An assessment of *Cuminum cyminum* (Boiss) essential oil, NaCl, bile salts and their combinations in probiotic yogurt

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Abstract

This article is prepared to investigate the impacts of *Cuminum cyminum* essential oil (CEO), NaCl, bile salts, and their combinations on the viability of *Lactobacillus casei* in probiotic yogurt.

The water distillation method was used to extract the CEO, and GC/MS was used to determine its constituents. Then, the CEO's antibacterial activity, together with NaCl and bile salts, was investigated via the microdilution technique by determining the minimum inhibitory concentration (MIC) against *L. casei.* Further, the stress effects of 50% MIC on CEO, NaCl, and bile salts were examined by comparing the stress treatments with the control in terms of the *L. casei* population, pH, acidity, and syneresis percentage in probiotic yogurt during storage in the refrigerator for 28 days.

According to the results, the *L. casei* population and pH decreased in all the treatments during the storage time, such that the intensity of the decrease in the control and CEO treatments was lesser than in other stress treatments ($P<0.05$). The acidity and percentage of syneresis during the storage time increased for all the treatments, with the increase being less in control and CEO than in the other stress treatments ($P<0.05$). The control and CEO treatments scored the highest in the sensory evaluation ($P<0.05$).

Applying stresses below the MIC resulted in the survival of *L. casei* in the recommended amount (10^5–10^6 CFU mL^{-1}) in the probiotic yogurt until the end of 28 days.

Keywords: *Cuminum cyminum*, probiotic, yogurt, *Lactobacillus casei,* Stress, Essential oil

Introduction

Today, particular attention is being given to functional foods, which have nutritional value as well as positive effects on human health. People are excited about eating products containing probiotics. However, products containing probiotics are amongst the ones in which there have been health allegations. These allegations are even advertised in the media during the last few years (Zendeboodi *et al*., 2020). Various studies on probiotics for humans' advantages have focused on the innovative formulation, and some even provided valuable information on probiotics linked to health and well-being. (Roobab *et al*., 2020). Probiotics are microorganisms that improve the gut microbial balance. These often include the *Lactobacillus* and *Bifidobacterium* species,

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as they have a historically prolonged and reliable value. Also, identified as generally recognized as safe (GRAS), all are the predominant inhabitants in the human intestines. (Al-Okbi and Mohamed, 2012; Lucatto *et al*, 2020). *Lactobacillus casei* is a gram-positive, mesophilic, microaerophilic, catalase-negative, and spore-free bacterium and a facultative hetero-formative bacterium with high acid production capacity (Fontana *et al*., 2018). *L. casei* is a probiotic strain linked with antihypertensive antioxidant, antihypocholesterolemic and anticarcinogenic, characteristics (Balthazar *et al*., 2018; Garcia *et al.,* 2019).

Fermented milk, such as yogurts, has the potential to act as a medium for producing value-adding materials because of its favorable sensory attributes, nutritional properties, and high-grade harmony, and ample content of essential nutrients; besides, yogurt consumption enhances gut macrobiotic activity, mitigates immune responses, and increases gastrointestinal functionality by adjusting lactose intolerance. Technologists and manufacturers have reviewed distant fortifications by combining probiotics and nutraceutical compounds (Alizadeh Khaledabad *et al*., 2020; Lucatto *et al*., 2020).

Today, probiotic yogurt is the most popular and widely consumed probiotic product in the world. The survival of probiotic bacteria in yogurt and similar products is an important challenge during storage in probiotic products. The minimum acceptable concentration of probiotic strains for beneficial and therapeutic effects should be at least 10^6–10^7 CFU g^{-1} or mL in the final product (Azizkhani and Parsaeimehr, 2018). The main problem in production was maintaining the survival rate of probiotic strains during storage of product with due attention to high acidity, oxygen stress, and nutrient deficiencies. The main reasons for reducing the viability of probiotic strains in the stomach were low pH and bile salts in the intestines (El-Shafei *et al*., 2010). Many studies have been carried out on the viability of probiotics under the stomach's acidic conditions, bile salts of the small intestine (Sahadeva *et al*., 2011), and survival rate of probiotics were studied in the cold storage of foods (Mortazavian *et al*., 2007). Various methods such as microencapsulation, the addition of prebiotics and essential oils (Capela *et al*., 2006), and different procedures were used to increase the survival rate of probiotic strains during storage of functional products applied stresses were less than the minimum inhibitory concentration (MIC). They produced resistance-inducing genes (Maragkoudakis *et al*., 2006). Some essential oils improve probiotics' viability, and others may also decrease the viability of probiotics (Calsamiglia *et al*., 2007).

In many cases, the viability of probiotic bacteria is not sufficient, and it is necessary to evaluate the viability of probiotic bacteria in yogurt by applying different procedures to increase the resistance of bacterial cells against stresses. Hence, the usage of herbal essential oils and probiotic strains in dairy products is a new strategy to overcome pathogenic bacteria and stimulate probiotics. This study aimed to evaluate the effects of stresses less than the MIC of *Cuminum cyminum* Boiss. essential oil (CEO), NaCl (NC), and bile salts (BS) or a combination of them on the viability of probiotic *L. casei* and later we monitored through the storage time, physicochemical and sensory properties of probiotic yogurt.

Materials and Methods

Materials

Cow's milk (3% fat) was supplied from Pegah, Tehran Plant, and Starter culture of yogurt (*Streptococcus thermophilus* and *Lactobacillus bulgaricus* sub spp. *delbrukii*) was purchased from Hanson Company, Denmark.

Extraction and analysis of the essential oil

The *Cuminum cyminum* was collected from the Kerman province of Iran, and the Iranian Institute approved its scientific name of Botanical Garden Research. The plant›s essential oil was extracted by the water vapor distillation method, and then it was analyzed by colorimeter attached to a mass spectrometer (Model HP-6890, USA). HP-5MS capillary column with 30 m length, 0.25 mm inner diameter, and 0.32 µm inner layer thicknesses was used. The regulated programming for identification and quantification was set up as follows: the temperature was elevated from 60 to 265°C, with a flow rate of 2.5°C per min, and then the column was maintained at 265°C for 30 min. The injection room temperature was 250°C, and the flow rate of helium as a carrier gas was 1 mm/min. Finally, the flame ionization detector (FID) identified the essential oil components with an electrical capacity of 70 eV and an ionization source temperature of 250°C.

Preparation of inoculums

Lactobacillus casei (ATCC39392) was supplied from the Microbial Collection of Pasteur Institute of Iran. It remained in laboratory samples in a glycerol stock at –70°C and transferred in Brain Heart Infusion (BHI) broth (Merck, Germany) at 37°C without shaking. Working cultures were prepared from stock cultures in two successive transfers (1% inoculum) in BHI broth at 37°C for 18 h. *L. casei* cells were inoculated from working cultures to BHI broth. After 18 h incubation at 37°C, optical density (OD) (absorbance) of 0.1 at 600 nm,

using a Spectronic 20 spectrophotometer (Varian, USA) was applied for determining the population of *L. casei.* Cell concentration of *L. casei* was 2.2×10^{10} CFU mL^{-1} for inoculation. The enumeration of *L. casei* was performed according to the serial dilution method and cultivating on (BHI) agar (Merck, Germany) after incubation for 24 h at 37°C.

Determination of minimum inhibitory concentration (MIC) of *Cuminum cyminum* Boiss. essential oil, NaCl, and bile salt

A 96-well plate with a volume of 300 µL was used in this experiment. Sequential concentrations of essential oil of *C. cyminum* Boiss (0, 500, 1000, 2000, 3000, 4000, 5000, 7500, and 10. 000 mg L^{-1}), bile salt (0%, 0.05%, 0.02%, 0.03%, 0.06%, 0.07%, 0.08%, 0.1%, 0.2% and 0.3%) and NaCl (0%, 1%, 2%, 3%, 4% and 5%) were used in De Man, Rogosa and Sharpe agar (MRS) broth (Merck, Germany). Media contained 5% DMSO and were transferred to 96-well plates. Then, 250 µL of different concentrations of essential oil, NaCl, and bile salts along with 20 µL of *L. casei* suspension (5×10^6 CFU mL^{-1}) were added to all well. The contents of each well were mixed with a shaker for 2 min. The microplates were closed by Parafilm and then incubated for 24 h at 37°C in an anaerobic jar (Merck, Germany). At the end of incubation time, turbidity or non-turbidity was evaluated in the wells.

Adaptation and challenging conditions

Cultures were at the logarithmic phase. Bacterial cells were separated by centrifugation (Hettich, Germany) and resuspended in fresh BHI broth (non-adapted control culture). The inoculation content of *L. casei* was 1×10^{10} CFU mL^{-1}. Adaptation time was conducted in the same medium at 37°C (I) for 120 min with 1 mL included in 100 mL of 5% DMSO (CEO), (II) 10 g per 100 mL in Mueller Hinton broth medium (Merck, Germany) (NC), (III) 0.05 g per 100 mL in Mueller Hinton broth medium (BS), (IV) 1 mL in 100 mL containing 5% DMSO plus 10 g per 100 mL in Mueller Hinton broth medium (CEONC), (V) 1 mL in 100 mL containing 5% DMSO plus 0.05 g per 100 mL in Mueller Hinton broth medium (CEOBS), (VI) 10 g per 100 mL plus 0.05 g per 100 mL in Mueller Hinton broth medium (NCBS) and (VII) 1 mL in 100 mL containing 5% DMSO, 10 g per 100 mL plus 0.05 g per 100 mL in Mueller Hinton broth medium (CEONCBS). After centrifugation, adapted and non-adapted cells were inoculated to yogurt (10^8 per mL). Enumeration of *L. casei* was conducted by serial dilution method (Most Probable Number) at 0, 7, 14, 21, and 28 days of storage to evaluate survival rate. All the plates were incubated at 37°C for 48 h. Furthermore, all experiments have been done twice.

Physicochemical properties of yogurt samples

The experiments for physicochemical properties were pH, acidity%, and syneresis%. pH and titratable acidity concluded based on the method described by Yangilar and Yildiz (2018). Moreover, the percentage of syneresis was averaged according to the method defined by Wacher-Rodarte *et al*. (1993). Five milliliters of the yogurt sample was centrifuged at 2.208 g for 20 min at 4°C, and the volume of isolated whey was calculated after 1 min. Lastly, we displayed the Syneresis rate (%) as the separated whey volume per 100 g of yogurt (Wacher-Rodarte *et al*., 1993).

Sensory evaluation

Sensorial tests were executed based on a 5-point Hedonic scale. The lowest score was intensely disliked, and the highest score was 5 as remarkably like samples (Shahdadi *et al*., 2015). Sensory properties were measured as follows: flavor, texture, and overall acceptability. Sensory evaluations were carried out during 28 days of storage. Ten trained panelists performed judgments.

Analytical study

All experiments were performed in completely randomized design as triplicates and the result was reported as mean ± SD. The comparisons of data mean were performed by Tukey test. Two-way ANOVA was used for determination of significance or non-significance of data ($P<0.05$).

Results

The chemical combination of *Cuminum cyminum* Boiss. essential oil

Accordingly, 15 compounds were identified that constitute 100% of the essential oils in total. The most abundant essential oil component was Propanal, 2-methyl-3-phenyl. The concentration of them was 24.2%. Next, Gamma-Terpinene, Phenylethanediol, and 2-Beta-Pinene had the highest level of essential oil components, with 18.94%, 18.88%, and 12.59%, respectively (Table 1).

MIC results

The MIC values of the CEO, NC, and BS against *L. casei* were 1%, 4%, and 0.3% (v/v), respectively.

Table 1. GC/MS results of *Cuminum cyminum* (Boiss) essential oil

No.	Name	Area (%)
1	α-Pinene	1.12
2	Sabinene	0.74
3	2-Beta-Pinene	12.59
4	β-Myrcene	0.93
5	l-Phellandrene	1.10
6	Cymene	6.91
7	Limonene	1.73
8	Gamma-terpinene	18.94
9	(E)-4-(Cyclohex-1'-enyl)but-2-en-1-ol	1.88
10	Propanal, 2-methyl-3-phenyl-	24.22
11	2-Caren-10-al	8.80
12	Phenylethanediol	18.89
13	Gamma-cadinene	0.57
14	Trans-beta-farnesene	0.72
15	Carotol	0.86

Survival of *Lactobacillus casei*

The results showed that the interaction effects of treatment and storage time on the *L. casei* population (Figure 1) were significant (P<0.05). The viability of *L. casei* decreased in all treatments except for control and sample under CEO stress at 7 days of storage time (P<0.05). The viability of *L. casei* of all samples significantly decreased during 28 days of storage (P<0.05). Following 28 days of storage time, the highest survival rate of *L. casei* was detected for CEO (6.05±0.03 log CFU mL^{-1}) and control (6.01±0.02 log CFU mL^{-1}) treatments, which was significantly different from others (P<0.05). No significant difference was observed within NC and BS treatments (P>0.05). Nevertheless, the lowest survival rate of *L. casei* was observed in the stress condition treated with CEONCBS at all days of storage time (P<0.05).

pH

The data for pH (Figure 2) showed that the effect of time and type of samples were significant (P<0.05). The pH of all the yogurt samples decreased significantly during the storage period (P<0.05), and higher pH was attributed to samples under stress (except for the CEO) (P<0.05). At the end of 28 days of storage, the highest and lowest pH values for CEONCBS and the control treatments were 4.23±0.06 and 3.91±0.06, respectively (P<0.05). The comparison among all stress treatments showed the highest and lowest pH of samples were attributed to CEO and CEONCBS during storage time, respectively (P<0.05). No significant difference was observed between NC and BS treatments on the same day of storage (except on day 14) (P>0.05).

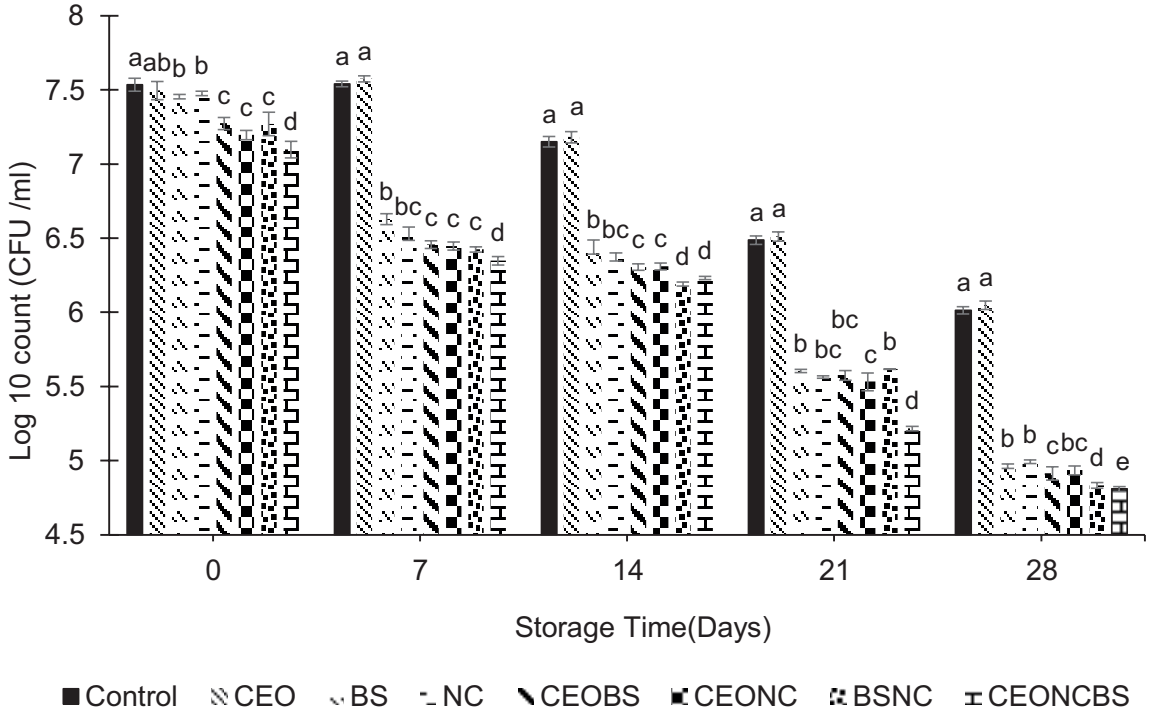

Figure 1. Effect of *Cuminum cyminum* essential oil (CEO), NaCl (NC), bile salts (BS), and their combinations on the survival of *L. casei* ATCC-39392 (log CFU mL^{-1}). Deviation bars designate the standard error of the method (n = 3).

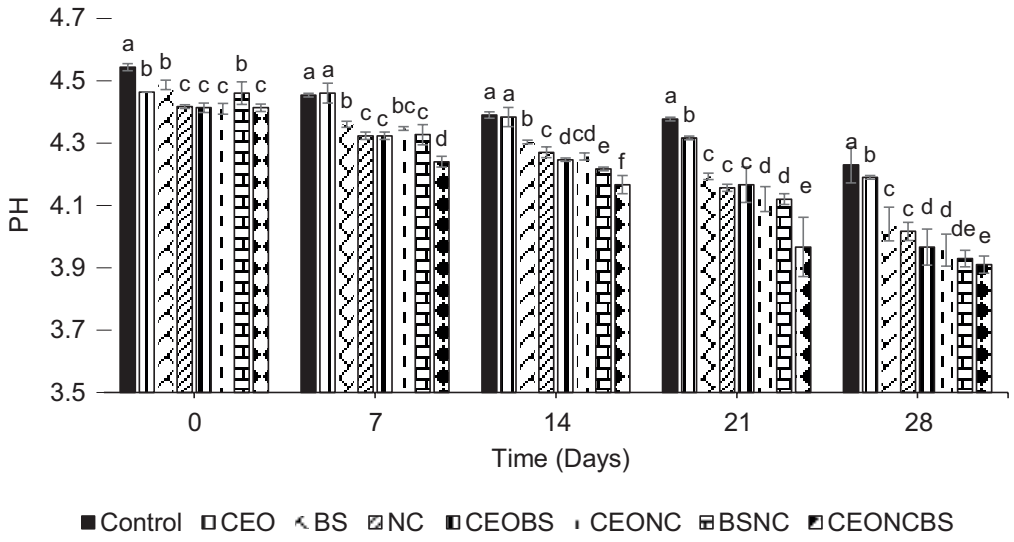

Figure 2. Effect of *Cuminum cyminum* essential oil (CEO), NaCl (NC), bile salts (BS), and their combinations on changes in pH of probiotic yogurt. Deviation bars designate the standard error of the method (n = 3).

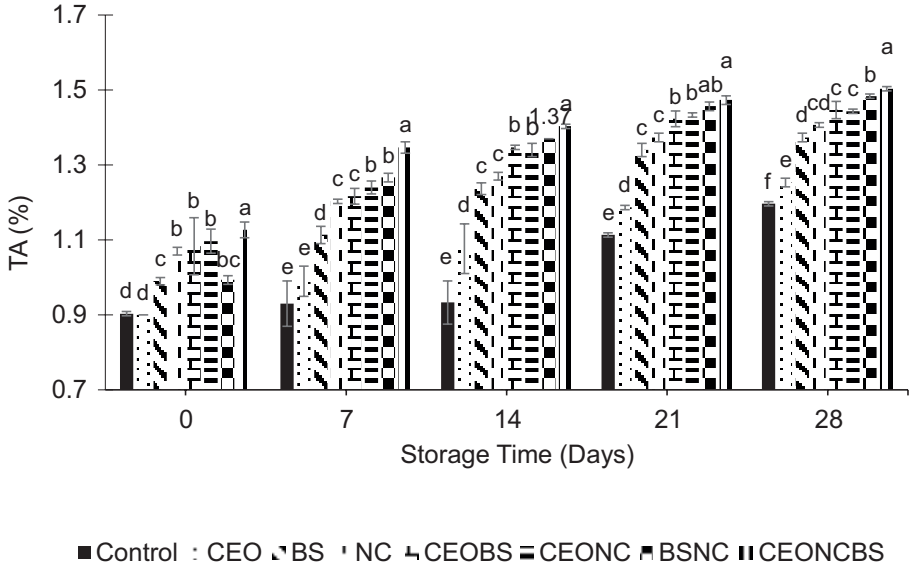

Figure 3. Effect of *Cuminum cyminum* essential oil (CEO), NaCl (NC), bile salts (BS), and their combinations on acidity changes of probiotic yogurt. Deviation bars designate the standard error of the method (n = 3).

Titratable acidity (TA%)

The titratable acidity (Figure 3) results showed that interaction between group and time was significant (P<0.05). The acidity of all samples significantly increased during storage time (P<0.05). The acidity of the control sample was less than those of stress treatments (P<0.05). Treatments CEO and CEONCBS had the highest and lowest acidity, respectively (P<0.05). After 28 days of the storage time, the lowest and highest acidity values were related to treatments under the stress of CEONCBS (1.19±0.008) and control (1.50±0.006), respectively. No significant difference was observed between NC and BC treatment during 28 days of storage (P>0.05).

Syneresis

Results showed that exchange within-group and time was notable (P<0.05). As shown in Figure 4, the amount of syneresis significantly rose in all samples during storage time (P<0.05). The increase in intensity for control and CEO treatments was less than that for stress treatments (P<0.05). Among the stressful treatments, the treatments under stress with CEO and CEONCBS had the lowest and highest percentage of syneresis, respectively, during 28 days of storage (P<0.05). No significant difference was observed between treatments with NC and BC on the same day of storage time (P>0.05). After 28 days of storage time, the lowest and the

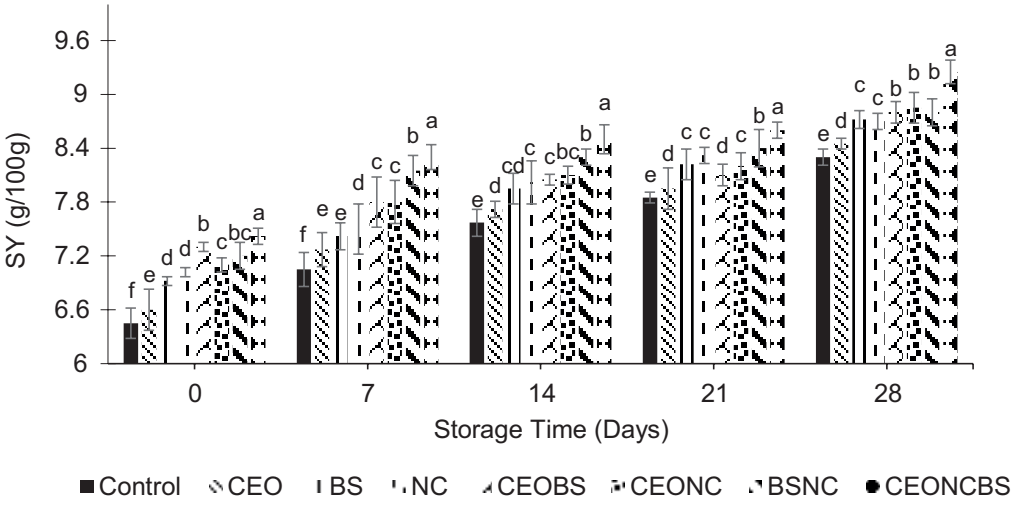

Figure 4. Effect of *Cuminum cyminum* essential oil (CEO), NaCl (NC), bile salts (BS), and their combinations on syneresis changes in probiotic yogurt. Deviation bars designate the standard error of the method (n = 3).

highest percentages of syneresis were observed for control (8.30±0.09) and stress with CEONCBS (9.25±0.13) treatments, respectively.

Sensory properties

Table 2 showed the results of sensory characteristics (flavor, texture, and general acceptance) during 28 days of storage time. The interaction between group and time on sensory characteristics was significant (P<0.05). The sensory scores of examples decreased during storage time. A more severe drop in sensory score was observed for stress treatments except for the CEO (P<0.05). Flavor scores for all treatments showed there were no significant differences among all samples on the first day of storage (P>0.05). The most leading and lowest scores were attributed to control (4±0) and CEONCBS (3.11±0.33) at 28 days of storage time, respectively (P<0.05). The texture feature results showed no significant difference within samples on the first day of storage (P>0.05). The highest and lowest scores were attributed to control (4.33±0.50) and CEONCBS (3.22±0.44) at 28 days of storage time, respectively (P < 0.05). Overall acceptance of samples indicated no significant difference among all samples on the first day of storage (P > 0.05). The highest and lowest scores were attributed to control (4±0) and CEONCBS (3.220 ± 0.44) at 28 days of storage time, respectively (P < 0.05).

Discussion and Conclusion

The effect of MIC of the essential oils and salts in return to the survival of *L. casei* was quite high; the inhibitory effect of concentrations less than MIC was also seen on pathogenic bacteria. Hence, the usage of concentrations of essential oils, NaCl, and bile salts less than MIC may also eliminate pathogens without harm to probiotics (Calsamiglia *et al*., 2007).

The viability of probiotic strains during production, food storage, and passage through the gastrointestinal tract is a major challenge in fermented dairy products. Researchers reported that enumeration of probiotic strains for beneficial and therapeutic effects should be at least 10^6 to 10^7 CFU g^{-1} or mL in products (Azizkhani and Parsaeimehr, 2018). Treatments with stresses of NC, BS, CEONC, CEOBS, and CEONCBS maintained viability until 21 days of storage. However, control with CEO sample-maintained viability up to 28 days of storage time. The survival rate of *L. casei* in control and stress treatments with the CEO increased at 7 days of storage. A rise in the survival rate of *L. casei* was observed to reduce pH at 7 days of storage. The increased survival rate of *Lactobacillus acidophilus* LA5, *Lactobacillus fermentum*, and *Bifidobacterium* Bb-12 in yogurt at 7 days of storage was reported by Azizkhani and Parsaeimehr . The bacterial population decline was attributed to the accumulation of organic acid during growth and fermentation. The main reasons for reducing pH are converting lactose to lactic acid, type of starter culture, duration of storage, and fermentation temperature (Singh *et al*., 2011). Several studies reported a decline in probiotic strains' survival rate during storage time (Azizkhani and Parsaeimehr, 2018; Yangilar and Yildiz, 2018). Indeed, decreased pH of products during storage was due to activation of the beta-galactosidase enzyme at 0–5°C, as well as post-acidification. pH values decreased to 4.2. The viability of probiotic bacteria is affected by increased

Table 2. Sensory properties of probiotic yogurt samples with *Cuminum cyminum* essential oil (CEO), NaCl (NC), bile salts (BS), and their combinations.

Sensory properties	Yogurt samples	Storage time (Days)				
		0	7	14	21	28
Flavor	Control	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	4.67 ± 0.50^{a}	4 ± 0^{a}
	CEO	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	4.75 ± 0.44^{a}	4 ± 0^{a}
	BS	5 ± 0^{a}	4 ± 0^{b}	4 ± 0^{b}	4 ± 0^{b}	3.33 ± 0.50^{b}
	NC	5 ± 0^{a}	4 ± 0^{b}	4±0^{b}	3.78 ± 0.44^{b}	3.33 ± 0.50^{b}
	CEOBS	5 ± 0^{a}	4 ± 0^{b}	4 ± 0^{b}	3.78 ± 0.44^{b}	3.33 ± 0.50^{b}
	CEONC	5 ± 0^{a}	4.33 ± 0.50^{b}	3.78 ± 0.44^{b}	3.78 ± 0.44^{b}	3.33 ± 0.50^{b}
	NCBS	4.67 ± 0.50^{a}	4 ± 0^{b}	3.78 ± 0.44^{b}	3.22 ± 0.44^{c}	3.11 ± 0.33^{b}
	CEONSBS	4.75 ± 0.44^{a}	4.33 ± 0.50^{b}	4.33 ± 0.50^{b}	3.22 ± 0.44^{c}	3.11 ± 0.33^{b}
Texture	Control	5 ± 0^{a}	5 ± 0^{a}	5 ± 0^{a}	4.67 ± 0.23^{a}	4.33 ± 0.50^{a}
	CEO	5 ± 0^{a}	5 ± 0^{a}	4.56 ± 0.53^{b}	4.33 ± 0.50^{bc}	4.33 ± 0.50^{a}
	BS	5 ± 0^{a}	4 ± 0^{b}	4.33 ± 0.50^{bc}	4.33 ± 0.50^{bc}	3.44 ± 0.53^{b}
	NC	5 ± 0^{a}	4.33 ± 0.50^{b}	4.33 ± 0.50^{bc}	4.33 ± 0.50^{bc}	3.22 ± 0.44^{b}
	CEOBS	4.56 ± 0.53^{a}	4 ± 0^{b}	4 ± 0^{c}	4 ± 0^{c}	3.44 ± 0.55^{b}
	CEONC	5 ± 0^{a}	4 ± 0^{b}	4 ± 0^{c}	4 ± 0^{c}	3.44 ± 0.44^{b}
	NCBS	4.67 ± 0.50^{a}	4.33 ± 0.50^{b}	4 ± 0^{c}	3.22 ± 0.44^{d}	3.44 ± 0.44^{b}
	CEONSBS	4.67 ± 0.50^{a}	4 ± 0.70^{b}	3.78 ± 0.44^{b}	3.22 ± 0.44^{d}	3.22 ± 0.44^{b}
Overall acceptability	Control	5 ± 0^{a}	5 ± 0^{a}	4.89 ± 0.33^{a}	4.67 ± 0.50^{a}	4 ± 0^{a}
	CEO	5 ± 0^{a}	5 ± 0^{a}	4.56 ± 0.53^{a}	4.33 ± 0.50^{ab}	3.78 ± 0.44^{ab}
	BS	5 ± 0^{a}	4 ± 0^{b}	4 ± 0^{b}	4 ± 0.70^{b}	3.22 ± 0.44^{b}
	NC	5 ± 0^{a}	4 ± 0.70^{b}	4 ± 0^{b}	4 ± 0^{b}	3.22 ± 0.44^{b}
	CEOBS	4.75 ± 0.44^{a}	4 ± 0^{b}	4 ± 0^{b}	4 ± 0^{b}	3.22 ± 0.44^{b}
	CEONC	5 ± 0^{a}	4 ± 0^{b}	4 ± 0^{b}	4 ± 0^{b}	3.22 ± 0.44^{b}
	NCBS	4.67 ± 0.50^{a}	4.33 ± 0.50^{b}	4 ± 0^{b}	3.56 ± 0.53^{c}	3.22 ± 0.44^{b}
	CEONSBS	4.67 ± 0.50^{a}	4 ± 0.70^{b}	4.33 ± 0.50^{b}	3.22 ± 0.44^{c}	3.22 ± 0.44^{b}

Each observation is a mean ± SD of three replications. In each column and row, means with the same letters had no significant difference at $P > 0.05$.

hydrogen ions compared to lactate ions (Kailasapathy, 2006). Compared to treatments with stress, treatment with CEO had higher population viability of *L. casei* than others during storage time ($P < 0.05$). The susceptibility of microorganisms to essential oils depends on the details of essential oil and the type of microorganisms. The antimicrobial activity of essential oils is complicated due to their volatility, insolubility in water, and complex chemical structure (Calsamiglia *et al.*, 2007). The antibacterial effects of *Cuminum cyminum* Boiss. essential oil on different microorganisms depends on the concentration and composition of nutrients, storage temperature, and nature of the organism's metabolites (Mahmoudi, 2013). The survival rate of *Lactobacillus acidophilus* in bioyogurt containing different concentrations of the essential oils, *Mentha piperita* and *Ziziphoraclinopodioides*, was significantly reduced 7 days of storage time at 4°C. One of the main criteria for selecting probiotic bacteria is resistance to NaCl and bile salts (Sarabi Jamab and Niazmand, 2009). According to the obtained results, treatments under stress with 0.15% BS were to be effective in the food at end of the 21st day. However, the survival rate of *L. casei* in BS stress treatment was significantly decreased from the 21st to the 28th day of storage time. It was less than the acceptable limit ($P < 0.05$). Probiotic bacteria have different mechanisms of protection against stress, one of which is the bile hydrolysis system. The resistance of some strains to bile salts is associated with the bile salt hydrolysis activity. Therefore, the hydrolysis of the bile salts will reduce their toxicity and side effects (Sahadeva *et al.*, 2011). According to Taranto *et al.* (2006), *Lactobacillus delbrucium* subsp. *Bulgaricus* treated with different concentrations of thiorodoxylate (one of the bile salts) showed different levels of activity of the hydrolysis system (Taranto *et al.*, 2006). The researchers reported that in some bacterial cells, the bile hydrolysis system's activity was significantly stronger than others, which resulted in cells showing greater resistance to higher concentrations and longer exposure times to these bile salts (Taranto *et al.*, 2006). When probiotic bacteria are exposed to bile salts, cellular homeostasis disorders occur. Destruction of lipid membranes and cell

membrane proteins leads to bacteria's death (Sahadeva *et al*., 2011). The survival rate of *L. casei* with considering salt stress was within acceptable limit until the end of 21 days of storage, and these findings are following other research that studied the viability of probiotic strain more than 2% of concentration (Fortin *et al.*, 2011). Other researchers reported that probiotic strains' viability decreased in samples with high concentrations of salt (4%) (Hekmat *et al*., 2009).

The suitable pH for commercial yogurt is 4.5. pH enhanced shelf life of the yogurt maintained mild taste and optimum appearance. Undesirable pH (less than 4), which had been resulted by *Lactobacillus bulgaricus,* produced large amounts of lactic acid, acetaldehyde, and byproducts from proteolytic activity (Mahmoudi *et al*., 2014). The main reason for the lower pH of CEO, BS, and NC salts compared to the control sample was due to the presence of some phenolic compounds in the *C. cyminum* Boiss essential oil, which may have inhibitory effects on the growth of *L. casei* and changed pH values (Mahmoudi, 2013). Salt also affected pH and acidity values by influencing the growth of microorganisms, which may cause lactic acid production during storage time (Fortin *et al*., 2013). Some studies reported that probiotic yogurt's pH decreased during storage time (Mahmoudi, 2013 ; Azizkhani and Pasaeimehr, 2018).

The acidification degree of probiotic yogurt is a crucial process control model that directly impacts the gel intensity and the commercially available fermentation period (Alizadeh Khaledabad *et al*., 2020). The production of organic acids by lactic acid bacteria was the main reason for increased acidity during storage time (Mahmoudi *et al*., 2014). Salt also influenced the acidity of samples because it impacted the growth of microorganisms, which influenced lactic acid production during storage time (Fortin *et al*., 2013). The consumption of lactose by lactic acid bacteria led to lactic acid production, and then the acidity of samples increased. The production of lactic acid in yogurt is the main factor in producing a unique flavor. Due to casein instability, conversion of the colloidal calcium phosphate complex to soluble calcium phosphate, calcium excretion, and casein coagulation take place at pH 4_4.6 (Ramasubramanian *et al*., 2008).

Significant factors such as heterogeneity, high acidity, storage, breakage of protein strand, and structural rearrangement that will induce yogurt's whey to leakage are recognized as substantial defects (Yildiz and Ozcan, 2020). The main reason for increased syneresis in probiotic yogurt during storage might be the activation of microorganisms of starter culture and their effects on long-chain biopolymers, which could be an important factor in reducing the softness and enhancing the syneresis of the yogurt during storage (Kailasapathy, 2006). At the same time, Akgun (2018) found that the syneresis rate in probiotic yogurt samples increased during storage at 4°C. The consistency of yogurt increased by stabilizers, an increase in the amount of milk casein concentration, and a reduction of acidification rate (Everett and Mcleod, 2005). Samples with stress under essential oil had a lower syneresis percentage than others. The main reason for lower syneresis was acidification of yogurt containing herbal essential oils, which might cause high-strength gels, low permeability, a fine protein mesh, and higher water uptake. Consequently, the syneresis of probiotic yogurt was decreased (Ozer *et al*., 2007).

In the overall acceptability of food products by consumers, sensory characteristics represent a vital role. Researches affirm that flavor is the first criterion for food acceptance, followed by health considerations as the second rule (Alizadeh Khaledabad *et al*., 2020). The reduction of flavor scores in all samples during storage might be related to increasing acidity and reduced starter bacteria activity, which induced flavor components (Yangilar and Yildiz, 2018). The percentage of syneresis causes a change in the firmness of the sample. The firmness of yogurt decreased with increasing of syneresis. Indeed, the percentage of syneresis was inversely correlated to yogurt's firmness (Ayar and Gurlin, 2014). Treatments that contained more probiotic counts had better flavor and texture than treatments with less probiotic counts, so the overall acceptance scores of yogurts with more probiotic counts were higher than the yogurt with less probiotic counts (under stress). Proteolytic strains of *Lactobacillus* could produce taste through carbohydrate metabolism, proteolysis, and low lipolysis processes. Enzymes of *Lactobacillus* hydrolyzed casein and produced large and medium bioactive peptides. Proteolytic enzymes may subsequently degrade these peptides from starter bacteria, non-acidic lactic acid probiotic bacteria to small peptides and free amino acids, which are the major contributors to taste in dairy products (Everett and Mcleod, 2005; Grom *et al*, 2020). All in all, the highest and lowest sensory scores were attributed to control and CEONCBS at 28 days of storage time, respectively. According to Silva *et al*. (2018), salt is also expressed as a factor affecting the formation and development of aroma compounds and impacts the dairy product's sensory properties. Won-Young *et al*. (2020) described that olive leaf extract to yogurt reduces the yogurt's sensory score.

According to the results, the pH and population of *L. casei* decreased for all the treatments during the storage time, while their acidity and percentage of syneresis increased. The enumeration of *L. casei* was in the range of the recommended amount (10^6_10^7 CFU mL^{-1}) for probiotic yogurt with stresses less than MIC during 28

days of storage. Therefore, it is possible to produce novel functional products such as probiotic yogurt containing herbal essential oil. The usage of combined *C. cyminum* Boiss, NaCl, and bile salts in cool conditions and MIC up to 50% during 21 days of storage is recommended for probiotic yogurt containing *L. casei*.

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References

Akgun, A., Yazici, F., and Gulec, H.A., 2018. The combined effect of probiotic cultures and incubation final pH on the quality of buffalo milk yogurt during cold storage. Journal of Food Science and Nutrition. 6(2): 492–502. https://doi.org/10.1002/fsn3.580

Alizadeh Khaledabad, M., Ghasempour, Z., Moghaddas Kia, E., and Rezazad Bari, M., 2020. Probiotic yogurt functionalised with microalgae and Zedo gum: chemical, microbiological, rheological and sensory characteristics. International Journal of Dairy Technology. 73(1): 67–75. https://doi.org/10.1111/1471-0307.12625

Al-Okbi, S.Y., and Mohamed, D.A., 2012. Preparation and evaluation of functional foods in adjuvant arthritis. Grasas y aceites international journal of fats and oils. 63(4): 394–402. https://doi.org/10.3989/gya.130811

Ayar, A. and Gurlin, E., 2014. Production and sensory, textural, physico-chemical properties of flavored spreadable yogurt. Life Science Journal. 11(4)**:** 58–65.

Azizkhani, M. and Parsaeimehr, M., 2018. Probiotics survival, antioxidant activity and sensory properties of yogurt flavored with herbal essential oils. International Food Research Journal. 25(3): 921–927.

Balthazar, C.F., Silva, H.L., Esmerino, E.A., Rocha, R.S., Moraes, J., Carmo, M.A., *et al*. 2018. The addition of inulin and *Lactobacillus casei* 01 in sheep milk ice cream. Food Chemistry. 246: 464–472. https://doi.org/10.1016/j.foodchem.2017.12.002

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L. and Ferret, A., 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science. 90(6): 2580–2595. https://doi.org/10.3168/jds.2006-644

Capela, P., Hay, T.K.C. and Shah, N.P., 2006. Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yogurt and freeze-dried yogurt. Food Research International. 39(2): 203–211. https://doi.org/10.1016/j.foodres.2005.07.007

El-Shafei, K., Tawfik, N.F., Dabiza, N.M.A., Sharaf, O.M. and Effat, B.A., 2010. In vitro assessment of gastrointestinal viability of potentially probiotic *Lactobacilli*. Journal of American Science. 6(11): 357–367.

Everett, D.W. and McLeod, R.E., 2005. Interactions of polysaccharide stabilisers with casein aggregates in stirred skim-milk yogurt . International Dairy Journal. 15(11): 1175–1183. . https://doi.org/10.1016/j.idairyj.2004.12.004

Fontana, A., Zacconi, C. and Morelli, L., 2018. Genetic signatures of dairy *Lactobacilluscasei* Group. Frontiers in microbiology. 9: 2611. https://doi.org/10.3389/fmicb.2018.02611

Fortin, M.H., Champagne, C.P., St-Gelais, D., Britten, M., Fustier, P. and Lacroix, M., 2011. Effect of time of inoculation, starter addition, oxygen level and salting on the viability of probiotic cultures during Cheddar cheese production. International Dairy Journal. 21(2): 75–82. https://doi.org/10.1016/j.idairyj.2010.09.007

Garcia, C., Bautista, L., Rendueles, M., and Diaz, M., 2019. A new synbiotic dairy food containing lactobionic acid and Lactobacillus casei. International Journal of Dairy Technology, 72(1): 47–56. https://doi.org/10.1111/1471-0307.12558

Grom, L., Rocha, R., Balthazar, C., Guimarães, J., Coutinho, N., Barros, C., *et al.,* 2020. Postprandial glycemia in healthy subjects: Which probiotic dairy food is more adequate? Journal of Dairy Science. 103(2): 1110–1119. https://doi.org/10.3168/jds.2019-17401

Hekmat, S., Soltani, H. and Reid, G., 2009. Growth and survival of *Lactobacillus reuteri* RC-14 and *Lactobacillusrhamnosus* GR-1 in yogurt for use as a functional food. Innovative Food Science and Emerging Technologies. 10(2): 293–296. https://doi.org/10.1016/j.ifset.2008.10.007

Kailasapathy, K., 2006. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yogurt . LWT-Food Science and Technology. 39(10): 1221–1227. https://doi.org/10.1016/j.lwt.2005.07.013

Lucatto, J. N., Silva Buzanello, R. A., Mendonça, S. N. T. G., Lazarotto, T. C., Sanchez, J. L., Bona, E., *et al*., 2020. Performance of different microbial cultures in potentially probiotic and prebiotic yogurts from cow and goat milks. International Journal of Dairy Technology. 73(1): 144–156. https://doi.org/10.1111/1471-0307.12655

Mahmoudi, R., 2013. Improvement the hygienic quality and organoleptic properties of bioyogurt using *Cuminum cyminum* L. essential oil. Journal of Agroalimentary Processes and Technologies. 19(4): 405–412.

Mahmoudi, R., Zare, P., Hassanzadeh, P. and Nosratpour, S., 2014. Effect of T eucrium polium essential oil on the physicochemical and sensory properties of Probiotic yogurt . Journal of Food Processing and Preservation. 38(3): 880–888. https://doi.org/10.1111/jfpp.12042

Maragkoudakis, P.A., Zoumpopoulou, G., Miaris, C., Kalantzopoulos, G., Pot, B. and Tsakalidou, E., 2006. Probiotic potential of *Lactobacillus* strains isolated from dairy products. International Dairy Journal. 16(3): 189–199. https://doi.org/10.1016/j.idairyj.2005.02.009

Mortazavian, A.M., Ehsani, M.R., Mousavi, S.M., Rezaei, K., Sohrabvandi, S. and Reinheimer, J.A., 2007. Effect of refrigerated storage temperature on the viability of probiotic microorganisms in yogurt. International Journal of Dairy Technology. 60(2): 123–127. https://doi.org/10.1111/j.1471-0307.2007.00306.x

Ozer, B., Kirmaci, H.A., Oztekin, S., Hayaloglu, A. and Atamer, M., 2007. Incorporation of microbial transglutaminase into non-fat

yogurt production. International Dairy Journal. 17(3): 199–207. https://doi.org/10.1016/j.idairyj.2006.02.007

Ramasubramanian, L., Restuccia, C. and Deeth, H.C., 2008. Effect of calcium on the physical properties of stirred probiotic yogurt. Journal of Dairy Science. 91(11): 4164–4175. https://doi.org/10.3168/jds.2008-1354

Roobab, U., Batool, Z., Manzoor, M.F., Shabbir, M.A., Khan, M.R., and Aadil, R.M,. 2020. Visualization of food probiotics with technologies to improve their formulation for health benefits and future perspectives, Current Opinion in Food Science. 32: 17–28. https://doi.org/10.1016/j.cofs.2020.01.003

Sahadeva, R.P.K., Leong, S.F., Chua, K.H., Tan, C.H., Chan, H.Y., Tong, E.V., *et al*., 2011. Survival of commercial probiotic strains to pH and bile. International Food Research Journal. 18(4): 1515–1522.

Sarabi-Jamab, M. and Niazmand, R., 2009. Effect of essential oil of Mentha piperita and Ziziphora clinopodioides on *Lactobacillusacidophilus* activity as bioyogurt starter culture. American-Eurasian Journal of Agricultural and Environmental Science. 6(2): 129–131.

Shahdadi, F., Mirzaie, H., Kashaninejad, M., Khomeiri, M., Ziaiifar, A.M. and Akbarian, A., 2015. Effects of various essential oils on chemical and sensory characteristics and activity of probiotic bacteria in drinking yogurt. Agricultural Communications. 3(1): 16–21.

Silva, H.L.A., Balthazar, C.F., Silva,R., Vieira, A.H., Costa, R.G.B., Esmerino, E.A., *et al*., 2018. Sodium reduction and flavor enhancer addition in probiotic prato cheese: contributions of quantitative descriptive analysis and temporal dominance of sensations for sensory profiling. Journal of Dairy Science. 101(10): 8837–8846. https://doi.org/10.3168/jds.2018-14819

Singh, G., Kapoor, I.P.S. and Singh, P., 2011. Effect of volatile oil and oleoresin of anise on the shelf life of yogurt. Journal of Food Processing and Preservation. 35(6):778–783. https://doi.org/10.1111/j.1745-4549.2011.00528.x

Taranto, M.P., Perez-Martinez, G., and de Valdez, G.F., 2006. Effect of bile acid on the cell membrane functionality of lactic acid bacteria for oral administration. Research in Microbiology. 157(8): 720–725. https://doi.org/10.1016/j.resmic.2006.04.002

Wacher-Rodarte, C., Galvan, M.V., Farres, A., Gallardo, F., Marshall, V.M. and Garcia-Garibay, M., 1993. Yogurt production from reconstituted skim milk powders using different polymer and non-polymer forming starter cultures. Journal of Dairy Research. 60(2): 247–254. https://doi.org/10.1017/S0022029900027564

Won-Young Cho, Da-Hee Kim, Ha-Jung Lee, Su-Jung Yeon and Chi-Ho Lee., 2020. Quality characteristic and antioxidant activity of yogurt containing olive leaf hot water extract, CyTA - Journal of Food. 18:1: 43–50. https://doi.org/10.1080/19476337.2019.1640797

Yangilar, F. and Yildiz, P.O., 2018. Effects of using combined essential oils on quality parameters of bio yogurt. Journal of Food Processing and Preservation. 42(1): e13332. https://doi.org/10.1111/jfpp.13332

Yildiz, E. and Ozcan, T., 2020. Functional and textural properties of vegetable- fibre enriched yogurt . International Journal of Dairy Technology. 72: 199–207. https://doi.org/10.1111/1471-0307.12566

Zendeboodi F., Khorshidian N., Mortazavian A.M., da Cruz A.G., 2020. Probiotic: conceptualization from a new approach, Current Opinion in Food Science. 32: 103–120. https://doi.org/10.1016/j.cofs.2020.03.009