,8-cineole (23.47%), α-pinene (21.74%), berbonone (7.57%), camphor (7.21%), and eucalyptol (4.49%).

Table 1. Chemical Composition of REO

No.	Compound	Retention Index	Content (%)
1	Tricyclene	926	1.51
2	α-pinene	938	15.11
3	Camphene	944	6.65
4	Verbenene	962	0.81
5	β-pinene	976	2.58
6	3-octanone	987	1.92
7	β-myrcene	991	2.93
8	α-phellandrene	1002	0.47
9	α-terpinene	1018	0.52
10	1,8-cineole	1038	14.17
11	γ-terpinene	1060	0.62

No.	Compound	Retention Index	Content (%)
12	α-terpinolene	1089	0.86
13	Linalool	1106	3.12
14	Chrysanthenone	1123	1.91
15	Camphor	1147	10.17
16	Trans-pinocamphone	1160	1.19
17	Isoborneol	1156	1.47
18	Borneol	1167	7.55
19	Terpinen-4-ol	1177	1.32
20	α-terpineol	1191	2.15
21	Verbenone	1205	4.51
22	Piperitone	1246	3.96
23	Bornyl Acetate	1286	6.60
24	Neryl Acetate	1365	0.19
25	Methyl Eugenol	1401	1.14
26	β-caryophyllene	1418	1.97
27	α-humulene	1460	0.21
28	Caryophyllene Oxide	1587	1.18
29	Widdrol	1597	0.70
30	α-bisabolol	1683	0.50
Total	-	-	97.99

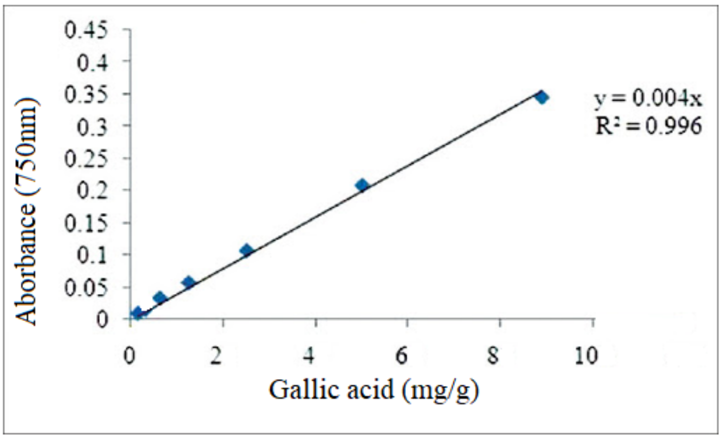

Figure 1. Gallic Acid Standard Curve for Total Phenolic Content

Microbiological Activity

Table 2 shows the microbiological counts of *B. bifidum*, *L. bulgaricus*, and *S. thermophilus* in various yogurt samples at the temperature of 4°C. Accordingly, the count of the probiotic bacteria and starter cultures significantly reduced over 21 days of storage at the temperature of 4°C ($P<0.05$). In the present study, the viability of *S. thermophilus* and *L. bulgaricus* showed the maximum reduction of 0.80 and 0.98 log 10 CFU/g, respectively in the control samples during storage, while the bacteria in the supplemented samples (Y1, Y2, and Y3) decreased less significantly (*S. thermophilus*: 0.55-0.79 log 10 CFU/g; *L. bulgaricus*: 0.39-0.67 log 10 CFU/g).

The results obtained by Sarabi-Jamab and Niazmand (31) showed a significant decline in the *L. acidophilus* activity after exposure to the EOs of *Mentha piperita* and *Ziziphora clinopodioides* in yogurt. In addition, Jimborean et al. (32) reported that the yogurt incorporated with orange EOs showed the increased growth of lactic acid bacteria depending on the biologically active compounds obtained from the orange peels. The chemical composition of EOs, their phenolic profiles, and secreted metabolites by the starter cultures could cause a wide range of effects on bacterial growth. Notably, phenolic compounds could be converted into more active derivatives (e.g., aglycones) in certain conditions, which are more likely to elevate the starter culture activity (33). Phenolic compounds possess variable extents of antimicrobial properties (34).

The main constituent of REO is α-pinene, which is one of the common monoterpenoids emitted from numerous aromatic plant species. According to the information in Table 2, the REO decreased the cell counts of the starter cultures and probiotics. In another study, Singh et al. (35) reported the growth inhibitory activity of α-pinene, with an ability to induce oxidative stress through increasing the generation of reactive oxygen species. Additionally, the type of bacteria could determine antimicrobial properties.

In the current research, Bifidobacterium demonstrated a lower viable cell count in the presence of the REO, with the value estimated at 0.71-3.02 log 10 CFU/g during storage compared to the control samples (no additives: 0.47 log 10 CFU/g). The minimum viability of *B. bifidum* was 6 log 10 CFU/g in the yogurt samples on day 21. In this regard, Yangilar et al. (20) reported that the separate use of ginger and chamomile EOs in the yogurt matrix maintained the cell counts of *B. bifidum* over 6 log 10 CFU/g up until 14 days, which was followed by a decline. However, Illupapalayam et al. (36) observed that in yogurt containing LA5, the addition of spices (e.g., cardamom, nutmeg) had a negative impact on the viable count of BB-12 compared to controls.

Table 2. Survival of Starter and Probiotic Bacteria in Yogurt Supplemented with REO

Yogurt Sample	Storage Time (day)	*B. bifidum* (log 10 CFU/g)	*L. bulgaricus* (log 10 CFU/g)	*S. thermophilus* (log 10 CFU/g)
Control	1	8.18±0.01^{f}	6.92±0.05^{d}	7.01±0.00^{e}
	7	8.32±0.03^{g}	7.17±0.02^{e}	6.64±0.09^{c}
	14	7.98±0.09^{e}	6.10±0.01^{b}	6.21±0.03^{b}
	21	7.71±0.02^{d}	6.12±0.02^{b}	6.03±0.01^{ab}
Y1	1	8.15±0.01^{f}	7.15±0.00^{e}	6.67±0.02^{c}
	7	8.00±0.07^{e}	7.16±0.01^{e}	6.60±0.03^{c}
	14	7.78±0.05^{d}	6.03±0.02^{b}	6.22±0.01^{b}
	21	7.44±0.05^{c}	6.41±0.05^{c}	6.00±0.08^{ab}
Y2	1	8.08±0.01^{e}	7.00±0.02^{de}	6.32±0.05^{b}
	7	7.71±0.05^{d}	6.61±0.01^{d}	6.60±0.01^{c}
	14	7.64±0.07^{cd}	6.22±0.07^{b}	6.02±0.05^{ab}
	21	5.77±0.06^{a}	6.21±0.03^{b}	5.88±0.06^{a}
Y3	1	8.03±0.01^{e}	6.05±0.01^{b}	6.28±0.00^{b}
	7	7.47±0.02^{c}	6.16±0.04^{b}	5.16±0.09^{a}
	14	6.70±0.07^{b}	5.55±0.04^{a}	5.79±0.07^{a}
	21	5.01±0.03^{a}	5.50±0.06^{a}	5.89±0.02^{a}

The mean values followed by various superscript small letters in the same column are significantly different (P<0.05). Yogurt samples included control (probiotic yogurt without EO), Y1 (probiotic yogurt with 1% REO), Y2 (probiotic yogurt with 2% REO), and Y3 (probiotic yogurt with 3% REO).

The Physical and Chemical Properties of the Yogurt Samples

According to the findings of the current research, acidity enhanced in the control and REO samples during refrigerated storage over 21 days (P<0.05). On the other hand, pH decreased in all the samples within 21 days of storage at the temperature of 4ºC (P<0.05) (Table 3). No further reduction was observed in pH than 0.30. In this regard, Ghalem and Zouaoui (14) reported that the yogurt samples containing 0.36 g/l of *Chamaemelum* spp. extract or 0.29 and 0.36 g/l of *Lavandula* spp. oil showed increased acidity during the storage period. Furthermore, Moritz et al. (37) observed significantly higher TA in the yogurt samples containing clove and mint EOs.

According to Yangilar et al. (20), yogurt samples supplemented with ginger EO (0.2%) or combined ginger (0.2%) and chamomile EOs (0.2%) had a descending trend in pH and an increasing trend in TA during 28 days, which may have resulted from the accumulation of acetic acid, acetaldehyde, formic acid, and lactic acid. In another investigation, Joung et al. (38) stated that yogurt samples supplemented with *Nelumbo nucifera* had the lowest pH compared to the other supplemented yogurts with the use of herbal extracts. In the mentioned study, most of the yogurt samples reached a pH of approximately 4.3-4.5 after 21 and 28 days of cold storage, which in turn culminated the inhibition of *S. thermophilus* growth and metabolism (39). In the present study, the pH status and REO constituents prevented the growth of the starter cultures and acid production during the storage.

According to the literature, lactic acid production is partly correlated with the viable counts of probiotic strains (15). This was evident in the present study in the cell counts of the probiotic bacteria, indicating a descending trend after the storage period. In this regard, Shahdadi et al. (40) also reported that EOs reduced the lactic acid production by probiotics, which in turn affected the survival of the probiotic bacteria.

Table 3. Effects of REO on TA, pH, and Syneresis of Probiotic Yogurt Samples

Yogurt Sample	Storage Time (day)	TA (%)	pH	Syneresis (%)
Control	1	0.82±0.01^{b}	4.64±0.06^{a}	3.93±0.14^{d}
	7	0.85±0.01^{b}	4.53±0.01^{ab}	4.59±0.41^{c}
	14	0.89±0.03^{c}	4.42±0.01^{bc}	4.81±0.33^{c}
	21	0.91±0.03^{c}	4.34±0.08^{c}	5.35±0.16^{b}
Y1	1	0.82±0.04^{b}	4.65±0.01^{a}	3.80±0.29^{d}
	7	0.83±0.07^{b}	4.52±0.01^{ab}	3.99±0.23^{d}
	14	0.88±0.02^{c}	4.48±0.01^{bc}	5.55±0.11^{b}
	21	0.91±0.01^{c}	4.35±0.06^{c}	6.22±0.10^{b}
Y2	1	0.81±0.01^{b}	4.64±0.01^{a}	3.80±0.10^{d}
	7	0.82±0.01^{b}	4.54±0.01^{b}	4.39±0.23^{c}
	14	0.85±0.05^{b}	4.47±0.01^{bc}	5.87±0.47^{b}
	21	0.89±0.01^{c}	4.36±0.01^{c}	6.42±0.41^{b}
Y3	1	0.79±0.06^{a}	4.65±0.01^{a}	3.80±0.08^{d}
	7	0.82±0.01^{b}	4.57±0.01^{b}	5.69±0.37^{b}
	14	0.85±0.01^{b}	4.47±0.01^{bc}	6.92±0.29^{a}
	21	0.88±0.01^{c}	4.38±0.01^{c}	7.59±0.11^{a}

Mean values followed by different superscript small letters in the same column are significantly different (P<0.05). Yogurt samples included control (probiotic yogurt with no EO), Y1 (probiotic yogurt with 1% REO), Y2 (probiotic yogurt with 2% REO), and Y3 (probiotic yogurt with 3% REO).

Syneresis or whey separation creates balance between the attraction and repulsion forces within the casein network, as well as the rearrangement capacity of the network bonds (41). Syneresis is an important influential factor consumer satisfaction (42). Evidence attests to low syneresis during the storage period possibly due to the contracting effects of low pH on casein particles, which in turn improves the resistance of yogurt to syneresis (43). According to the information in Table 3, syneresis remarkably increased throughout storage at the temperature of 4ºC in the lowest to highest order in the control (no EO; 1.42%), Y1 (1% REO; 2.42%), Y2 (2% REO; 2.62%), and Y3 samples (3% REO; 3.79%). In a similar study, Panesar and Shinde (44) stated that the syneresis rate elevated from 4.7% to 8.3% (v/w) over 28 days of storage. Furthermore, Han et al. (45) reported that milk gel syneresis increased with the addition of a commercial grape extract, which was an abundant source of phenolic compounds. In the present study, syneresis increased with the elevated EO concentration, which was possibly due to the structural difference in the gels caused by the phenolic compounds.

Polyphenols could enhance rearrangements and increase the pore size in the gel matrix, thereby leading to higher syneresis (14). The phenolic compounds in the milk matrix are more likely to interact with the hydrophobic surface of the milk protein, which in turn decreases the hydrophobic interactions between the amino acid side chains. A drop in the number of the available hydrophobic functional groups could be associated with low water binding (45).

Rheology

Table 4 shows the flow behavior of the yogurt samples. The samples exhibited thixotropic non-Newtonian behavior, which resulted from the breakage of the gel when a shear force was used (46). The gel network often resists against the shear force, and after the use of a critical shear rate, breakdown occurs, and the area between the rising and descending flow curves is indicative of the thixotropic effect (47). According to the information in Table 4, the thixotropy of the control samples was higher compared to the supplemented samples. In addition, the values of the hysteresis area were notably attenuated with the increased content of the EO and storage period (P<0.05).

Table 4. Rheological Parameters in Herschel-Bulkley Model for Probiotic Yogurt with REO

	Storage Time (day)	Study Group			
		Control	Y1	Y2	Y3
N-value	1	0.501 (0.001)$^{d, B}$	0.238 (0.001)$^{b, A}$	0.234 (0.001)$^{a, A}$	0.232 (0.002)$^{a, A}$
	7	0.509 (0.003)$^{c, B}$	0.238 (0.002)$^{a, A}$	0.236 (0.003)$^{a, A}$	0.236 (0.001)$^{a, A}$

	Storage Time (day)	Study Group			
		Control	Y1	Y2	Y3
	14	0.498 (0.001)$^{c, B}$	0.230 (0.001)$^{a, A}$	0.230 (0.002)$^{a, A}$	0.230 (0.003)$^{a, A}$
	21	0.413 (0.004)$^{c, A}$	0.231 (0.003)$^{a, A}$	0.229 (0.002)$^{a, A}$	0.231 (0.004)$^{a, A}$
Consistency Index ($\times 10^2$ Pa.s^n)	1	8.24 (1.05)$^{a, B}$	10.81 (1.29)$^{b, c, A}$	11.33 (1.01)$^{c, A}$	11.94 (0.83)$^{d, A}$
	7	8.00 (2.02)$^{a, A}$	10.72 (1.52)$^{b, c, A}$	11.20 (0.99)$^{c, A}$	11.90 (1.00)$^{d, A}$
	14	8.45 (1.46)$^{a, C}$	10.94 (1.06)$^{b, B}$	11.48 (1.27)$^{c, B}$	12.02 (1.14)$^{d, B}$
	21	8.32 (3.08)$^{a, B}$	10.86 (1.02)$^{b, AB}$	11.37 (1.73)$^{c, AB}$	11.96 (1.63)$^{d, A}$
Apparent Viscosity ($\times 10^2$ Pa.s)	1	0.86 (0.01)$^{c, B}$	0.61 (0.00)$^{b, B}$	0.55 (0.01)$^{a, b, B}$	0.49 (0.00)$^{a, B}$
	7	0.80 (0.02)$^{c, A}$	0.57 (0.01)$^{a, b, B}$	0.52 (0.02)$^{a, b, B}$	0.44 (0.01)$^{a, B}$
	14	0.78 (0.01)$^{c, A}$	0.51 (0.01)$^{a, b, AB}$	0.49 (0.02)$^{a, b, AB}$	0.40 (0.00)$^{a, AB}$
	21	0.75 (0.00)$^{c, A}$	0.46 (0.15)$^{a, b, A}$	0.46 (0.02)$^{a, b, A}$	0.35 (0.03)$^{a, A}$
Thixotropic Index (Pa.s^{-1})	1	21.50 (1.16)$^{c, B}$	13.75 (1.10)$^{b, A}$	8.70 (1.17)$^{a, B}$	6.36 (0.12)$^{a, B}$
	7	20.06 (1.13)$^{c, AB}$	12.47 (1.11)$^{b, A}$	8.06 (0.12)$^{a, AB}$	5.19 (0.15)$^{a, AB}$
	14	18.40 (1.12)$^{b, A}$	11.73 (1.10)$^{a, b, A}$	6.96 (0.17)$^{a, AB}$	6.38 (0.18)$^{a, B}$
	21	19.64 (1.10)$^{c, AB}$	11.05 (1.15)$^{b, A}$	5.78 (1.14)$^{a, A}$	3.79 (1.10)$^{a, A}$

Mean values followed by different superscript small letters in the same row and capital letters in the same column in the same variable are significantly different (P<0.05). Yogurt samples included control (probiotic yogurt with no EO), Y1 (probiotic yogurt with 1% REO), Y2 (probiotic yogurt with 2% REO), and Y3 (probiotic yogurt with 3% REO).

Sensory Evaluation

Table 5 shows the assessment of the aroma, odor, texture, and overall acceptance of the yogurt samples. According to the obtained results, the control samples and those supplemented with 1% REO had the highest mean scores of flavor, odor, texture, and overall acceptability. Evidently, the high total phenolic content strongly affected the gustatory perception (48). This is in line with the study by Vanegas-Azuero et al. (3). The addition of plant extracts to yogurt may ameliorate the organoleptic attributes as well, such as sourness, bitterness, flavor, viscosity, and texture. Moreover, the properties of yogurt texture play a key role in the development of products, quality control and consumer satisfaction (49). The study by Ghalem and Zouaoui (14) indicated that supplementation with *Chamaemelum* spp. extract (0.29-0.36 g/l) in yogurt samples was associated with significantly higher scores of textures compared to the *Lavandula* spp. oil (0.29-0.36 g/l). in another research, Moritz et al. (37) reported no significant difference between various concentrations of cinnamon EO in this regard, while Jimborean et al. (32) reported that the addition of orange EO to yogurt resulted in higher acceptability scores.

Other studies have also been focused on the addition of various EOs to yogurt. Table 6 shows a brief comparison of these reports.

Table 5. Sensory Attributes of Control and Supplemented Yogurt Samples with 5-point Hedonic Scale

	Storage Time (day)	Study Group			
		Control	Y1	Y2	Y3
Flavor	1	4.9 (0.03)$^{b, A}$	4.2 (0.04)$^{b, A}$	3.9 (0.08)$^{b, A}$	3.0 (0.01)$^{a, A}$
	21	4.7 (0.00)$^{b, A}$	4.0 (0.01)$^{b, A}$	3.9 (0.06)$^{b, A}$	3.0 (0.00)$^{a, A}$
Odor	1	4.5 (0.01)$^{b, A}$	4.5 (0.09)$^{b, A}$	3.0 (0.04)$^{a, A}$	2.0 (0.01)$^{a, A}$
	21	4.4 (0.01)$^{b, A}$	4.5 (0.01)$^{b, A}$	3.1 (0.00)$^{a, A}$	2.1 (0.00)$^{a, A}$
Texture	1	5.0 (0.01)$^{b, A}$	5.0 (0.01)$^{b, A}$	4.1 (0.08)$^{b, A}$	4.1 (0.00)$^{b, A}$
	21	5.0 (0.08)$^{b, A}$	5.0 (0.01)$^{b, A}$	4.1 (0.01)$^{b, A}$	4.1 (0.00)$^{b, A}$
Overall Acceptability	1	4.8 (0.01)$^{b, A}$	4.1 (0.01)$^{b, A}$	3.0 (0.01)$^{a, A}$	2.1 (0.00)$^{a, A}$
	21	4.7 (0.01)$^{b, A}$	4.0 (0.06)$^{b, A}$	2.9 (0.01)$^{a, A}$	2.0 (0.01)$^{a, A}$

Mean values followed by different superscript small letters in the same row and capital letters in the same column in the same variable are significantly different (P<0.05). Yogurt samples included control (probiotic yogurt with no EO), Y1 (probiotic yogurt with 1% REO), Y2 (probiotic yogurt with 2% REO), and Y3 (probiotic yogurt with 3% REO).

Table 6. Comparison of Various Studies on Addition of Different EOs to Yogurt

EO Type	Result	Year	Reference
Oregano Methanolic	Less Molds and Yeasts	2019	1
Garlic EO	Antibacterial Effects against Foodborne Pathogens	2019	50
Cinnamon EO	Antimicrobial Activity against Microbial Spoilage	2015	51
Thymus kotschyanus	Inactivation of Foodborne Pathogens	2014	52
Lavandula and Chamaemelum EO	Improvement of Microbiological Quality	2013	53
Anise Volatile Oil	Effective Control of Growth of Spoilage Microorganisms	2011	54

Conclusion

According to the results, REO has a notable potential to deliver *Bifidobacterium bifidum* in sufficient population in yogurt, as well as starter cultures. The lowest pH and highest acidity were observed in the samples supplemented with 1% REO. In addition, the optimal viscosity and sensory scores of probiotic yogurts were observed in the samples supplemented with 1% REO. The statistical analyses also revealed that during the refrigerated storage, the pH, sensory properties, bacterial counts, and viscosity reduced, while the acidity values increased.

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Conflicts of interest

None declared.

References

1. Shahbazi Y, Shavisi N. Effects of Oregano Methanolic Extract on the Chemical, Microbial, and Sensory Properties of Yogurt. Journal of Nutrition Fasting and Health. 2019; 7(3): 138-45.
2. Ghorban Shiroodi S, Mohammadifar MA, Ghorbani Gorji E, Ezzatpanah H, Zohouri N. Influence of gum tragacanth on the physicochemical and rheological properties of kashk. J Dairy Res. 2012; 79(1): 93-101.
3. Vanegas-Azuero AM, Gutiérrez LF. Physicochemical and sensory properties of yogurts containing sacha inchi (Plukenetia volubilis L.) seeds and β-glucans from Ganoderma lucidum. J Dairy Sci. 2018; 101(2): 1020-33.
4. Roesch R, Juneja M, Monagle C, Corredig M. Aggregation of soy/milk mixes during acidification. Food Res Int. 2004; 37(3): 209-15.
5. Singh M, Kim S, Liu SX. Effect of Purified Oat β-Glucan on Fermentation of Set-Style Yogurt Mix. J Food Sci. 2012; 77(8): E195-201.
6. Williams EB, Hooper B, Spiro A, Stanner S. The contribution of yogurt to nutrient intakes across the life course. Nutr Bull. 2015;40(1): 9-32.
7. Guarner F, Khan AG, Garisch J, Eliakim R, Gangl A, Thomson A, et al. Probiotics and prebiotics. World Gastroenterology Organisation Global Guidelines; 2011.
8. Lee YK, Salminen S. Handbook of probiotics and prebiotics. Hoboken: John Wiley & Sons; 2009.
9. Mitsuoka T, Kaneuchi C. Ecology of the bifidobacteria. Am J Clin Nutr. 1977; 30(11): 1799-810.
10. Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C. Review article: bifidobacteria as probiotic agents–physiological effects and clinical benefits. Aliment Pharmacol Ther. 2005; 22(6): 495-512.
11. Donkor O, Henriksson A, Vasiljevic T, Shah NP. Effect of acidification on the activity of probiotics in yoghurt during cold storage. Int Dairy J. 2006; 16(10): 1181-9.
12. Dave RI, Shah NP. Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of probiotic bacteria in yoghurts made with commercial starter cultures. Int Dairy J. 1997; 7(6-7):435-43.
13. Güler Z, Gürsoy-Balcı AC. Evaluation of volatile compounds and free fatty acids in set types yogurts made of ewes', goats' milk and their mixture using two different commercial starter cultures during refrigerated storage. Food Chem. 2011; 127(3): 1065-71.
14. Ghalem BR, Zouaoui B. Evaluation of the quality of steamed yogurt treated by Lavandula and Chamaemelum species essential oils. J Med Plant Res. 2013; 7(42): 3121-6.
15. Da Silva DF, Tenório Junior NN, Gomes RG, dos Santos Pozza MS, Britten M, Matumoto-Pintro PT. Physical, microbiological and rheological properties of probiotic yogurt supplemented with grape extract. J Food Sci Technol. 2017; 54(6): 1608-15.
16. Al-Otaibi M, El.Demerdash H. Improvement of the quality and shelf life of concentrated yoghurt (labneh) by the addition of some essential oils. Afr J Microbiol Res. 2008; 2(7): 156-61.

17. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol. 2004; 94(3): 223-53.
18. El–Nawawy M, El–Kenany Y, Abd El Ghaffar E. Effect of some herb plants on the use of yoghurt culture. Paper presented at: Annals of Agriculture Science, 7th conference of agriculture research, Ain Shams university of Cairo, Egypt; 15–17 December 2008.
19. Golestan L, Seyedyousefi L, Kaboosi H, Safari H. Effect of Mentha spicata L. and M entha aquatica L. essential oils on the microbiological properties of fermented dairy product, kashk. Int J Food Sci Technol. 2016; 51(3): 581-7.
20. Yangilar F, Yildiz PO. Effects of using combined essential oils on quality parameters of bio-yogurt: Yangilar and Yildiz. J Food Process Preserv. 2017; 42(2): e13332.
21. Genena AK, Hense H, Smânia Junior A, Souza SM. Rosemary (Rosmarinus officinalis): a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. Food Sci Technol. 2008; 28(2): 463-9.
22. Hussain AI, Anwar F, Chatha SAS, Jabbar A, Mahboob S, Nigam PS. Rosmarinus officinalis essential oil: antiproliferative, antioxidant and antibacterial activities. Braz J Microbiol. 2010; 41(4): 1070-8.
23. AOAC. Official methods of analysis, 16th ed. Association of official analytical chemists, arlington, va, USA. 1995.
24. Amatayakul T. The improvement of physical properties of yoghurts by varying casein/whey protein ratio and EPS-producing starter cultures, Victoria University; 2005.
25. Karsheva M, Paskov V, Tropcheva R, Georgieva R, Danova S. Physicochemical parameters and rheological properties of yoghurts during the storage. Journal of Chemical Technology and Metallurgy. 2013; 48(5): 483-8.
26. Vinderola CG, Reinheimer JA. Culture media for the enumeration of Bifidobacterium bifidum and Lactobacillus acidophilus in the presence of yoghurt bacteria. Int Dairy J. 1999; 9(8): 497-505.
27. Eissa EA, Yagoub AEA, Babiker EE, Mohamed Ahmed IA. Physicochemical, microbiological and sensory characteristics of yoghurt produced from camel milk during storage. Electronic Journal of Environmental, Agricultural and Food Chemistry. 2011; 10(6): 2305-13.
28. Bajalan I, Rouzbahani R, Ghasemi Pirbalouti A, Maggi F. Quali-quantitative variation of essential oil from Iranian rosemary (Rosmarinus officinalis L.) accessions according to environmental factors. Journal of Essential Oil Research. 2018; 30(1): 16-24.
29. Jaimand K, Rezaee MB, Homami S. Comparison Extraction Methods of Essential oils of Rosmarinus officinalis L. in Iran By Microwave Assisted Water Distillation; Water Distillation and Steam Distillation. Journal of Medicinal Plants and By-products. 2018; 7(1): 9-14.
30. Jalali-Heravi M, Moazeni RS, Sereshti H. Analysis of Iranian rosemary essential oil: Application of gas chromatography–mass spectrometry combined with chemometrics. J Chromatogr A. 2011; 1218(18): 2569-76.
31. Sarabi-Jamab M, Niazmand R. Effect of essential oil of Mentha piperita and Ziziphora clinopodioides on Lactobacillus acidophilus activity as bioyoghurt starter culture. American-Eurasian Journal of Agricultural and Environmental Science. 2009; 6(2): 129-31.
32. Jimborean MA, Salanta LC, Tofana M, Pop CR, Rotar AM, Fetti V. Use of essential oils from citrus sinensis in the development of new type of yogurt. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca Food Science and Technology. 2016; 73(1): 24-27.
33. Sun-Waterhouse D, Zhou J, Wadhwa SS. Drinking yoghurts with berry polyphenols added before and after fermentation. Food Control. 2013; 32(2): 450-60.
34. Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. Int J Food Microbiol. 2000; 56(1): 3-12.
35. Singh HP, Batish DR, Kaur S, Arora K, Kohli RK. α-Pinene Inhibits Growth and Induces Oxidative Stress in Roots. Ann Bot. 2006; 98(6): 1261-9.
36. Illupapalayam VV, Smith SC, Gamlath S. Consumer acceptability and antioxidant potential of probiotic-yogurt with spices. LWT - Food science and technology. 2014; 55(1): 255-62.
37. Feniman Moritz CM, Mores Rall VL, Saeki MJ, Junior AF. Inhibitory effect of essential oils against Lactobacillus rhamnosus and starter culture in fermented milk during its shelf-life period. Braz J Microbiol. 2012; 43(3): 1147-56.
38. Joung JY, Lee JY, Ha YS, Shin YK, Kim Y, Kim SH, et al. Enhanced Microbial, Functional and Sensory Properties of Herbal Yogurt Fermented with Korean Traditional Plant Extracts. Korean J Food Sci Anim Resour. 2016; 36(1): 90-9.
39. Robinson RK. Yogurt types and manufacture. London, UK: Academic Press; 2002.
40. Shahdadi F, Mirzaie H, Kashaninejad M, Khomeiri M, Ziaiifar AM, Akbarian A. Effects of various essential oils on chemical and sensory characteristics and activity of probiotic bacteria in drinking yoghurt. Agricultural Communications. 2015; 3(1): 16-21.
41. Matumoto-Pintro P, Rabiey L, Robitaille G, Britten M. Use of modified whey protein in yoghurt formulations. Int Dairy J. 2011; 21(1): 21-6.
42. Gürsoy A, Durlu-Özkaya F, Yildiz F, Aslim B. Set type yoghurt production by exopolysaccharide producing turkish origin domestic strains of Streptococcus thermophilus (W22) and Lactobacillus delbrueckii ssp. bulgaricus (B3). Kafkas Universitesi Veteriner Facultesi Dergisi. 2010; 16: S81-6.
43. Lucey JA, Singh H. Formation and physical properties of acid milk gels: a review. Food Res Int. 1997; 30(7): 529-42.

44. Panesar PS, Shinde C. Effect of Storage on Syneresis, pH, Lactobacillus acidophilus Count, Bifidobacterium bifidum Count of Aloe vera Fortified Probiotic Yoghurt. Current Research in Dairy Sciences. 2012; 4(1): 17-23.
45. Han J, Britten M, St-Gelais D, Champagne CP, Fustier P, Salmieri S, et al. Polyphenolic compounds as functional ingredients in cheese. Food Chem. 2011; 124(4): 1589-94.
46. Pereira EPR, Cavalcanti RN, Esmerino EA, Silva R, Guerreiro LRM, Cunha RL, et al. Effect of incorporation of antioxidants on the chemical, rheological, and sensory properties of probiotic petit suisse cheese. J Dairy Sci. 2016; 99(3): 1762-72.
47. Bourne M. Food texture and viscosity: concept and measurement. 2nd Edition. Amsterdam: Elsevier Science & Technology Books; 2002.
48. Cerretani L, Salvador MD, Bendini A, Fregapane G. Relationship between sensory evaluation performed by Italian and Spanish official panels and volatile and phenolic profiles of virgin olive oils. Chem Percept. 2008; 1(4): 258-67.
49. Tarakci Z. Influence of kiwi marmalade on the rheology characteristics, color values and sensorial acceptability of fruit yogurt. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 2010; 16(2): 173-8.
50. Nazaria M, Ghanbarzadeh B, Samadi Kafil H, Zeinalie K, Hamishehkar H. Garlic essential oil nanophytosomes as a natural food preservative: Its application in yogurt as food model.Colloid Interface Sci Commun. 2019; 30: 20-9.
51. Feniman Moritz CM, Mores Rall VL, Júri Saeki M, Júnior AF. Assessment of antimicrobial activity of cinnamon (Cinnamomum zeylanicum) essential oil in yogurt. Acta Scientiarum. Technology. 2015; 37(1): 12-26.
52. Mahmoudi R, Gajarbeygi P, Mahmodzadeh F, Hassanzadeh M, Kiyania R, Asadi Nadari MR. Quality of yogurt blended with thymus kotschyanus essential oil. Malaysian Journal of Science. 2014; 33(2): 176-82.
53. Ghalem BR, Zouaoui B. Evaluation of the quality of steamed yogurt treated by Lavandula and Chamaemelum species essential oils. Journal of Medicinal Plants Research. 2013: 7(42): 3129-34.
54. Singh G, Singh Kapoor IP, Singh P. Effect of volatile oil and oleoresin of anise on the shelf life of yogurt. J Food Process Preserv. 2011; 35(6): 778-83.