



The possibility of producing synbiotic yogurt containing mint extracts

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Abstract

The improvement of synbiotic yogurt with multiple health benefits has developed to be a new trend. This study aimed to produce a low-fat synbiotic yogurt fermented by probiotic (*Lactobacillus acidophilus*) and prebiotic (Inulin) in addition to the alcoholic and aqueous extracts of mint, besides *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

Four treatments were manufactured, Plain Yogurt (PY), Synbiotic Yogurt (SY), Mint Alcoholic extract + Synbiotic Yogurt (MCSY) and (Mint Aqueous extract + Synbiotic Yogurt (MASY), and were stored up to 28 days at 4 °C. Chemical composition, pH, titratable acidity, LAB viable counts and sensory characteristics were measured during storage periods. (MCSY) treatment was the best during and at the end of storage periods, it was the lowest in its moisture content, the higher in its protein, fat and ash content compared to the rest treatments. (MCSY) treatment was significantly ($p < 0.05$) the lowest in its pH (4.00) and the highest in its titratable acidity (1.23) followed by (MASY), (SY) and (PY) treatments at the end of storage periods. The survival of LAB decreased along with the storage periods, (MCSY) treatment was the higher significantly ($p < 0.05$) in LAB counts (log 9.47 cfu/ml) after 28 days followed by (MASY), (SY) and (PY) treatments. All treatments' sensory characteristics reduced throughout storage periods, the best treatment was (MCSY) with acceptable taste, texture, odor, color and overall acceptance properties followed by (MASY) and (SY) treatments which were close along the storage periods. Results indicate that mint alcoholic extract, inulin, probiotic (*Lactobacillus acidophilus*) effectively improved the synbiotic yogurt properties.

Keywords: Inulin, synbiotic yogurt, mint alcoholic extract, mint aqueous extract, viable counts, *Lactobacillus acidophilus*

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INTRODUCTION

Recently, demand for diversity and quality of food has increased, and developing healthy synbiotic yogurt has become a new trend. Yogurt is a well-known dairy product consumed in the world for its desirable characteristics and health benefits after its ingestion (Wang *et al.*, 2012).

Yoghurt is made of milk and produced by lactic acid bacteria. Two starters are used in production of yogurt, including *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Unfortunately, none of the two mentioned bacteria can survive the digestive tract. Thus, yogurt doesn't provide probiotic properties since the beneficial bacteria destroyed under the acidic conditions of the stomach. For this reason, yogurt should be supplemented with acid-resistant beneficial bacteria (Tewari *et al.*, 2019).

Synbiotics composed of both probiotic and prebiotic. Probiotic is beneficial bacteria that protect the host from diseases and defined as "live microorganisms' supplements which beneficially affect the host by

improving the microbial balance of intestinal" while prebiotic is indigestible carbohydrate which stimulates the growth of the good bacteria (Khurana and Kanawjia, 2007). Synbiotics possess many benefits such as immune-stimulating properties, anti-allergic, antimicrobial and anticancer, improve minerals and nutrient absorption (Cadioux *et al.*, 2008).

Mint is grown traditionally in central Asia and Europe to produce the dry leaves and essential oils (Hay & Waterman, 1993). The components of mint may use as cooking herb or in salads (fresh herbs) and as a tea (dry leaves) (Baratta *et al.*, 1998; Lu & Foo, 2001) and in foods as aromatic substances, cosmetics, pharmaceutical, functional food and nutraceuticals industries. Mint has been demonstrated significant antioxidant activity (Lu & Foo, 2001) and antimicrobial activity (Marino *et al.*, 2001; Anaeto, et al, 2017). for both the extract and essential oils, and anti-cancer (Akdogan

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et al., 2004). Producing yogurt flavored with mint may contribute to developing dairy products that contain phytochemicals. Thus, the aim of this study is to explore the potential effect of adding mint extracts to synbiotic yogurt (inulin + *L. acidophilus*) on the chemical composition, pH, titratable acidity, LAB counts and sensory characteristics.

MATERIAL AND METHODS

Mint was purchased from Basrah city local markets.

Alcoholic extract was conducted according to the method of Elmastas *et al.* (2015) with some modification. A hundred grams of mint were added to 500 ml of ethyl alcohol (100%) and mixed well. Mixture put at (30°C) for 24 hrs. in vertical shaker, the mixture was filtered by using filter paper (Whatman No.1), to dispose of the solvent, a rotary vacuum evaporator was used to concentrate the filtrate at (40°C), left at laboratory temperature until it gets viscous and dry, then it was kept in dark bottles and placed in refrigerator.

Aqueous extract was prepared by using the modified method of Case (2005). Ten grams of mint were soaked in 100 ml of hot (95°C) distilled water, left a day in refrigerator at 4°C. Mint extract was placed in a rotary shaker at 100 rpm for 1hrs., the extract then filtered with filter paper (Whatman No.1), lyophilized at (-47.5°C). A powder of frozen mint extract was gained by using freeze-drier, then stored at 4°C.

Skim milk was purchased from Basrah markets (regilaite).

Starter cultures were mixture (1:1) of *Lactobacillus bulgaricus* and *Streptococcus thermophiles* (Chr. Hansen, Denmark).

Probiotic bacteria (*Lactobacillus acidophilus*) from LGGTM (Finland).

Prebiotic (Inulin) from NOW Foods products (USA).

Yogurt manufacturing Yogurt was manufactured according to Guler-Akin (2005). The reconstitution powder of skim milk with water was done at (30°C) using magnetic stirrer, the milk then pasteurized (80°C for 15 minutes), cooled to (42-45 °C), then adding (starter cultures, probiotic (*Lactobacillus acidophilus*) and prebiotic (Inulin) to the samples). Milk then divided into four treatments: (A) Plain yogurt, (B) Synbiotic yogurt (*Lactobacillus acidophilus* 10⁹ cfu/ml+ 1% Inulin), (C) 1% w/v mint alcoholic extract yogurt + Synbiotic, and (D) 1% w/v mint aqueous extract + Synbiotic yogurt. Samples were kept at 43 °C until (4.3-4.5) pH was gained. The treatments of yogurt were placed in the refrigerator at (4 °C) for four weeks, treatments were examined during study periods of (0, 7, 14, 21 and 28) days.

Yogurt chemical and physical tests

Moisture was determined depending on A.O.A.C (2005).

Ash was determined depending to A.O.A.C (2008).

Total nitrogen was determined as described by Uaboi-Egbenniet *et al.* (2010) by using semi-micro Kjeldahl method by digesting 0.2 g of sample by using concentrated sulfuric acid, then distilling it using Kjeldahl apparatus, titrating it, then the total protein ratio was calculated by multiplying the total nitrogen value by 6.38 (the coefficient).

Fat percentage was determined depending on the method of Egan *et al.* (1988).

pH was determined by placing the pH meter sensor into yogurt sample.

Titratable acidity was determined according to A.O.A.C. (2008).

Microbial content

Lactic Acid Bacteria (LAB) Enumeration Was conducted by using MRS agar.

Sensory evaluation of Yogurt:

Sensory evaluation of yogurt treatments was carried out by 5 panelists. The scores ranged from 1-5, 1 is extremely bad and 5 is excellent. The evaluated characteristics of yogurt treatments were: taste, texture, odor, color and overall acceptance (Mosiyani *et al.*, 2017).

Statistical design and analysis: Analyzing the data was done by using Completely Randomized Design (CRD)

While the the lowest significant LSD at 0.05 was used to compare the mean parameters (SPSS, 2009).

RESULTS AND DISCUSSION

Results in **Fig. 1** show the chemical composition of yogurt treatments during storage periods of 28 days. Results revealed that moisture content at zero time was significantly ($p < 0.05$) higher (90.35%) in (PY) treatment followed by (SY), (MASY) and (MCSY) which were (87.95%, 87.90% and 87.80%), respectively. All yogurt treatments reduced significantly ($p < 0.05$) in their moisture content gradually along with storage periods. After 28 days, the lowest moisture content was in (MCSY) yogurt treatment (86.82%) followed by (MASY), (SY) and (PY) which were (86.94%, 87.00% and 89.31%) respectively.

Treatments RLSD: Moisture 0.108: Protein 0.8110: Fat 0.02600: Ash 0.3240

Protein content at zero time was higher (4.90) for (MCSY) treatment and differ significantly ($p < 0.05$) from (PY) treatment, and followed by (MASY), (SY) and (PY) which were 4.88, 4.86 and 3.98, respectively. Protein content increased to reach 5.12 which was the higher (non-significant $p < 0.05$) for (MCSY) followed by (MASY), (SY) and (PY) which were (5.10, 5.09 and 4.32) respectively after 28 days. For Fat, results were 0.169, 0.29, 0.32 and 0.30 for (PY), (SY), (MCSY) and (MASY) respectively at zero time and reached over 28 days to be 0.48 for (MCSY) which was the highest treatment significantly ($p < 0.05$) followed by 0.45, 0.43 and 0.35

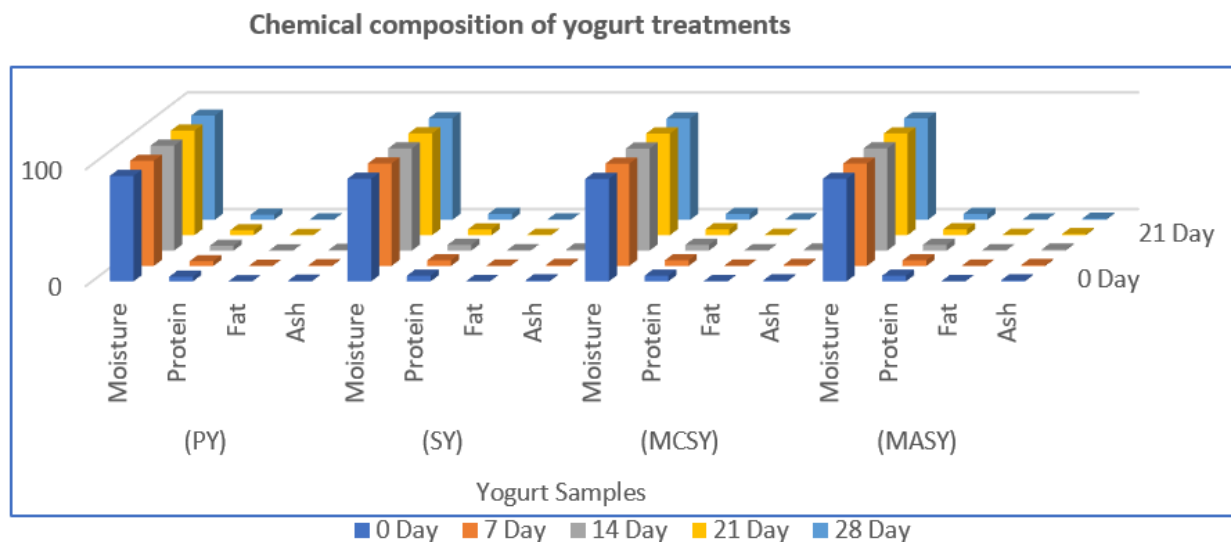


Fig. 1. Chemical composition of yogurt treatments

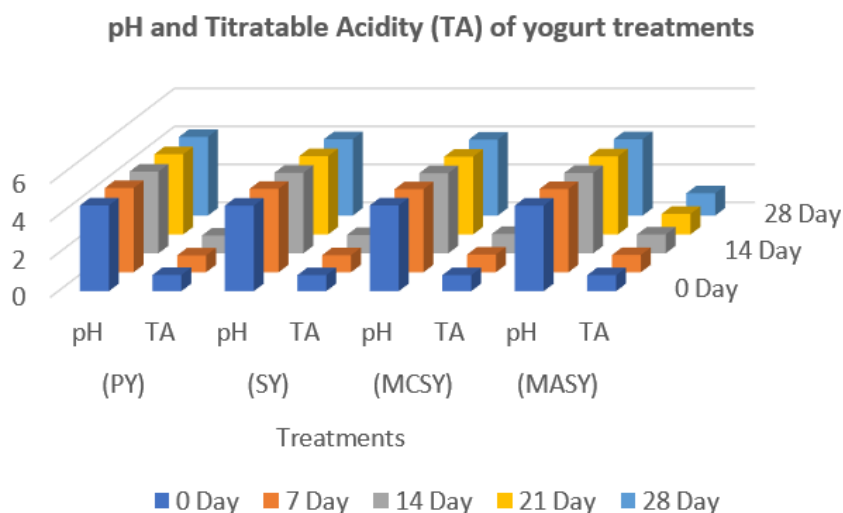


Fig. 2. pH and Titratable Acidity (TA) of yogurt treatments

for (MASY), (SY) and (PY) respectively. While ash results were 0.55, 0.91, 0.93 and 0.92 for (PY), (SY), (MCSY) and (MASY), respectively at zero time and increased at the end of storage period to reach 1.05 for (MCSY) which was the higher treatment non-significantly followed by 1.04, 1.03 and 0.75 respectively for (MASY), (SY) and (PY) treatments.

The previous results of low-fat yogurt treatments show that (MCSY) treatment was the best treatment compared with the rest treatments regarding the chemical composition followed by (MASY), (SY) and (PY) treatments, respectively. Moisture content for all treatments was close to the results of IHEMEJE *et al.* (2015) and MADADLOU *et al.* (2005) who attributed the reason behind the high moisture content was the low-fat milk that used in yogurt manufacturing. The reduction in moisture content along with the progress of storage periods may be attributed to the evaporation and the continuous decrease in yogurt pH during storage

periods. The mint extracts affected positively on the chemical composition of (MCSY) and (MASY) treatments, this finding agrees with (SATPUTE *et al.*, 2018) who figured out that the yogurt moisture content was positively affected by mint content. (MCSY) and (MASY) treatments were higher in their protein, fat and ash content, and this may be attributed to the incorporation of mint content of protein and this increased the level of minerals compared with (SY) and (PY) treatments (SATPUTE *et al.*, 2018).

Treatments RLSD: pH 0.0160: Titratable acidity 0.0200

Fig. 2 illustrates the pH and titratable acidity values of yogurt treatments. pH values at zero time were non-significant ($p < 0.05$) 4.51, 4.50, 4.51 and 4.50 for (PY), (SY), (MCSY) and (MASY) treatments and reduced gradually during storage periods to reach 4.00 for (MCSY) treatment which was significantly ($p < 0.05$) the

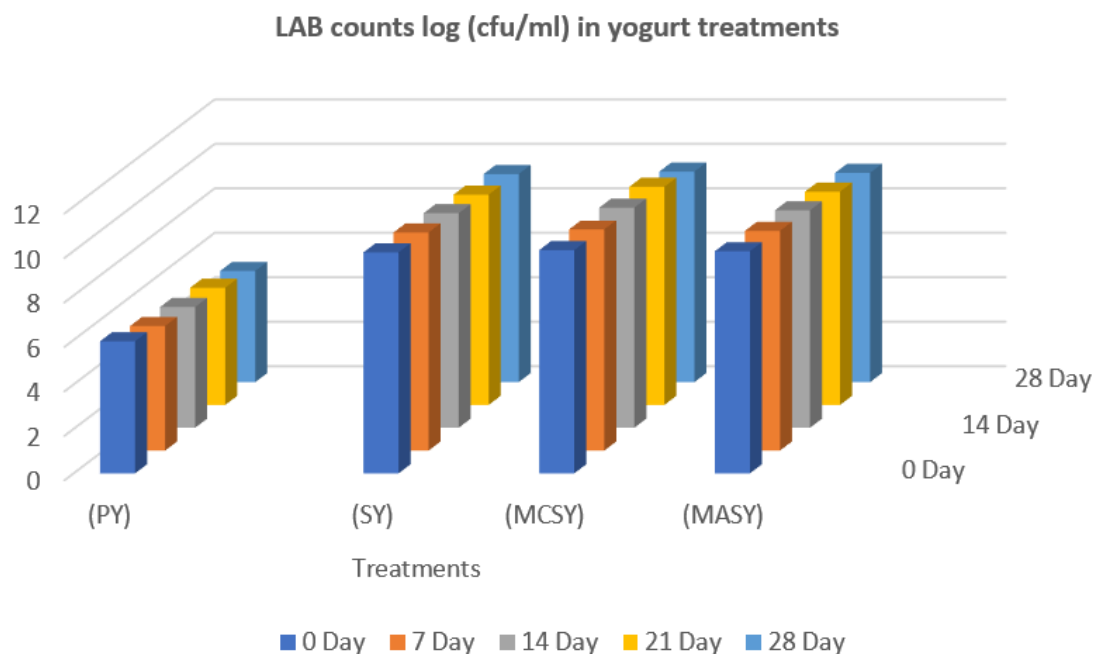


Fig. 3. LAB counts log (cfu/ml) in yogurt treatments

lowest pH followed by 4.02, 4.02 and 4.14 for (MASY), (SY) and (PY) treatments, respectively after 28 days. While the values of titratable acidity were non-significant ($p < 0.05$) and close at zero time (0.85, 0.84, 0.84 and 0.84) for (PY), (SY), (MCSY) and (MASY) respectively, and recorded the highest values for (MCSY) and (MASY) treatments which were 1.23 and 1.17, respectively which differ significantly ($p < 0.05$) with (SY) and (PY) treatments which were 1.13 and 1.07 respectively at the end of storage periods. The addition of inulin affected on reducing pH values and increasing titratable acidity values in inulin-treated treatments. The results of pH values agree with Shaghghi *et al.*, (2013) who found that the lowest value of acidity was found for samples containing inulin when they manufactured symbiotic yogurt by using different prebiotics. While acidity results agreed with the finding of Ozcan and Kurtuldu (2014) who reported that the addition of prebiotic (β -glucan) to yogurt affected significantly on the titratable acidity. Symbiotic yogurt treatments were higher in their acidity values and lower in their pH values comparing with the plain yogurt, and this might be attributed to the variation in microbial population and the species kind of (LAB) in yogurt treatments. The presence of mint extracts may have improved the proteolytic activity of *L. acidophilus* in (MCSY) and (MASY) treatments and led to pH reduction in these two treatments.

LAB count results of yogurt treatments within storage periods are presented in **Fig. 3**. At zero time, (PY) treatment was significantly ($p < 0.05$) the lowest in LAB counts, which was log 5.94 cfu/ml, while for the rest treatments, (MCSY) was the higher log (10.04) cfu/ml followed by (MASY) and (SY) treatments which were log

9.99 cfu/ml and log 9.94 cfu/ml, respectively. All yogurt treatments reduced in LAB counts at the end of the storage periods, the highest LAB count was significantly ($p < 0.05$) log 9.47 cfu/ml for (MCSY) treatment followed by (MASY) and (SY) treatments which were log 9.41 cfu/ml and log 9.36 cfu/ml, respectively and differ significantly ($p < 0.05$) from (PY) treatment which was log 5 cfu/ml. Results show that synbiotic yogurt treatment supplemented with an alcoholic mint extract was the highest in its LAB counts in all storage periods and this agrees with Marhamatizadeh *et al.*, (2011) who investigated the effect of adding spearmint on LAB growth, they demonstrated that *Lactobacillus acidophilus* and *Bifidobacterium bifidum* counts increased along with increasing spearmint concentration in probiotic milk and yoghurt, which means that mint addition, affected positively on LAB count in manufactured synbiotic yogurt of the current study.

Treatments RLSD: pH 0.0160: Titratable acidity 0.0200

The minimum acceptable probiotics level in probiotic products should be between 10^6 - 10^7 cfu/g to provide therapeutic properties (Boylston *et al.*, 2004) and this in agreement with study results for probiotic counts of synbiotic yogurt treatments which was higher than these limits. Study results show that inulin addition, affected positively on LAB counts and this is similar to Shu *et al.*, (2018) who found that prebiotic (stachyose) had a positive effect on *L. acidophilus* growth along with fermentation periods and its counts increased significantly, and similar to Mohebbi and Ghodusi (2008) who figured out that inulin affected positively on stimulating probiotics growth. Same finding, that Inulin addition to low fat synbiotic yogurt increased *L.*

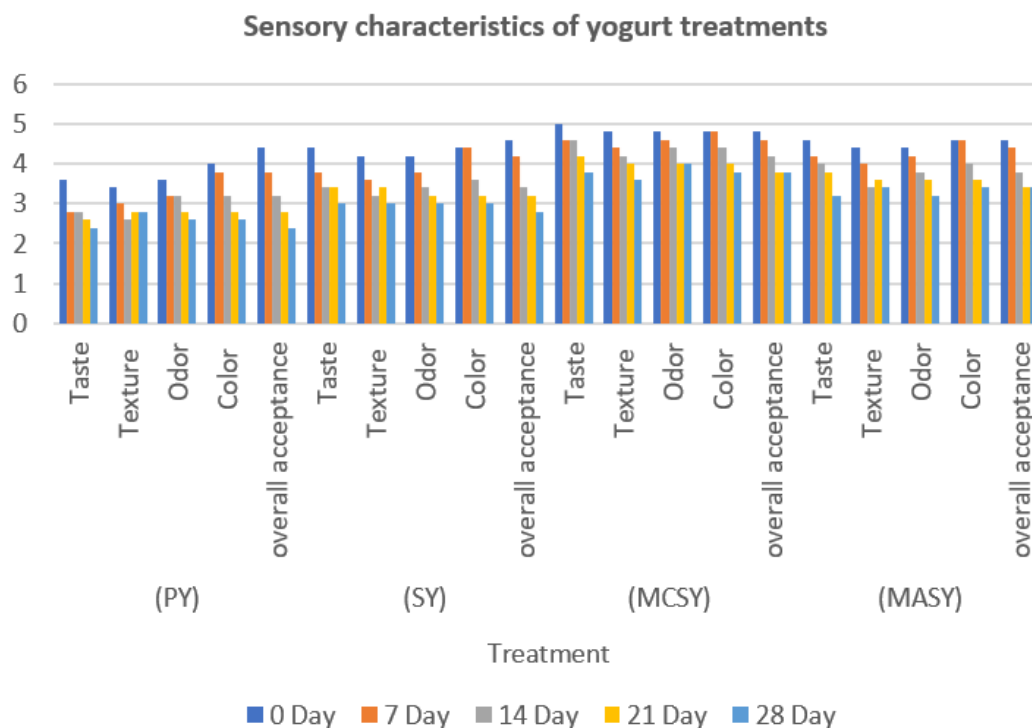


Fig. 4. Sensory characteristics of yogurt treatments

acidophilus and *L. delbrueckii ssp. bulgaricus* viability (Mazloomi *et al.*, 2011).

The addition of mint extracts to (MCSY) and (MASY) increased LAB counts significantly compared with the rest treatments and this in correspondence with Marhamatizadeh *et al.*, (2012) who observed a positive correlation between the increased concentrations of garlic in the milk and *Lactobacillus acidophilus* counts. The results were close to Marhamatizadeh *et al.*, (2013) who found that *Lactobacillus acidophilus* count was log 9 cfu/ml in day 14 when they produced a probiotic yogurt fortified with olive leaf extract.

Sensory scores for taste, texture, odor, color and overall acceptance of yogurt treatments are shown in **Fig. 4**. Along the storage periods, the highest scores were for (MCSY) treatment which was significant ($p < 0.05$) comparing with (SY) and (PY) treatments in most storage periods and non-significant ($p < 0.05$) with (MASY) in some storage periods, while (MASY) and (SY) treatments were close in their sensory scores along the storage periods with a slight significant preference for (MASY) treatment. All yogurt treatments' sensory scores reduced unevenly along with storage periods and that might be attributed to lactic acid production and aromatic compound such as acetone, acetaldehyde and diacetyl (Kaminarides *et al.*, 2007). The addition of probiotic, prebiotic and mint extracts affected positively on sensory scores of synbiotic yogurt treatments, and this agrees with the finding, that many desirable sensory properties will be gained by using probiotic species in dairy products (Hekmat *et al.*, 2009).

Treatments RLSD: Taste 0.0160: Texture 0.44: Odor 0.32: Color 0.32: Over all acceptance 0.32

Figure (4) Sensory characteristics of yogurt treatments

CONCLUSION

Study finding agrees with Mosiyani *et al.*, (2017) who showed that adding plant extract contributed in increasing (taste, texture and flavor) sensory scores of probiotic yogurt comparing with plain yogurt. The results agree with Joung *et al.* (2016) who figured out that yogurt containing herbal extract has better sensory properties than plain yogurt. The superiority of raffinose-treated yogurt regarding the organoleptic properties was over the yogurt without raffinose (Marinaki, 2016). Also, the inulin-treated sample was significantly ($p < 0.05$) higher in scores compared to control sample (Shaghghi *et al.*, 2013). The prebiotics, inulin and lactulose were the most acceptable samples in synbiotic yogurt production using different probiotic lactobacilli and prebiotic (Golob *et al.*, 2004; Shaghghi *et al.*, 2013).

Many researchers have studied probiotic, prebiotic and synbiotic yogurts which have many health privileges and to gain these privileges, yogurt should have probiotic counts between 10^6 - 10^7 cfu/ml when it is consumed. LAB counts in synbiotic yogurt treatments of the current study were more than these limits, in addition to that, adding of mint extracts gave desirable characteristics and content for synbiotic yogurt treatments. The alcoholic extract of mint was more effective when added to synbiotic yogurt in regard of

chemical composition, pH, titratable acidity, LAB counts and sensory characteristics compared with the aqueous extract of mint. Using of pharmaceutical plant extracts

with synbiotic yogurts may be a potential synbiotic yogurt have more desirable characteristics compared with plain yogurt.

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