Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition
Nutrition & Food Security Research Center

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2020; 5(4): 296-305 Website: jnfs.ssu.ac.ir

The Effect of Cuminum Essential Oil on Rheological Properties and Shelf Life of Probiotic Yoghurt

Hamed Ahari; PhD [1], Ramona Massoud; PhD [2] & Anousheh Sharifan; PhD [*1]

[1] *Department of Food Sciences and Technology, Sciences and Research Branch, Islamic Azad University, Tehran, Iran.*
[2] *Department of Food Sciences and Technology, Standard organization, Tehran, Iran.*

ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received:7 Dec 2019
Revised: 21 Jun 2020
Accepted: 21 May 2020

****Corresponding author:***
sharyfana@gmail.com
Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Postal code: 1477893855
Tel: +226 70 35 62 27

ABSTRACT

Background: Antimicrobial and antioxidant properties of the essential oils (EOs) are important in food industry. This study investigated the effects of EO from *Cuminum cyminum* on the preservation of yogurt containing *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium bifidum*. **Methods:** The yogurt samples' biological, physicochemical, and sensorial characteristics were evaluated at three levels of 1%, 2% and 3% of EO during the storage (7, 14, and 21 days). **Results:** The bacterial activity declined significantly in all samples during the storage ($P < 0.05$). Adding EO decreased the rate of bacterial growth of both probiotic and starter strains over 21 days. The titratable acidity (TA) and syneresis were increased ($P < 0.05$), while the pH levels were decreased ($P < 0.05$) during the storage. The highest viscosity was 8600 mili paskal secent (mPa.s) in control sample in the first day and 5700 mPa.s for 1% cuminum cyminum essential oil (CEO) on the 21st day. The CEO had no effects on flavor, odor, and overall acceptability. **Conclusion:** Generally, the high level of phenols influenced the biological, physicochemical, and rheological properties of bioyogurts positively.

Keywords: *Yoghurt; Probiotic; Cuminum; Essential oil*

Introduction

The literature showed that consumption of fermented dairy products had therapeutic and nutritional values (Vanegas-Azuero and Gutiérrez, 2018). Probiotics have beneficial effects that can improve the intestinal microflora (Güler and Gürsoy-Balcı, 2011). *Lactobacillus* and *Bifidobacterium* are the most well-known probiotic strains (Lee and Salminen, 2009). The viability of probiotics in yogurt is frequently less than 6 log CFU/g during the storage (Massoud *et al.*, 2015, Sarkar, 2008). On the other hand, the final flavor of yogurt is changed due to the addition of nonvolatile acids (lactic and pyruvic), volatile acids (butyric and acetic), miscellaneous compounds (amino acids), and carbonyl compounds (acetaldehyde and diacetyl) (Güler and Gürsoy-Balcı, 2011, Massoud *et al.*, 2014). Additionally, other factors such as microbial growth, texture, and flavor may be affected in the presence of phenolic compounds because of their interactions with proteins during the fermentation and storage (Da Silva *et al.*, 2017, Massoud *et al.*, 2016).

This paper should be cited as: Ahari H, Massoud R, Sharifan A. ***The Effect of Cuminum Essential Oil on Rheological Properties and Shelf Life of Probiotic Yoghurt.*** *Journal of Nutrition and Food Security (JNFS)*, 2020; 5(4): 296-305.

Essential oils (EOs), defined as aromatic and oily liquids, are extracted from different plants (Otaibi and Demerdash, 2008). The antimicrobial properties of EOs would enhance the shelf life of certain foods (El–Nawawy *et al.*, 1998). The EOs inhibits the growth of pathogens that may contaminate food and microorganisms responsible for food spoilage. Golestan et al. showed the antibacterial effects of *Mentha spicata* and *Mentha aquatic* EOs against *Staphylococcus aureus*, *Lactobacillus reuteri*, *Bifidobacterium animalis*, and *Clostridium perfringens* in kashk (a fermented dairy product) (Golestan *et al.*, 2016). Yangilar et al. successfully produced probiotic yogurt with ginger and chamomile EO (0.2 and 0.4%). The probiotic yogurt and the yogurt with ginger EO (0.4%) contained the highest rate of probiotics (*B. lactis* BB-12) (Yangilar and Yildiz, 2018). Some compounds from rosemary are responsible for the antimicrobial activity specially α-pinene, bornylacetate, and 1, 8-cineole (Hussain *et al.*, 2010).

The aim of the present study was to determine the effects of EO extracted from *Cuminum cyminum* (CEO) on the pH values, survival of microorganisms, syneresis, rheological parameters, and sensory characteristics of bioyogurt containing *Bifidobacterium bifidum* during fridge storage.

Materials and Methods

Plant Material and EO Extraction: *Cuminum cyminum* L. (Green Cumin) was purchased from a local plant shop in Isfahan, Iran. The seeds of cumin were dried at 25 ˚C. Dried seeds of cumin were hydrodistilled for 3 hours with 500 ml of distilled water. The extracted CEO was collected and stored in tightly sealed glass vials in a refrigerator at 4 ˚C.

Gas Chromatography-Mass Spectrometry Analysis of the EOs: The chemical composition of the EO was investigated by a gas chromatograph (Agilent Technologies, USA) associated with a mass spectrophotometer (Agilent Technologies, USA) with HP-5Ms capillary column (30.0m×0.25mm×0.25 µm). The initial temperature was 50 ºC for 2 minutes that subsequently elevated to 200 ºC at a rate of 3.5 ºC/min.

Primary culture preparation: *Bifidobacterium bifidum* PTCC 1644 was obtained from the Iranian Research Organization for Science and Technology. Lyophilized bacteria were transferred into a tube containing 10 ml of MRS broth that were incubated at 37 °C for 24 hours. The bacteria were then cultivated on MRS-bile agar (Merck, Germany) and anaerobically incubated at 37 °C for 48 hours.

Preparation of Bifidobacterium bifidum yogurt containing the CEO: *The* raw milk (2.5% fat) was divided into seven 250 ml containers and heated to 85 °C for 20 min. *Bifidobacterium bifidum* (140 µl), yogurt starter cultures YC-x11 (120 µl) (CHR Hansen, Denmark) with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* added to each container after cooling to 44±1 °C. Afterwards, the CEO (1%, 2%, and 3%) was added into the containers and homogenized. The yogurt samples were placed in an incubator at 43 ± 1 ºC. Incubation ended when the samples reached pH = 4.6.

Bacterial count: 1 ml of the yogurt samples was diluted using 9 ml of sterile peptone water (Merck, Germany). Thereafter, 0.1 ml of dilutions was put on MRS-bile, MRS and M17 agars. The first two plates were incubated at 35-37 °C for three days under the anaerobic conditions, while the latter was subjected to incubation for 2 days at 35–37 ºC. The colonies were counted applying a colony counter (Vinderola and Reinheimer, 1999).

pH and acidity measurement: *The* pH values were measured using a pH meter (Swiss, Metrohm 632). Acidity based on lactic acid amount was measured with titration method using 0.1 N sodium hydroxide and phenolphthalein (ISIRI 695).

Syneresis: 10 g of yogurt was put into the centrifuge tubes and weighted (W_0). The supernatant was removed after centrifuging at 350 G at 10 °C for half an hour. The syneresis of yogurt was calculated based on the following formula (Amatayakul, 2005):

$\% Rs = We / Wg \times 100$

Where, we is the weight of whey released from yogurt and Wg is the initial weight of yogurt.

Rheological properties: The flow behavior parameters, including apparent viscosity, consistency index, and flow index were measured using co-axial cylinders (Rheostress 300, ThermoHaake, Germany). The analysis was performed in triplicate with the shear rate varying from 0 to 22 s^{-1} in up-down-up steps. Thixotropic index refers to the area between the upward and downward shear stress curves. The Herschell-Bulkley model was utilized for fitting data (Karsheva *et al.*, 2013):

$$\sigma=\sigma_0+k\dot{\gamma}^{n}$$

Where, σ is the shear stress (Pa), σ_0 is the yield stress (Pa), k is the consistency index ($Pa.s^n$), γ is the shear rate (s^{-1}), and n is the flow index (dimensionless). The apparent viscosity, η, was described as the ratio of shear stress, σ, to shear rate, γ.

Sensory evaluation: 10 experienced panelists evaluated the aroma, flavor, texture, and overall acceptance. All organoleptic attributes were rated by a five-point hedonic scale from one to five; strongly dislike (1) to strongly like (5) (Ahmed, 2011).

Data analysis: The cell counts and physiochemical properties were expressed as mean ± standard deviation (SD) of three replicates using SPSS (version 20.0, SPSS Inc.). Analysis of variance test and Duncan's multiple range tests were used to examine the significant difference. A P-value of less than 0.05 was considered as highly significant.

Results

The chemical composition of the cuminum cyminum essential oil (CEO): The compounds of the CEO are shown in **Table 1**. The major 3 constituents of the CEO out of 18 were Cumin aldehyde (55%), γ-terpinene (14.01%), and Limonene (10.04%). The mean total phenol content of the CEO was 31.41 ± 0.25 mg gallic acid/g.

Bacterial activity**:** Microbiological counts of *B. bifidum*, *L. bulgaricus*, and *S. thermophilus* are presented in **Table 2**. The probiotic bacteria and starter cultures significantly were reduced over 21 days of storage at 4 °C ($P < 0.05$). In this study, the viability of *S. thermophiles* and *L. bulgaricus* showed the maximum decrease of 0.80 and 0.98 log_{10} CFU/g in the control sample during the storage time, respectively.

Titrable acidity (TA) and pH: The addition of EO did not change the TA and pH of control. Storage period had significant changes in both TA and pH ($P < 0.05$) (**Table 3**). TA enhanced in all samples during refrigeration over 21 days and pH decreased in all samples within 21-day storage at 4 ºC ($P < 0.05$). Treatment with 2% and 3% CEO exhibited a significantly lower pH and higher TA.

Syneresis*:* It is determined by the balance between attraction and repulsion forces in casein network (Amatayakul, 2005) . In **Table 3**, syneresis remarkably increased throughout the storage at 4 ºC in the following order (lowest to highest): Control (1.42%), Y1 (1.73%), Y2 (2.64%), and Y3 (2.82%).

Flow behavior: The flow behavior of all yogurt samples is summarized in **Table 4**. The samples showed pseudoplastic fluid characteristics owing to the flow behavior index (n) values below 1 ($R^2 > 0.99$). Adding the EO reduced n values considerably from 0.501 (control sample) to 0.240 (sample with 3% CEO) on the first day ($P< 0.05$). The refrigerator storage only changes n values of the control sample significantly ($P < 0.05$). The higher concentrations of EO did not change the flow behavior towards the dilatant fluid (n > 1). The consistency index notably increased from 8.24×10^{-2} Pascal secend ($Pa.s^n$) (control sample) to 10.63×10^{-2} $Pa.s^n$ (sample with 1% CEO) on the first day of storage ($P < 0.05$).

Sensory evaluation: The overall scores of aroma, odor, texture, and acceptance for the yogurt samples are demonstrated in **Table 5**. The control samples had the highest mean score of flavor, odor, texture, and acceptability. The yogurt samples containing 2% and 3% CEO represented the lowest sensory scores. The refrigerator storage did not affect the organoleptic properties significantly ($P > 0.05$).

Table 1. The chemical composition of the cuminum cyminum essential oil (CEO)

Compound	Retention index	Peak area
α-Thujene	930	1.05
α-pinene	938	0.47
Sabenene	981	0.37
β-pinene	987	5.38
Myrcene	995	1.07
α-phellandrene	1000	0.94
p-cymene	1013	0.60
Limonene	1028	10.04
1,8-cineole	1031	0.10
γ-terpinene	1072	14.01
m-cymenene	1085	0.20
trans-pinocarveol	1130	0.07
Cumin aldehyde	1167	55.00
p-cymene-8-ol	1182	0.40
α-terpineol	1188	0.20
Myrtenol	1195	0.10
o-cumenol	1196	0.10
Carvacrol	1299	5.40

Table 2. The survival of the starter and probiotic bacteria in the different yogurts during storage

Yogurt samples	Storage time (days)	*B. bifidum*	*L. bulgaricus*	*S. thermophilus*
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	8.16 ± 0.01^{f}	6.62 ± 0.05^{cd}	7.12 ± 0.01^{f}
	7	8.01 ± 0.07^{e}	6.84 ± 0.09^{d}	7.14 ± 0.01^{f}
	14	7.80 ± 0.09^{d}	6.72 ± 0.01^{d}	6.72 ± 0.00^{cd}
	21	7.48 ± 0.01^{c}	6.98 ± 0.06^{d}	6.88 ± 0.03^{d}
Y2	1	8.09 ± 0.01^{e}	6.84 ± 0.04^{d}	7.04 ± 0.02^{e}
	7	7.74 ± 0.07^{d}	7.19 ± 0.04^{e}	7.17 ± 0.05^{f}
	14	7.24 ± 0.06^{c}	7.03 ± 0.00^{de}	6.84 ± 0.06^{d}
	21	6.01 ± 0.09^{a}	6.82 ± 0.03^{d}	6.95 ± 0.04^{e}
Y3	1	8.01 ± 0.02^{e}	7.25 ± 0.08^{ef}	6.94 ± 0.01^{e}
	7	7.53 ± 0.06^{cd}	8.00 ± 0.06^{g}	7.60 ± 0.01^{g}
	14	6.84 ± 0.05^{b}	7.72 ± 0.01^{f}	6.80 ± 0.02^{d}
	21	5.85 ± 0.07^{a}	7.50 ± 0.06^{f}	6.82 ± 0.00^{d}

Mean and standard deviation values with different letters are significantly different ($P < 0.05$). Probiotic yogurt with no essential oil: Y1 (1% cuminum cyminum essential oil), Y2 (2% cuminum cyminum essential oil and Y3 (3% cuminum cyminum essential oil)

Table 3. The effect of essential oils and storage on the titrable acidity , pH, and syneresis of the probiotic yogurt samples

Yogurt samples	Storage time (day)	*Titrable acidity* (%)	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	0.79 ± 0.05^{a}	4.62 ± 0.05^{a}	4.01 ± 0.50^{d}
	7	0.83 ± 0.01^{b}	4.51 ± 0.04^{ab}	4.54 ± 0.71^{c}
	14	0.87 ± 0.01^{c}	4.40 ± 0.01^{bc}	4.36 ± 0.00^{c}
	21	0.90 ± 0.01^{c}	4.35 ± 0.01^{c}	5.74 ± 0.83^{b}
Y2	1	0.79 ± 0.01^{a}	4.61 ± 0.01^{a}	3.75 ± 0.41^{d}
	7	0.82 ± 0.01^{b}	4.55 ± 0.01^{b}	4.82 ± 0.00^{c}
	14	0.83 ± 0.01^{b}	4.41 ± 0.09^{bc}	5.09 ± 0.85^{c}
	21	0.88 ± 0.02^{c}	4.37 ± 0.01^{c}	6.39 ± 0.08^{b}
Y3	1	0.78 ± 0.01^{a}	4.63 ± 0.01^{a}	4.05 ± 0.50^{d}
	7	0.81 ± 0.01^{b}	4.51 ± 0.01^{ab}	5.79 ± 0.50^{b}
	14	0.84 ± 0.07^{b}	4.42 ± 0.08^{bc}	6.13 ± 0.25^{b}
	21	0.87 ± 0.07^{c}	4.39 ± 0.01^{bc}	6.87 ± 0.14^{a}

Mean and standard deviation values with different letters are significantly different ($P < 0.05$). Probiotic yogurt with no essential oil: Y1 (1% cuminum cyminum essential oil), Y2 (2% cuminum cyminum essential oil and Y3 (3% cuminum cyminum essential oil)

Table 4. Rheological parameters from the Herschel–Bulkley model and thixotropic index for the yogurt with the essential oils during storage

Variables	Storage time (day)	Study groups			
		Control	Y1	Y2	Y3
Behavior index	1	0.50 ± 0.001^{d,B}	0.24 ± 0.002^{c,A}	0.24±0.004^{bc,A}	0.24 ± 0.002^{b,A}
	7	0.50 ± 0.003^{c,B}	0.24 ± 0.001^{b,A}	0.24 ± 0.001^{b,A}	0.24 ± 0.003^{b,A}
	14	0.49 ± 0.001^{c,B}	0.24 ± 0.001^{b,A}	0.24 ± 0.002^{b,A}	0.24 ± 0.001^{b,A}
	21	0.41 ± 0.004^{c,A}	0.24 ± 0.003^{b,A}	0.23±0.003^{ab,A}	0.23 ± 0.001^{b,A}
Consistency index (×10^{2} Pascal-secnds)	1	8.24 ± 1.05^{a,B}	10.63 ± 2.12^{b,B}	11.27 ± 2.05^{c,A}	10.71 ± 1.00^{b,AB}
	7	8.00 ± 2.02^{a,A}	10.22 ± 1.04^{b,A}	11.25 ± 2.17^{c,A}	10.60 ± 1.08^{b,A}
	14	8.45 ± 1.46^{a,C}	10.89 ± 1.43^{b,C}	11.39 ± 1.26^{c,B}	10.98 ± 2.01^{b,C}
	21	8.32 ± 3.08^{a,B}	10.71 ± 2.35^{b,BC}	11.25 ± 2.07^{c,A}	10.85 ± 2.73^{b,B}
Apparent viscosity (×10^{2} Pa.s)	1	0.86 ± 0.01^{c,B}	0.69 ± 0.02^{b,B}	0.49 ± 0.01^{a,B}	0.42 ± 0.02^{a,B}
	7	0.80 ± 0.02^{c,A}	0.64 ± 0.01^{b,AB}	0.45 ± 0.00^{a,AB}	0.39 ± 0.02^{a,B}
	14	0.78 ± 0.01^{c,A}	0.60 ± 0.02^{b,A}	0.41 ± 0.03^{a,A}	0.34 ± 0.00^{a,AB}
	21	0.75 ± 0.00^{c,A}	0.57 ± 0.00^{b,A}	0.38 ± 0.01^{a,A}	0.30 ± 0.01^{a,A}
Thixotropic index (Pa.s^{-1})	1	21.50 ± 1.16^{c,B}	14.70 ± 1.11^{b,B}	9.25 ± 0.12^{ab,B}	7.25 ± 1.18^{a,A}
	7	20.06 ± 1.13^{c,AB}	13.78 ± 1.08^{b,AB}	9.12 ± 0.12^{ab,B}	6.15 ± 1.14^{a,A}
	14	18.40 ± 1.12^{b,A}	14.18 ± 1.17^{b,B}	8.13 ± 1.03^{a,AB}	6.04 ± 1.12^{a,A}
	21	19.64 ± 1.10^{c,AB}	11.45 ± 0.17^{b,A}	6.28 ± 0.06^{a,A}	7.00 ± 0.12^{a,A}

Mean and standard deviation values with different letters are significantly different ($P < 0.05$). Probiotic yogurt with no essential oil: Y1 (1% cuminum cyminum essential oil), Y2 (2% cuminum cyminum essential oil and Y3 (3% cuminum cyminum essential oil)

Table 5. Sensory attributes of the control and supplemented yogurts according to a 5-point hedonic scale during the storage

Variables	Storage time (day)	Study groups			
		Control	Y1	Y2	Y3
Flavor	1	4.9 ± 0.03^{b,A}	2.6 ± 0.07^{a,A}	1.3 ± 0.06^{a,A}	1.0 ± 0.04^{a,A}
	21	4.7 ± 0.00^{b,A}	2.8 ± 0.01^{a,A}	1.4 ± 0.05^{a,A}	1.0 ± 0.04^{a,A}
Odor	1	4.5 ± 0.01^{b,A}	2.0 ± 0.05^{a,A}	1.0 ± 0.07^{a,A}	1.0 ± 0.07^{a,A}
	21	4.4 ± 0.01^{b,A}	2.1 ± 0.04^{a,A}	1.1 ± 0.00^{a,A}	1.0 ± 0.01^{a,A}
Texture	1	5.0 ± 0.01^{b,A}	4.1 ± 0.00^{b,A}	3.1 ± 0.00^{a,A}	3.1 ± 0.00^{a,A}
	21	5.0 ± 0.08^{b,A}	4.0 ± 0.01^{b,A}	3.0 ± 0.06^{a,A}	2.8 ± 0.00^{a,A}
Overall acceptability	1	4.8 ± 0.01^{b,A}	3.1 ± 0.00^{a,A}	2.1 ± 0.06^{a,A}	1.1 ± 0.07^{a,A}
	21	4.7 ± 0.01^{b,A}	2.9 ± 0.01^{a,A}	1.9 ± 0.01^{a,A}	1.0 ± 0.00^{a,A}

Mean and standard deviation values with different letters are significantly different ($P < 0.05$). Probiotic yogurt with no essential oil: Y1 (1% cuminum cyminum essential oil), Y2 (2% cuminum cyminum essential oil and Y3 (3% cuminum cyminum essential oil)

Discussion

Some studies were conducted over the chemical composition of EO obtained from *Cuminum cyminum* indigenous in Iran. The main component of EO is cumin aldehyde (46.10%) and β-pinene (10.93%) (Mehdizadeh *et al.*, 2017). Mohammadpour et al. identified 28 compounds in the CEO with α-pinene (29.2%), Limonene (21.7%), 1, 8-cineole (18.1%), and Linalool (10.5%) (Mohammadpour *et al.*, 2012). The CEO's chemical composition was determined with almost similar main constituents of Cumin aldehyde 25.2%, γ-terpinene 19.0%, ρ-mentha-1, 4-dien -7-al 16.6%, and ρ-mentha-1, and 3-dien-7-al 13.0% (Derakhshan *et al.*, 2010).

Many studies reported the effects of different additives on the bacterial activity in yogurt. Blackcurrant polyphenol extract and cyanidin (cyanidin 3-*o*-*β*-glucopyranoside chloride) were added to yogurt (Sun-Waterhouse *et al.*, 2013) .The findings indicated that some bindings took place between phenolic compounds and milk proteins or polysaccharides in the yogurt (Papadopoulou and Frazier, 2004). The interaction between carbonyl compounds and phenolic compounds might also occur and form carbonyl–phenol bonds (Hidalgo *et al.*, 2017). In this study, adding EOs with different phenolic profiles altered the starter culture activity during the storage. This finding was in accordance with the study by (Da Silva *et al.*, 2017, Hadad Khodaparast *et al.*, 2007). revealed that the survival of yogurt starter culture decreased significantly by increase of the concentrations of *Ziziphora clinopodioides* EO, (Hadad Khodaparast *et al.*, 2007). Jimborean et al. reported that the yogurt incorporated with orange EOs showed an increased growth of the lactic acid bacteria (Jimborean *et al.*, 2016). Phenolic compounds possess different antimicrobial properties (Rauha *et al.*, 2000). Additionally, the bacteria type may be related to the antimicrobial properties (Lee *et al.*, 2006). *Bifidobacterium* demonstrated a lower viability in the presence of CEO: 0.68-2.16 $\log_{10}$ CFU/g during storage. Marhamatizadeh et al. reported a relation between the growth of *B. bifidum* and the concentrations of olive and dill extract (Marhamatizadeh *et al.*, 2013). In another study, they indicated that the bioavailability of lactic acid bacteria improved in the presence of coffee extract (Marhamatizadeh *et al.*, 2014) .de Lancey et. al. noted that the presence of green tea extracts increased the survival of *L. paracasei*, *L. acidophilus*, and *B. animalis* ssp. *lactis* during the incubation at 37 ºC for three days (de Lacey *et al.*, 2014) .

There are some studies on the effect of EOs on physical and chemical characteristics of yogurt. Ghalem and Zouaoui reported that the yogurt samples containing 0.36 g/l of *Chamaemelum* spp. extract showed an increased level of TA during the storage (Ghalem and Zouaoui, 2013b). Moritz et al. observed significantly higher TA in yogurt samples containing mint EOs (Moritz *et al.*, 2012). Yangilar and Yildiz reported that the yogurt samples with ginger and chamomile EOs had higher acidity levels (Yangilar and Yildiz, 2018) .Joung et al. reported that yogurt samples with *Nelumbo nucifera* had lower pH (Joung *et al.*, 2016). Furthermore, pH varied within the range of 4.08 and 4.66 in yogurt samples with *Chamaemelum* extract. In another study, they showed unchanged levels of pH in the yogurt samples with *R. officinalis* EO during the storage period (Ghalem and Zouaoui, 2013a). The lactic acid production correlated to the viable counts of probiotic strain and prevented the growth of starter cultures due to acid production during the storage (Moritz *et al.*, 2012). Shahdadi et al. also reported similar results; EOs reduced the lactic acid production, which in turn increased the survival of the probiotics (Shahdadi *et al.*, 2015) .

The synersis is the most important parameter that influenced consumer's acceptance level (Gürsoy *et al.*, 2010). Synersis is possibly due to the effect of low pH on casein particles, which improves the resistance of yogurt (Lucey and Singh, 1997) .Other factors resulting in synersis include high incubation temperature, low dry matter content, or high storage temperature (Lucey, 2004) .In a study, the synersis rate was elevated from 4.7% (v/w) to 8.3% (v/w) over 28 days (Panesar and Shinde, 2012a, b). In another study synersis increased by elevating the CEO concentration. The polyphenols would enhance rearrangements, which leads to a larger pore size in the gel matrix and higher synersis (Gürsoy *et al.*, 2010) .Phenolic compounds can establish strong links with the primary metabolites, including proteins and carbohydrates (McManus *et al.*, 1985).

The high flow behavior was also reported for the probiotic yogurt with grape extract (Da Silva *et al.*, 2017) .The higher concentrations of EO did not change the flow behavior towards the dilatant fluid ($n > 1$)(Da Silva *et al.*, 2017) .

According to **Table 4**, the EOs significantly mitigated the apparent viscosity of probiotic yogurts ($P < 0.05$), and the control sample showed the highest rate on the first day (i.e., 8600 mPa.s), while the sample containing 3% CEO had the lowest rate on the 21st day (i.e., 3000 mPa.s). The same trend was also observed by Vanegas-Azuero and Gutiérrez, 2018. Some reports pointed out that the positive contribution of the rearrangement of proteins increased during storage (Abu-Jdayil and Mohameed, 2002, Isleten and Karagul-Yuceer, 2006)

On the other hand, the results of thixotropic tests presented that the yogurt samples had a thixotropic non-Newtonian behavior. This phenomenon results from the breakage of the gel when a shear force is used (Bourne, 2002). The thixotropic characteristic of the control sample was higher than other samples. The values of the hysteresis area were notably affected by increasing the content of EOs during the storage period (**P** < *0.05*). Yogurts without any additive had notably higher sensory scores for color, appearance, flavor, texture, synersis, odor, acidity, and general acceptability than the controls (Vanegas-Azuero and Gutiérrez, 2018, Yangilar and Yildiz, 2018)

Conclusion

This study indicated that yogurt supplemented with CEO had a notable potential to deliver *Bifidobacterium bifidum* with sufficient population. The lowest pH and highest acidity rates belonged to groups with 1% CEO. Statistical analyses revealed that during the refrigerated storage, the bacterial counts, pH, sensory characteristics, and viscosity reduced, while the acidity values and sensory scores increased. Generally, the CEO contributed positively to the biological, physicochemical, and rheological properties of the bioyogurts, but not to the overall acceptance.

Acknowledgment

The authors acknowledge the Sciences and Research Branch of Islamic Azad University that cooperate for doing this work.

Conflicts of Interest

The team of authors declares that there is no conflict of interest.

Authors' contribution

All authors contributed to data gathering, analyzing, and writing the manuscript and final revising it.

References

Abu-Jdayil B & Mohameed H 2002. Experimental and modelling studies of the flow properties of concentrated yogurt as affected by the storage time. *Journal of Food Engineering*. **52 (4)**: 359-365.

Ahmed IAM 2011. Physicochemical, microbiological and sensory characteristics of yoghurt produced from camel milk during storage. *Electronic Journal of Environmental, Agricultural and Food Chemistry (EJEAFChe)*. **10 (6)**: 2305-2313.

Amatayakul T 2005. The improvement of physical properties of yoghurts by varying casein/whey protein ratio and EPS-producing starter cultures. Victoria University.

Bourne M 2002. Food texture and viscosity: concept and measurement. Elsevier.

Da Silva DF, et al. 2017. Physical, microbiological and rheological properties of probiotic yogurt supplemented with grape extract. *Journal of Food Science and Technology*. **54 (6)**: 1608-1615.

de Lacey AL, Pérez-Santín E, López-Caballero M & Montero P 2014. Survival and metabolic activity of probiotic bacteria in green tea. *LWT-Food Science and Technology*. **55 (1)**: 314-322.

Derakhshan S, Sattari M & Bigdeli M 2010. Effect of cumin (Cuminum cyminum) seed essential oil on biofilm formation and plasmid Integrity of Klebsiella pneumoniae. *Pharmacognosy Magazine*. **6 (21)**: 57.

El–Nawawy M, El–Kenany Y & Abd El–Ghaffar E 1998. Effect of some herb plants on the use of yoghurt culture. In *Annals of agriculture Sci. 7th. Conf. Agric. Dev. Res. Fac. Agric. Ain Shams University of Cairo, Egypt*, pp. 15-17.

Ghalem BR & Zouaoui B 2013a. Evaluation of the quality of steamed yogurt treated by Lavandula and Chamaemelum species essential oils. *Journal of Medicinal Plants Research*. **7 (42)**: 3121-3126.

Ghalem BR & Zouaoui B 2013b. Microbiological, physico-chemical and sensory quality aspects of yoghurt enriched with Rosmarinus officinalis oil. *African Journal of Biotechnology*. **12 (2)**.

Golestan L, Seyedyousefi L, Kaboosi H & Safari H 2016. Effect of M entha spicata L. and M entha aquatica L. essential oils on the microbiological properties of fermented dairy product, kashk. *International Journal of Food Science & Technology*. **51 (3)**: 581-587.

Güler Z & Gürsoy-Balcı AC 2011. 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 Chemistry*. **127 (3)**: 1065-1071.

Gürsoy A, Durlu-Özkaya F, Yildiz F & Aslim B 2010. 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*. **16**: S81-S86.

Hadad Khodaparast MH, Mehraban Sangatash M, Karazhyan R, Habibi Najafi MB & Beiraghi Toosi S 2007. Effect of essential oil and extract of Ziziphora clinopodioides on yoghurt starter culture activity. *World Sci. J.* **2**: 194-197.

Hidalgo FJ, Aguilar I & Zamora R 2017. Model studies on the effect of aldehyde structure on their selective trapping by phenolic compounds. *Journal of Agricultural and Food Chemistry*. **65 (23)**: 4736-4743.

Hussain AI, et al. 2010. Rosmarinus officinalis essential oil: antiproliferative, antioxidant and antibacterial activities. *Brazilian Journal of Microbiology*. **41 (4)**: 1070-1078.

Isleten M & Karagul-Yuceer Y 2006. Effects of dried dairy ingredients on physical and sensory properties of nonfat yogurt. *Journal of Dairy Science*. **89 (8)**: 2865-2872.

Jimborean MA, et al. 2016. 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*. **73 (1)**: 24-27.

Joung JY, et al. 2016. Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts. *Korean Journal for Food science of Animal Resources*. **36 (1)**: 90.

Karsheva M, Paskov V, Tropcheva R, Georgieva R & Danova S 2013. Physichochmical parameters and rheological properties of yoghurs during the storage. *Journal of Chemical Technology & Metallurgy*. **48 (5)**.

Lee HC, Jenner AM, Low CS & Lee YK 2006. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Research in Microbiology*. **157 (9)**: 876-884.

Lee YK & Salminen S 2009. Handbook of probiotics and prebiotics. John Wiley & Sons.

Lucey J & Singh H 1997. Formation and physical properties of acid milk gels: a review. *Food Research International*. **30 (7)**: 529-542.

Lucey JA 2004. Cultured dairy products: an overview of their gelation and texture properties. *International Journal of Dairy Technology*. **57 (2-3)**: 77-84.

Marhamatizadeh M, Ehsandoost E & Gholami P 2014. The effect of coffee extract on the growth and viability of Lactobacillus acidophilus and Bifidobacterium bifidum in probiotic milk and yoghurt. *Journal of Food Biosciences and Technology*. **4 (JFBT (Vol. 4-No. 1))**: 37-48.

Marhamatizadeh MH, Ehsandoost E, Gholami P & Mohaghegh MD 2013. Effect of olive leaf extract on growth and viability of Lactobacillus acidophilus and Bifidobacterium bifidum for production of probiotic milk and yoghurt. *Intl J Farm & Alli Sci*. **2 (17)**: 572-578.

Massoud R, Belgheisi S & Massoud A 2016. Effect of high pressure homogenization on improving the quality of milk and sensory properties of yogurt: a review. *International Journal of Chemical Engineering and Applications*. **7 (1)**: 66.

Massoud R, FADAEI NV & KHOSRAVI DK 2014. The effect of homogenization pressure and stages on the amounts of Lactic and Acetic acids of probiotic yoghurt.

Massoud R, Fadaei V, Khosravi-Darani K & Nikbakht HR 2015. Improving the viability of probiotic bacteria in yoghurt by homogenization. *Journal of Food Processing and Preservation*. **39 (6)**: 2984-2990.

McManus JP, et al. 1985. Polyphenol interactions. Part 1. Introduction; some observations on the reversible complexation of polyphenols with proteins and polysaccharides. *Journal of the Chemical Society, Perkin Transactions 2*.**(9)**: 1429-1438.

Mehdizadeh L, Ghasemi Pirbalouti A & Moghaddam M 2017. Storage stability of essential oil of cumin (Cuminum Cyminum L.) as a function of temperature. *International Journal of Food Properties*. **20 (sup2)**: 1742-1750.

Mohammadpour H, et al. 2012. Chemical composition and antifungal activity of Cuminum cyminum L. essential oil from Alborz mountain against Aspergillus species. *Jundishapur Journal of Natural Pharmaceutical Products*. **7 (2)**: 50.

Moritz CMF, Rall VLM, Saeki MJ & Fernandes Júnior A 2012. Inhibitory effect of essential oils against Lactobacillus rhamnosus and starter culture in fermented milk during its shelf-life period. *Brazilian Journal of Microbiology*. **43 (3)**: 1147-1156.

Otaibi MA & Demerdash HE 2008. Improvement of the quality and shelf life of concentrated yoghurt (labneh) by the addition of some essential oils. *African Journal of Microbiology Research*. **2 (7)**: 156-161.

Panesar P & Shinde C 2012a. Effect of storage on synersis, pH, Lactobacillus acidophilus count, Bifidobacterium bifidum count of Aloe vera fortified probiotic yoghurt. *Current Rsearch in Dairy Sciences*. **4 (1)**: 17-23.

Panesar P & SHINDE C 2012b. Effect of storage on synersis, pH, Lactobacillus acidophilus count, Bifidobacterium bifidum count of Aloe vera fortified probiotic yoghurt.

Papadopoulou A & Frazier RA 2004. Characterization of protein–polyphenol interactions. *Trends in Food Science & Technology*. **15 (3-4)**: 186-190.

Rauha J-P, et al. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology*. **56 (1)**: 3-12.

Sarkar S 2008. Effect of probiotics on biotechnological characteristics of yoghurt. *British Food Journal.*

Shahdadi F, et al. 2015. Effects of various essential oils on chemical and sensory characteristics and activity of probiotic bacteria in drinking yoghurt. *Agricultural Communications.* **3 (1)**: 16-21.

Sun-Waterhouse D, Zhou J & Wadhwa SS 2013. Drinking yoghurts with berry polyphenols added before and after fermentation. *Food Control*. **32 (2)**: 450-460.

Vanegas-Azuero A-M & Gutiérrez L-F 2018. Physicochemical and sensory properties of yogurts containing sacha inchi (Plukenetia volubilis L.) seeds and β-glucans from Ganoderma lucidum. *Journal of Dairy Science.* **101 (2)**: 1020-1033.

Vinderola C & Reinheimer J 1999. Culture media for the enumeration of Bifidobacterium bifidum and Lactobacillus acidophilus in the presence of yoghurt bacteria. *International Dairy Journal*. **9 (8)**: 497-505.

Yangilar F & Yildiz PO 2018. Effects of using combined essential oils on quality parameters of bio-yogurt. *Journal of Food Processing and Preservation.* **42 (1)**: e13332.