

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

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| ARTICLE INFO   | ABSTRACT  |
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| <p><i>Article type:</i><br/>Research Paper</p> <hr/> <p><i>Article History:</i><br/>Received: 14 Apr 2020<br/>Accepted: 16 Jun 2020<br/>Published: 09 Aug 2020</p> <hr/> <p><i>Keywords:</i><br/>Probiotic<br/>Bacterial activity<br/>Essential oil<br/>Flow behavior<br/>Yogurt</p> | <p><b>Introduction:</b> 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 <i>Rosmarinus officinalis</i> essential oil (REO) with variable phenol contents on the properties of probiotic yogurt containing <i>Bifidobacterium bifidum</i>.</p> <p><b>Methods:</b> Yogurt was prepared by adding <i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i> as the starter and the <i>Bifidobacterium bifidum</i> 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.</p> <p><b>Results:</b> The bacterial activity significantly declined in all the samples during 20 days of storage (P&lt;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&lt;0.05), which increasing increased titratable acidity and syneresis (P&lt;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.</p> <p><b>Conclusion:</b> 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.</p> |

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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  $\alpha$ -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  $\mu$ m) was employed for separation. The initial temperature was 50°C, which was maintained for two minutes and subsequently elevated to 200°C at the rate of 3.5°C/min. After a two-minute pause at the temperature of 200°C, the temperature increased to 280°C in steps of 7°C/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±1°C, 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):

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

where  $W_e$  is the weight of the whey released from the yogurt, and  $W_g$  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  $0-22 \text{ s}^{-1}$  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:

$$\sigma = \sigma_0 + k\gamma^n$$

In the formula above,  $\sigma$  is the shear stress (Pa),  $\sigma_0$  shows the yield stress (Pa),  $k$  is the consistency index ( $\text{Pa}\cdot\text{s}^n$ ),  $\gamma$  represents the shear rate ( $\text{s}^{-1}$ ), and  $n$  is the flow index. The apparent viscosity ( $\eta$ ) was also described as the ratio of shear stress ( $\sigma$ ) to the shear rate ( $\gamma$ ).

### 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  $35-37^\circ\text{C}$  for three days in anaerobic conditions, while the latter agar plate was subjected to incubation for two days at the temperature of  $35-37^\circ\text{C}$ . 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  $20^\circ\text{C}$  after 21 days of storage ( $4^\circ\text{C}$ ). 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  $\alpha$ -pinene (15.11%), 1,8-cineole (14.17%), and camphor (10.17%). The mean total phenol content of the REO was  $8.93 \pm 0.11$  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  $\alpha$ -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%),  $\gamma$ -terpinene (13.66%), and  $\beta$ -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%),  $\alpha$ -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   | $\alpha$ -pinene       | 938             | 15.11       |
| 3   | Camphene               | 944             | 6.65        |
| 4   | Verbenene              | 962             | 0.81        |
| 5   | $\beta$ -pinene        | 976             | 2.58        |
| 6   | 3-octanone             | 987             | 1.92        |
| 7   | $\beta$ -myrcene       | 991             | 2.93        |
| 8   | $\alpha$ -phellandrene | 1002            | 0.47        |
| 9   | $\alpha$ -terpinene    | 1018            | 0.52        |
| 10  | 1,8-cineole            | 1038            | 14.17       |
| 11  | $\gamma$ -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         |
| <b>Total</b> | -                   | -               | <b>97.99</b> |

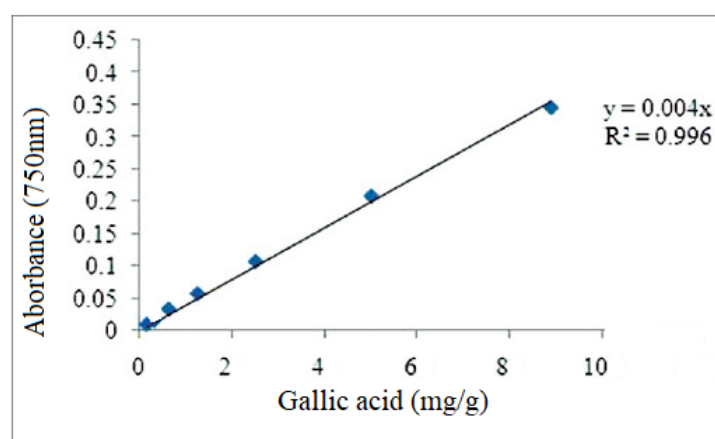


Figure 1. Gallic Acid Standard Curve for Total Phenolic Content

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

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 4°C ( $P < 0.05$ ) (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 <sup>b</sup> | 4.64±0.06 <sup>a</sup>  | 3.93±0.14 <sup>d</sup> |
|               | 7                  | 0.85±0.01 <sup>b</sup> | 4.53±0.01 <sup>ab</sup> | 4.59±0.41 <sup>c</sup> |
|               | 14                 | 0.89±0.03 <sup>c</sup> | 4.42±0.01 <sup>bc</sup> | 4.81±0.33 <sup>c</sup> |
|               | 21                 | 0.91±0.03 <sup>c</sup> | 4.34±0.08 <sup>c</sup>  | 5.35±0.16 <sup>b</sup> |
| Y1            | 1                  | 0.82±0.04 <sup>b</sup> | 4.65±0.01 <sup>a</sup>  | 3.80±0.29 <sup>d</sup> |
|               | 7                  | 0.83±0.07 <sup>b</sup> | 4.52±0.01 <sup>ab</sup> | 3.99±0.23 <sup>d</sup> |
|               | 14                 | 0.88±0.02 <sup>c</sup> | 4.48±0.01 <sup>bc</sup> | 5.55±0.11 <sup>b</sup> |
|               | 21                 | 0.91±0.01 <sup>c</sup> | 4.35±0.06 <sup>c</sup>  | 6.22±0.10 <sup>b</sup> |
| Y2            | 1                  | 0.81±0.01 <sup>b</sup> | 4.64±0.01 <sup>a</sup>  | 3.80±0.10 <sup>d</sup> |
|               | 7                  | 0.82±0.01 <sup>b</sup> | 4.54±0.01 <sup>b</sup>  | 4.39±0.23 <sup>c</sup> |
|               | 14                 | 0.85±0.05 <sup>b</sup> | 4.47±0.01 <sup>bc</sup> | 5.87±0.47 <sup>b</sup> |
|               | 21                 | 0.89±0.01 <sup>c</sup> | 4.36±0.01 <sup>c</sup>  | 6.42±0.41 <sup>b</sup> |
| Y3            | 1                  | 0.79±0.06 <sup>a</sup> | 4.65±0.01 <sup>a</sup>  | 3.80±0.08 <sup>d</sup> |
|               | 7                  | 0.82±0.01 <sup>b</sup> | 4.57±0.01 <sup>b</sup>  | 5.69±0.37 <sup>b</sup> |
|               | 14                 | 0.85±0.01 <sup>b</sup> | 4.47±0.01 <sup>bc</sup> | 6.92±0.29 <sup>a</sup> |
|               | 21                 | 0.88±0.01 <sup>c</sup> | 4.38±0.01 <sup>c</sup>  | 7.59±0.11 <sup>a</sup> |

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 4°C 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

| N-value | Storage Time (day) | Study Group                     |                                 |                                 |                                 |
|---------|--------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|         |                    | Control                         | Y1                              | Y2                              | Y3                              |
|         | 1                  | 0.501<br>(0.001) <sup>d,B</sup> | 0.238<br>(0.001) <sup>b,A</sup> | 0.234<br>(0.001) <sup>a,A</sup> | 0.232<br>(0.002) <sup>a,A</sup> |
|         | 7                  | 0.509<br>(0.003) <sup>c,B</sup> | 0.238<br>(0.002) <sup>a,A</sup> | 0.236<br>(0.003) <sup>a,A</sup> | 0.236<br>(0.001) <sup>a,A</sup> |

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

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                        |
| <b>Flavor</b>                | 1                  | 4.9 (0.03) <sup>b,A</sup> | 4.2 (0.04) <sup>b,A</sup> | 3.9 (0.08) <sup>b,A</sup> | 3.0 (0.01) <sup>a,A</sup> |
|                              | 21                 | 4.7 (0.00) <sup>b,A</sup> | 4.0 (0.01) <sup>b,A</sup> | 3.9 (0.06) <sup>b,A</sup> | 3.0 (0.00) <sup>a,A</sup> |
| <b>Odor</b>                  | 1                  | 4.5 (0.01) <sup>b,A</sup> | 4.5 (0.09) <sup>b,A</sup> | 3.0 (0.04) <sup>a,A</sup> | 2.0 (0.01) <sup>a,A</sup> |
|                              | 21                 | 4.4 (0.01) <sup>b,A</sup> | 4.5 (0.01) <sup>b,A</sup> | 3.1 (0.00) <sup>a,A</sup> | 2.1 (0.00) <sup>a,A</sup> |
| <b>Texture</b>               | 1                  | 5.0 (0.01) <sup>b,A</sup> | 5.0 (0.01) <sup>b,A</sup> | 4.1 (0.08) <sup>b,A</sup> | 4.1 (0.00) <sup>b,A</sup> |
|                              | 21                 | 5.0 (0.08) <sup>b,A</sup> | 5.0 (0.01) <sup>b,A</sup> | 4.1 (0.01) <sup>b,A</sup> | 4.1 (0.00) <sup>b,A</sup> |
| <b>Overall Acceptability</b> | 1                  | 4.8 (0.01) <sup>b,A</sup> | 4.1 (0.01) <sup>b,A</sup> | 3.0 (0.01) <sup>a,A</sup> | 2.1 (0.00) <sup>a,A</sup> |
|                              | 21                 | 4.7 (0.01) <sup>b,A</sup> | 4.0 (0.06) <sup>b,A</sup> | 2.9 (0.01) <sup>a,A</sup> | 2.0 (0.01) <sup>a,A</sup> |

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        |
| <i>Thymus kotschyanus</i>    | 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.

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