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Antimicrobial Activities of Probiotic Yogurts Flavored with Peppermint, Basil, and Zataria against *Escherichia coli* and *Listeria monocytogenes*

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HIGHLIGHTS

- Probiotic yogurts containing peppermint, basil, and zataria Essential Oils (EOs) were produced.
- EOs did not adversely affect the viability of probiotic bacteria in yogurt samples.
- Probiotic yogurts containing EOs had significant antimicrobial effects against *Escherichia coli* and *Listeria monocytogenes*.

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Acronyms and abbreviations
EO=Essential Oil
GC/MS=Gas Chromatography/Mass

ABSTRACT

Background: Yogurt is one of the most popular food diets with refreshing taste, nutritional, and health benefits. The objective of this study was to investigate the effect of three herbal Essential Oils (EOs) including peppermint, basil, and zataria on viability of the probiotic bacteria in yogurt samples and to evaluate the antimicrobial activities of the produced probiotic yogurts against *Escherichia coli* and *Listeria monocytogenes*.

Methods: Yogurt samples were produced using probiotic organisms *Lactobacillus acidophilus* LA5, *L. fermentum*, and *Bifidobacterium* Bb-12 besides the starter culture and added EOs, separately. The value of pH, viable counts of probiotics, and antimicrobial potential of yogurts at 0, 7, 14, and 28 days of storage at 4 °C were evaluated. The growth inhibition of *E. coli* and *L. monocytogenes* was determined *in vitro* using disk diffusion method. All data analyses were performed using SPSS, Inc, Chicago, IL software (v. 16.0).

Results: No significant difference was observed in pH value of treatment (EOs containing probiotic yogurts) and control samples during fermentation and storage period. The presence of EOs did not affect the total lactobacilli and *L. acidophilus* LA5 population in yogurt during 4 weeks of storage but the growth of *Bifidobacterium* Bb12 was retarded. Yogurt containing zataria exhibited the strongest inhibitory effect on the growth of *E. coli* and *L. monocytogenes* in comparison with other EOs treated and control yogurt ($p<0.05$). However, both control as well as treated yogurt showed inhibitory effect against *L. monocytogenes* stronger than *E. coli* ($p<0.05$).

Conclusion: The present findings suggested that adding zataria, basil, or peppermint EOs into probiotic yogurt formulation could improve the potential functionality of the product and also made an inhibitory effect against *L. monocytogenes* and *E. coli*.

Introduction

Acquiring unique food ingredients and flavours with enhanced health properties is one of the key global market trends (Netzel et al., 2007). Among food ingredients, yogurt is one of the most popular products, which is

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being enjoyed for its refreshing taste, nutrition, and health benefits. It possesses a high antagonistic and therapeutic value which provides higher levels of protein, carbohydrate, calcium, as well as certain B vitamins in comparison with milk (Abd-El Fattah et al., 2010). The properties of yogurt are due to the presence of lactic acid bacteria, which ferment lactose to lactic acid and so, improve the nutritional and therapeutic values of yogurt (Adolfsson et al., 2004).

Probiotics which are live beneficial microorganisms, when administered in adequate amounts, also have been shown to have numerous health benefits. More than 90% of probiotic products contain various species of lactobacilli and bifidobacteria (Shah, 2000). Foods containing viable probiotic microorganisms show several health benefits, for example reduction and prevention of diarrhea, improving the intestinal microbiota balance through antimicrobial effects, decreasing lactose intolerance symptoms and food allergy, improving immune potency, and antitumorigenic activities (Andersson et al., 2001; Isolauri et al., 2001; McFarland, 2006).

Recently, the use of different natural flavors in yogurt manufacturing has been attempted increasingly. Spices are a novel source of functional flavouring agents. There is now mounting scientific evidence of health benefits of plant Essential Oils (EOs), including antibacterial, antifungal, antioxidant, anti-inflammatory, as well as anticarcinogenic properties (Azhdarzadeh and Hojjati, 2016; Khorasany et al., 2016; Srinivasan, 2004; Tapsell et al., 2006). *Mentha piperita* L., a medicinally important plant belongs to the Lamiaceae family. Peppermint EO has moderate antibacterial effects against both Gram-positive and Gram-negative bacteria (Singh et al., 2015). The medicinally important constituents reported so far include menthol, menthon, and menthyl acetate representing nearly 70% of total EOs (Schmidt et al., 2009). *Ocimum basilicum* belonging to the Laminaceae family, collectively called basil, is widely cultivated and also extensively used in food industry, perfumery, cosmetics, pesticides, medicine, and traditional rituals because of its natural aromas, flavors, and other properties (Zheljazkov et al., 2007). Basil EOs and their principal constituents were found to exhibit antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria, yeast, as well as mold (Suppakul et al., 2003). *Zataria multiflora* Boiss is a plant belonging to the Laminaceae family that grows only in Iran, Pakistan, and Afghanistan. Zataria is extensively used in Iran as a flavoring agent in foods such as yogurt, cheese, and soup. The main constituents of the zataria EO are phenolic compounds, including carvacrol, thymol, and gamma-terpinene (Basti et al., 2007). Carvacrol and thymol are known to have wide spectra of antimicrobial activities and have been the subject of several studies (Azizkhani et al., 2013; Parsaeimehr et al., 2010). Combinations of probiotics with spices may provide further antimicrobial-therapeutic properties. However, since some spices are antimicrobials, they may affect probiotic viability. Some *in vitro* studies that tested spices on the growth of selected probiotics showed that spices significantly enhance the growth of probiotics while inhibiting pathogens (Be et al., 2009; Sutherland et al., 2009). Therefore, the objective of this study was to investigate the effect of three herbal Eos, including peppermint, basil, and zataria on viability of the probiotic bacteria in yogurt samples, and to evaluate the antimicrobial activities of the produced probiotic yogurts against *Escherichia coli* and *Listeria monocytogenes*.

Materials and methods

EOs, pathogenic bacteria, and chemicals

In this experimental study, three EOs, including peppermint (*M. piperita*), basil (*O. basilicum*), as well as zataria (*Z. multiflora* Boiss.) were obtained from Barij Company, Kashan, Iran. EOs were stored in airtight dark glass vials at 4 °C. Two studied pathogens, *E. coli* (ATCC 25922) and *L. monocytogenes* (ATCC 19118) were also obtained as lyophilized cultures from the Pasteur Research Institute, Tehran, Iran. All culture media and chemicals were analytical grade or the highest grade and were obtained either from Sigma-Aldrich or Merck (Germany).

Gas Chromatography (GC) and Gas Chromatography/Mass (GC/MS) spectrometry

EOs were analyzed by GC apparatus (Thermo Quest, UK). The chromatograph was equipped with a DB_5 capillary column (Aligent Technologies, USA; 30×0.25 mm ID×0.25 µm film thickness) and the data were acquired under the following conditions: initial temperature 50 °C; rate of temperature increase, 2.5 °C per min, final temperature of 265 °C and injector temperature of 250 °C. An injection volume of 0.5 µl was employed using the autosampler (autosampler 7693–100 positions, Agilent Technologies, USA). The carrier gas was helium and the split ratio was 120. The column head pressure was 24.9 kPa. An Agilent 6890 Flame Ionization Detector (Agilent Technologies, USA), operated at 200 Hz, was used. EOs were also analyzed by GC/MS (Thermo Quest Finnigan®, UK) using the same capillary column and analytical conditions indicated above. The MS was run in the electron ionization mode using ionization energy of 70 eV. Components were identified based on the comparison of their relative retention times and mass spectra with those of standards. Alkanes were used as reference points in the calculation of relative retention indices.

Probiotics and starter culture

The probiotic microorganisms *L. acidophilus* LA5, *L. fermentum*, and *Bifidobacterium* Bb-12 were selected for this study based on the preliminary studies (Be et al., 2009; Behrad et al., 2009). Freeze-dried starter culture (*Streptococcus thermophilus* and *L. delbrueckii* ssp. *bulgaricus*) and probiotic bacteria were obtained from Chr. Hansen, Ltd, Denmark.

Preparation of probiotic yogurt with EOs

According to the manufacturer's instruction for yogurt preparation, 2 L milk (local supplier), with the required fat content (3.9 g/100 ml) was mixed vigorously with 2% skimmed milk powder (Pajan Dairy Co., Iran) and 5% sucrose (Sigma-Aldrich, Germany) to ensure an efficient mixing. Then, remaining 2 L milk was added and mixed thoroughly. The mixture was heated at 85 °C for 30 min, then cooled to 43-45 °C in a water bath while stirring slowly with a hand stirrer and inoculated with the prepared yogurt starter culture (0.02 percent v/v). The mix was aliquoted into four equal portions (1 L each). Three portions (0.5 percent v/v) were added with either one of the EO containing probiotic yogurts (treatment groups) and one portion used without EOs (control group). Each portion was inoculated with probiotics *L. acidophilus* LA5 (0.02 percent w/v), *L. fermentum* (0.02 percent w/v), as well as *Bifidobacterium* Bb12 (0.02 percent w/v). The inoculated mixtures were aliquoted into cups and incubated at 40 °C for fermentation and terminated when pH reached 4.5 (approximately 6 h), then yogurt cups were cooled rapidly in a refrigerator at 4±1 °C for storage.

Determination of pH

The pH value of yogurts was determined every h at 20±1 °C during fermentation and storaged at 4 °C. Each yogurt sample (1 g) was mixed with distilled water (1:1), and pH was measured using a pH meter (Jenway, UK), calibrated routinely with fresh pH 4.0 and 7.0 standard buffers.

Enumeration of probiotic bacteria

Clindamycin was used as antibiotic for growth inhibition of lactic acid bacteria in the starter culture but allowing the growth of *L. acidophilus* LA5 and *L. fermentum*. MRS agar was prepared with addition of 0.05% clindamycin stock solution per L. Enumeration of total lactobacilli was carried out using MRS agar without clindamycin. Enumeration was conducted by aseptically mixing yogurt sample (1 ml) with 9 ml buffered peptone water. Peptone water was used as the diluents to perform serial dilutions. One ml of diluted yogurt was inoculated in each empty Petri dish followed by adding 15 ml melted (45 °C) MRS agar. After mixing thoroughly, each Petri dish was inverted and incubated in anaerobic condition in jar equipped with anaerogen sachets (Oxoid Deutschland, Germany) for 24-48 h at 37 °C to determine viable count on days 1, 7, 14, and 28 according to Illupapalayam et al. (2014).

To inhibit yogurt starter culture, but allowing the appropriate growth of *Bifidobacterium* Bb12, the combination of dicloxacillin (10%), lithium chloride (11%), as well as cysteine hydrochloride (10%) was used. The *Bifidobacterium* Bb12 viable cell counts were carried out by plating diluted yogurt samples using the MRS agar with addition of 0.5% dicloxacillin stock solution, 1% lithium chloride stock solution, as well as 0.5% cysteine hydrochloride stock solution per L of medium according to the pour plate method. Each plate was incubated anaerobically in jar containing anaerogen sachets at 37°C for 48 h to determine the viable microbial count on days 1, 7, 14, and 28.

Pathogen growth inhibition assay

Growth inhibition was evaluated by the filter paper disk diffusion method (Zaika, 1988) which conforms to the recommended standards of National Committee for Clinical Laboratory Standards (CLSI, 2015). Each EO-yogurt water extracts (25 µl) was aliquoted on standard 6 mm paper disks (Whatman, UK) which were then placed on PALCAM agar (Merck, Germany) inoculated with 0.1 ml *L. monocytogenes* suspension (10^3-10^4 CFU/ml) in the brain heart infusion broth (Arsalan and Ozdemir, 2008) and on CT-SMAC agar (Merck, Germany) inoculated with 0.1 ml *E. coli* suspension (10^4-10^5 CFU/ml) in the brain heart infusion broth (Mahmoudzadeh et al., 2015). The growth of bacteria was evaluated under anaerobic conditions at 37 °C, 24 h for *E. coli* and 48 h for *L. monocytogenes*. The inhibition zone around each disk was measured on days 0, 7, 14, and 28.

Statistical analysis

All data analyses were performed using SPSS, Inc, Chicago, IL software (v. 16.0). One way ANOVA with post-hoc mean separation using LSD was carried out for statistical analysis of the treatment and storage effect on the viable microorganisms and antimicrobial activity and pH over the 28-day period of storage. Results were presented as the mean and its standard error (±SE). All data obtained from in triplicate experiments.

Results

GC/MS analysis showed the identification of 25 components for peppermint EO, 23 for basil EO, and 12 for

that of zataria, representing more than >91% (v/v) of the oils in each case. The main components of peppermint EO were menthol (32.92%), menthone (31.11%), menthyl acetate (6.58%), and 1,8 cineole (3.98%); the ones for basil EO were linalool (58.63%), cadinol (10.01%), α-bergamotene (7.62%), as well as γ-cadinen (4.92%) whilst those of zataria EO were carvacrol (71.12%), gamma-terpinene (7.34%), alpha-pinene (4.26%), and eucaliptol (3.37%).

The pH value for control yogurt was approximately the same as pH of EO treated yogurts. The pH value for all yogurts was reduced (p<0.05) from the initial values of approximately 4.45 to between 3.95 and 4.09 by day 28 of storage. An overall decline of pH of yogurts occurred during refrigerated storage. The pH value for all yogurts was reduced (p<0.05) in comparison with control yogurt except for zataria treated samples (Fig. 1).

As shown in Fig. 2 and Fig. 3, the presence of basil or peppermint did not affect the viable count of *L. acidophilus* LA5 and total lactobacilli on day 0 in comparison with control group. But, the presence of zataria was resulted in a lower count of viable lactobacilli and also *L. acidophilus* LA5 in zataria treated yogurt (p<0. 05) on day 0 of storage. The viable count of *L. acidophilus* LA5 and lactobacilli was increased significantly from day 0 to day 7 of storage for all yogurts but was reduced in zataria treated sample (p<0.05). The viable *L. acidophilus* LA5 and lactobacilli count however was reduced from day 7 to day 28 of storage for all yogurts with the fastest rate occurring in control yogurt. However, the viable *L. acidophilus* LA5 count on day 28 of storage for basil treated yogurt (4.8×10^6 CFU/ml) was not significantly different from peppermint treated yogurt (p>0. 05). Similar to the results of total lactobacilli and *L. acidophilus* LA5 counts in EO-treated yogurts, the presence of basil and peppermint EOs did not significantly affect the viable count of *Bifidobacterium* Bb12 on day 0 in comparison with control yogurt (Fig. 4). But the presence of zataria EO was resulted in a lower *Bifidobacterium* Bb12 count on the same day of storage in comparison with all other yogurts (p<0.05). The viable count of *Bifidobacterium* Bb12 was increased from day 0 to day 14 in all yogurts. But from day 14 to day 28 of storage, all yogurts had a reduced viable count with the slowest rate occurring in basil treated yogurt (p<0.05). On day 28, the viable count of zataria treated yogurt (0.7×10^4 CFU/ml) was significantly lower than control yogurt and other treated samples. The basil-yogurt was resulted in significantly (p<0.05) higher viable *Bifidobacterium* count (2.92×10^4 CFU/ml) in comparison with control yogurt (1.64×10^4 CFU/ml).

During the whole period of storage, zataria treated yogurt exhibited the strongest inhibitory effect on the growth of *E. coli* and *L. monocytogenes* (Fig. 5 and Fig. 6) in comparison with other EO treated and control yogurt (p<0.05). However, both control and treated yogurt showed inhibitory effect against *L. monocytogenes* stronger than *E. coli* (p<0.05). The strongest inhibitory effects which was determined as diameter of inhibition zone (mm) were seen on day 14 of storage for both *E. coli* and *L. monocytogenes* as followed: zataria (15.5 and 16.7 mm), basil (9.5 and 9.9 mm), peppermint treated yogurt (9.4 and 10 mm), and control yogurt (8.4 and 8.8 mm).

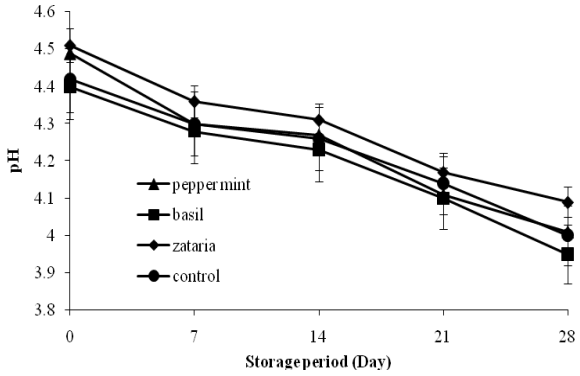

Fig. 1: pH value for EOs treated and control yogurts during refrigerated storage

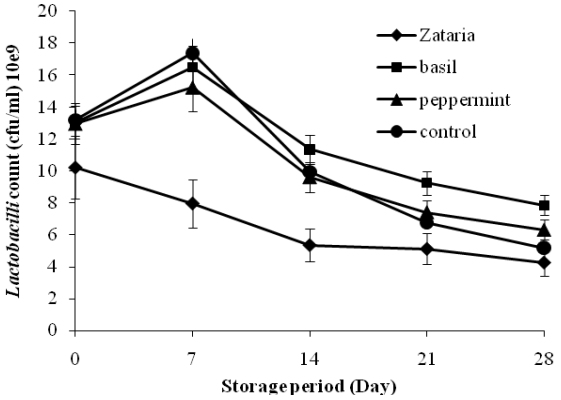

Fig. 2: Viable total of lactobacilli count in EOs treated and control yogurts during refrigerated storage

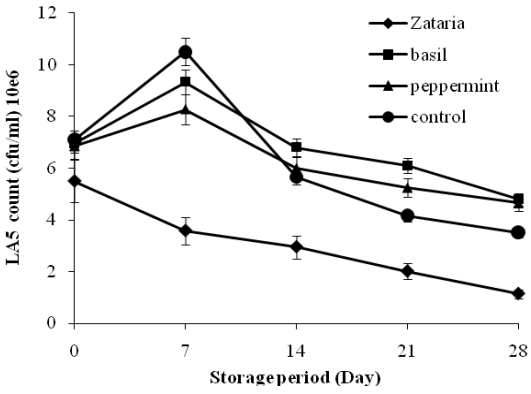

Fig. 3: Viable *L. acidophilus* LA5 count in EOs treated and control yogurts during refrigerated storage

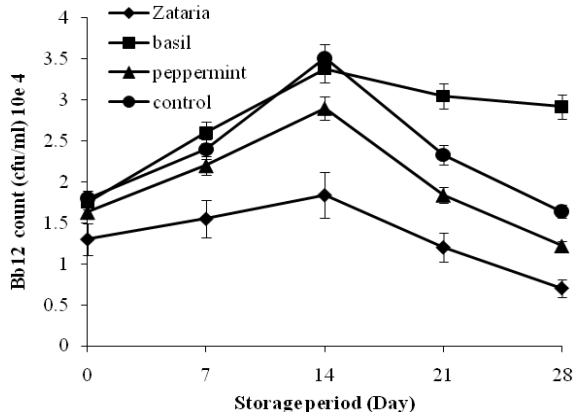

Fig. 4: Viable *Bifidobacterium* Bb12 count in EOs treated and control yogurts during refrigerated storage

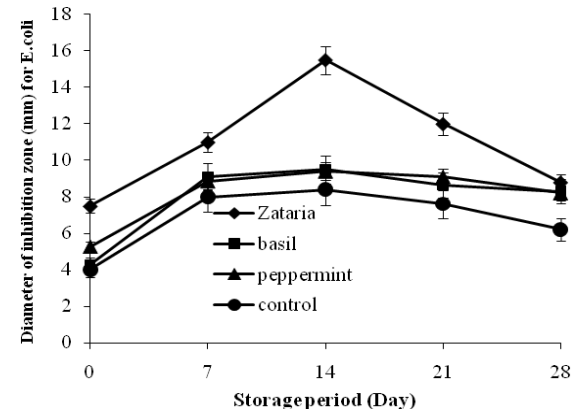

Fig. 5: Growth inhibition of *E. coli* in EOs treated and control yogurts during refrigerated storage

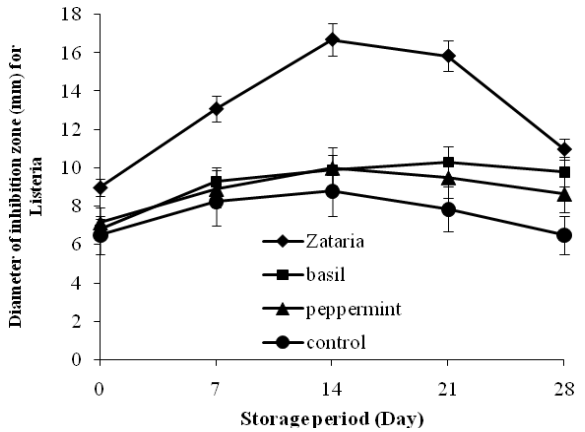

Fig. 6: Growth inhibition of *L. monocytogenes* in EOs treated and control yogurts during refrigerated storage

Discussion

Probiotic-yogurts with various flavours such as peach, blackberry, and strawberry have been developed by some researchers (Cruz et al., 2010; Gonzalez et al., 2011). Availability and cost effective nature of herbal EOs make them suitable food additives for enhancing the functionality of commercial yogurts. In the present study, we found effective antimicrobial activities against two main pathogenic bacteria in probiotic yogurts flavored with three herbal Eos, including peppermint, basil, and zataria. As shown in the results section, overall decline of pH in yogurts occurred during refrigerated storage. The presence of herbs did not make any changes in pH of herbal yogurts in comparison with control yogurt. Our results were in accordance with the findings of Behrad et al. (2009), who showed that pH in control yogurt was almost the same as pH of spice treated yogurts.

The survival of probiotic bacteria in yogurt or the other foods during refrigerated storage until consumption is a crucial factor in the field of probiotic products. According to several studies in this regard, to achieve optimal potential beneficial therapeutic effects, the number of probiotics in a product at the time of consumption should at least meet a suggested "therapeutic minimum" 10^5-10^6 CFU/g or ml of the final product for functionality and 10^8 CFU/g for presentation to the gut for all functional benefit (Cruz et al., 2012a; Cruz et al., 2012b; Granato et al., 2010). Although, the same volume of *L. acidophilus* LA5 and *Bifidobacterium* Bb12 was added at the time of yogurt production, but viable colonies for *Bifidobacterium* Bb12 at the first day of storage was quite different; however, for *L. acidophilus* LA5 and total lactobacilli a high viable count was maintained. The results for *L. acidophilus* LA5 as well as lactobacilli were comparable to the levels of other commercial probiotics such as *L. delbrueckii* ssp. *bulgaricus* with levels of 10^5 CFU/ml in probiotic stirred-type yogurt (Marafon et al., 2011). This indicated that freeze drying could kill *Bifidobacterium* Bb12 more than *L. acidophilus* LA5 and other lactobacilli prior to manufacture of the yogurt (Illupapalayam et al., 2014). In the present work, after 4 weeks of storage, all essential EOs treated yogurts contained an acceptable level of *L. acidophilus* LA5 (10^6 CFU/ml) and total lactobacilli (10^9 CFU/ml). These results were in accordance with Behrad et al. (2009) that concluded *L. acidophilus* LA5 were maintained in spice (cinnamon and liquorice) yogurt for 28 days storage at the level between 10^6-10^7 CFU/ml, but our results were in contrast to theirs in regard to *Bifidobacterium* Bb12. Several studies have reported that the survival of probiotic bifidobacteria is often low in yogurt (Gonzalez et al., 2011; Lourens-Hattingh and Viljoen, 2001; Shah, 2000), as seen in the present study. The increase in viable count of probiotics during the first 7 days was related to the marked reduction in pH recorded in all samples. In a similar way, the sharp was increased in viable count of *Bifidobacterium* Bb12 from day 7 to day 14 coincided with the approximately stable pH recorded (slight change

in pH) in all samples. These results showed that, the reduction in viable count which occurred in consistent manner in all yogurt samples might be attributed to the organic acid accumulation as the results of growth and fermentation as reported from Shah (2000). Altogether, the conversion of lactose to lactic acid, the composition of bacterial starter culture, duration of storage, and fermentation temperature could be the reason for the decrease in pH during storage (Singh et al., 2011). In this study, the viable numbers of probiotics were lower when zataria EO was used. This may be due to the main component of zataria EO (carvacrol>70%) with a great inhibitory effect on Gram-positive bacteria and so, zataria was considered as a strong antimicrobial (Zomorodian et al., 2011).

Zataria treated yogurt exhibited the strongest inhibitory effect on both pathogens in comparison with other EO treated and control yogurt during the storage period. The main constituents of zataria EO are phenolic compounds such as carvacrol, γ-terpinene, and α-pinene. The greater the content of phenolic compounds, the greater the antimicrobial activity exhibited by EO. The existence of some antimicrobial constituents such as linalool (Bassole et al., 2003), oxygenated carvacrol derivatives such as thymol methyl ether as well as carvacrol methyl ether (Rota et al., 2008), γ-terpinene, *p*-cymene (Al-Bayati, 2008; Gilles et al., 2010), α-pinene, and eucaliptol combined with other minor compounds might be involved in improving the overall antimicrobial activity of zataria EO in comparison with peppermint and basil EOs. Based on our results, water extract of EO treated yogurts exhibited inhibitory activity against *L. monocytogenes* stronger than *E. coli.* It seems that *L. monocytogenes* (Gram-positive) is more vulnerable than *E. coli* (Gram-negatives) in the presence of herbal EOs. The microstructure of the Gram-positives' cell wall facilitates the penetration of hydrophobic molecules into the cells so these components exert their effect on the cell wall and also within the cytoplasm. Phenolic compounds, which are also present in EOs, generally show antimicrobial activity against Gram-positive bacteria. But, Gram-negative bacteria possess more complex cell wall and as a result a stronger barrier. Their cell wall consists of a peptidoglycan layer with 2–3 nm thickness, which composes almost 20% of the dry weight of the cell. Also, this thin peptidoglycan layer is covered by an outer membrane that protects it (Kalemba and Kunicka, 2003; Nazzaro et al., 2013; Reichling et al., 2009).

Another result revealed that control yogurts (contained only probiotics) showed *in vitro* inhibitory activity against *L .monocytogenes* more than *E. coli.* As reported by several researchers, organic acids and antimicrobial substances produced by probiotic bacteria exert antimicrobial effect on Gram-positive pathogenic bacteria stronger than Gram-negatives (Jack et al., 1995; Maftah et al., 1993).

Conclusion

In conclusion, probiotic yogurt containing strains of *L. fermentum*, *L. acidophilus* LA5, and *Bifidobacterium* Bb12; and EOs of peppermint, basil, and zataria was successfully manufactured with viable probiotic counts up to the acceptable range and appropriate antimicrobial activity during 28 days. It is worth to note that the survival of lactobacilli in all yogurt samples was higher than *Bifidobacterium*. Also, both control and treated yogurt showed inhibitory effect against *L. monocytogenes* stronger than *E. coli*. The effectiveness of these herbal yogurts to halt the growth of *L. monocytogenes* and *E. coli* and also other pathogens needs to be further investigated under the real environment of the stomach and intestine. In addition, sensory profiling of yogurt with probiotics and EOs is needed for manufacturers considering incorporating the healthful ingredients into their products.

Conflicts of interest

The authors have declared that no conflict of interests exists.

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References

Abd-El Fattah S.M., Yahia Hassan A., Bayomn H.M., Eissa H.A. (2010). The use of lemongrass extracts as antimicrobial and food additive potential in yoghurt. *Journal of American Sciences*. 6: 582-594.

Adolfsson O., Meydani S.N., Russel R.M. (2004). Yoghurt and gut function. *The American Journal of Clinical Nutrition*. 80: 245-256.

Al-Bayati F.A. (2008). Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. *Journal of Ethnopharmacology*. 116: 403-406.

Andersson H., Asp N.G., Bruce A., Roos S., Wadstrom T., Wold A.E. (2001). Health effects of probiotics and prebiotics. A literature review on human studies. *Scandinavian Journal of Nutrition*. 45: 58-75.

Arsalan S., Ozdemir F. (2008). Prevalence and antimicrobial resistance of *Listeria* spp. in homemade white cheese. *Food Control*. 19: 360-363.

Azhdarzadeh F., Hojjati M. (2016). Chemical composition and antimicrobial activity of leaf, ripe and unripe peel of bitter

orange (*Citrus aurantium*) essential oils. *Nutrition and Food Sciences Research*. 3: 43-50.

Azizkhani M., Misaghi A., Basti A.A., Gandomi H., Hosseini H. (2013). Effects of *Zataria multiflora* Boiss. essential oil on growth and gene expression of enterotoxins A, C and E in *Staphylococcus aureus* ATCC 29213. *International Journal of Food Microbiology*. 163: 159-165.

Bassole I.H.N., Ouattara A.S., Nebie R., Ouattara C.A.T., Kabore Z.I., Traore S.A. (2003). Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso. *Phytochemistry*. 62: 209-212.

Basti A.A., Misaghi A., Khaschabi D. (2007). Growth response and modeling of *Zataria multiflora* Boiss. essential oil, pH and temperature on *Salmonella typhimurium* and *Staphylococcus aureus*. *LWT-Food Science and Technology*. 40: 973-981.

Be K., Gamlath S., Smith S.C. (2009). *In-vitro* antimicrobial effect of spices on probiotic bacteria. *Australasian Medical Journal*. 1: 113-140.

Behrad S., Yusof M.Y., Goh K.L., Baba A.S. (2009). Manipulation of probiotics fermentation of yogurt by cinnamon and licorice: effects on yogurt formation and inhibition of *Helicobacter pylori* growth *in vitro*. *World Academy of Science, Engineering and Technology*. 60: 590-594.

Clinical and Laboratory Standards Institute (CLSI). (2015). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 10th edition. CLSI document M07-A10.

Cruz A., Walter E., Rafael S., Faria J., Bolini H., Pinheiro H., Sant'Ana A.S. (2010). Survival analysis methodology to predict the shelf-life of probiotic flavoured yogurt. *Food Research International*. 43: 1444-1448.

Cruz A.G., Cadena R.S., Faria J.A., Bolini H.M., Dantas C., Ferreira M.M., Deliza R. (2012a). PARAFAC: adjustment for modelling consumer study covering probiotic and conventional yogurt. *Food Research International*. 45: 211-215.

Cruz A.G., Castro W.F., Faria J.A., Lollo P.C., Amaya-Farfan J., Freitas M.Q., Rodrigues D., Oliveira C.A.F., Godoy H.T. (2012b). Probiotic yogurts manufactured with increased glucose oxidase levels: postacidification, proteolytic patterns, survival of probiotic microorganisms, production of organic acid and aroma compounds. *Journal of Dairy Science*. 95: 2261-2269.

Gilles M., Zhao J., An M., Agboola S. (2010). Chemical composition and antimicrobial properties of essential oils of three Australian *Eucalyptus* species. *Food Chemistry*. 119: 731-737.

Gonzalez N.J., Adhikari K., Sancho-Madriz M.F. (2011). Sensory characteristics of peach-flavoured yogurt drinks containing prebiotics and synbiotics. *LWT-Food Science and Technology*. 44: 158-163.

Granato D., Branco G.F., Cruz A.G., Faria A.F.F., Shah N.P. (2010). Probiotic dairy products as functional foods. *Comprehensive Reviews in Food Science and Food Safety*. 9: 455-470.

Illupapalayam V.V., Smith S.C., Gamlath S. (2014). Consumer acceptability and antioxidant potential of probiotic-yogurt with spices. *LWT-Food Science and Technology*. 55: 255-262.

Isolauri E., Sutas Y., Kankaanpaa P., Arvilommi H., Salminen S. (2001). Probiotics: effects on immunity. *The American Journal of Clinical Nutrition*. 73: 444s-450s.

Jack R.W., Tagg J.R., Ray B. (1995). Bacteriocins of Gram-positive bacteria. *Microbiological Reviews*. 59: 171-200.

Kalemba D., Kunicka A. (2003). Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*. 10: 813-829.

Khorasany S., Azizi M.H., Barzegar M., Hamidi Esfahani Z. (2016). A study on the chemical composition and antifungal activity of essential oil from *Thymus caramanicus*, *Thymus daenensis* and *Ziziphora clinopodiaides*. *Nutrition and Food Sciences Research*. 3: 35-42.

Lourens-Hattingh A., Viljoen B.C. (2001). Yogurt as probiotic carrier food. *International Dairy Journal*. 11: 1-17.

Maftah A., Renault D., Vignoles C., Hechard Y., Bressollier P., Ratinaud M.H., Cenatiempo Y., Julien R. (1993). Membrane permeabilization of *Listeria monocytogenes* and mitochondria by the bacteriocin mesentericin Y105. *Journal of Bacteriology*. 175: 3232-3235.

Mahmoudzadeh M., Hosseini H., Hedayati M., Mousavi Khanghah A., Chaves R.D., Azizkhani M. (2015). Establishment of a method for describing *stx* genes expression of *E. coli* O157:H7 in ground beef matrix during refrigerated storage. *Journal of Food Safety*. 36: 220-226.

Marafon A.P., Sumi A., Granato D., Alcantara M.R., Tamime A., de Olivera M.N. (2011). Effects of partially replacing skimmed milk powder with dairy ingredients on rheology, sensory profiling, and microstructure of probiotic stirred-type yogurt during cold storage. *Journal of Dairy Science*. 94: 5330-5340.

McFarland L.V. (2006). Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. *The American Journal of Gastroenterology*. 101: 812-822.

Nazzaro F., Fratianni F., De Martino L., Coppola R., De Feo V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*. 6: 1451-1474.

Netzel M., Netzel G., Tian Q., Schwartz S., Konczak I. (2007). Native Australian fruits-a novel source of antioxidants for food. *Innovative Food Science and Emerging Technology*. 8: 339-346.

Parsaeimehr M., Basti A.A., Radmehr B., Misaghi A., Abbasifar A., Karim G., Rokni N., Motlagh M.S., Gandomi H., Noori N., Khanjari A. (2010). Effect of *Z. multiflora* Boiss. essential oil, nisin, and their combination on the production of enterotoxin C and a-hemolysin by *Staphylococcus aureus*. *Foodborne Pathogens and Disease*. 7: 299-305.

Reichling J., Schnitzler P., Suschke U., Saller R. (2009). Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties-an overview. *Forsch Komplementmed*. 16: 79-90.

Rota M.C., Herrera A., Martinez R.M., Sotomayor J.A., Jordan M.J. (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control*. 19: 681-687.

Schmidt E., Bail S., Buchbauer G., Stoilova I., Atanasova T., Stoyanova A., Krastanov A., Jirovetz L. (2009). Chemical composition, olfactory evaluation and antioxidant effects of essential oil from *Mentha x piperita*. *Natural Products Communications*. 4: 1107-1112.

Shah N.P. (2000). Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of Dairy Science*. 83: 894-907.

Singh G., Kapoor I.P.S., Singh P. (2011). Effect of volatile oil and oleoresin of anise on the shelf life of yogurt. *Journal of Food Processing and Preservation*. 35: 778-783.

Singh R., Shushni M.A., Belkheir A. (2015). Antibacterial and antioxidant activities of *Mentha piperita* L. *Arabian Journal of Chemistry*. 8: 322-328.

Srinivasan K. (2004). Spices as nutraceuticals with multi-beneficial health effects. *Journal of Herbs, Spices and Medicinal Plants*. 11: 137.

Suppakul P., Miltz J., Sonneveld K., Bigger S.W. (2003). Antimicrobial properties of basil and its possible application in food packaging. *Journal of Agriculture and Food Chemistry*. 51: 3197-3207.

Sutherland J., Miles M., Hedderley D., Li J., Devoy S., Sutton K., Lauren D. (2009). *In vitro* effects of food extracts on selected probiotic and pathogenic bacteria. *International Journal of Food Sciences and Nutrition*. 60: 717-727.

Tapsell L., Hemphill I., Cobiac L., Patch C., Sullivan D., Fenech M., Patch C.S., Roodenrys S., Keogh J.B., Clifton P.M., Williams P.G., Fazio V.A. (2006). Health benefits of herbs and spices: the past, the present, the future. *Medical Journal of Australia*. 185: 4-24.

Zaika L.L. (1988). Spices and herbs: their antimicrobial activity and its determination. *Journal of Food Safety*. 9: 97-118.

Zheljazkov V.D., Callahan A., Cantrell C.L. (2007). Yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in Mississippi. *Journal of Agriculture and Food*

Chemistry. 56: 241-245.

Zomorodian K., Saharkhiz M.J., Rahimi M.J., Bandegi A., Shekarkhar G., Bandegani A., Pakshir K., Bazargani A.(2011). Chemical composition and antimicrobial activities of the essential oils from three ecotypes *of Zataria multiflora*. *Pharmacognosy Magazine*. 7: 53-59.