

EFFECTS OF ARTICHOKE (*CYNARA SCOLYMUS* L.) EXTRACT ADDITION ON MICROBIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF PROBIOTIC YOGURT

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ABSTRACT

In this study, the effects of addition of artichoke (*Cynara scolymus* L.) leaf extract into yogurt (0 or 0.5%) on biochemical parameters (pH, titrable acidity) and the viability of probiotic bacteria (*Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12) during fermentation and over 28 days of refrigerated storage (4°C) were investigated. Moreover, the amounts of syneresis, total phenolic content, antioxidant activity and sensory attributes of yogurts at the end of fermentation were assessed. Yogurts contained the two yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*: ABY) or only *S. thermophilus* (ABT) as adjunct culture to probiotics. Yogurts containing *Cynara scolymus* L. (ABT-C and ABY-C) had faster acidity increase, shorter incubation time and greater final titrable acidity than control yogurts (ABT and ABY). Also, yogurts containing *Cynara scolymus* L. had lower syneresis, higher total phenolic content and greater antioxidant activity. ABT-C yogurt had the ever greatest viability of probiotics. In case of samples sensory evaluation, generally, the highest total score was related to ABT yogurt whereas lowest total score belonged to ABT-C yogurt.

Keywords: Antioxidant activity, *Cynara scolymus* L., Phenolic, Probiotic, Syneresis, Yogurt

INTRODUCTION

Viability of probiotic microorganisms in the final product until the time of consumption is its most important qualitative parameter. Although there is no world-wide agreement on the minimum of viable probiotic cells per gram or milliliter of probiotic product until the time of consumption, generally, the values of 10^6 and 10^7 - 10^8 cfu/mL or cfu/g have been accepted as the minimum and satisfactory levels, respectively. In Japan, the "Fermented Milks and Lactic Acid Bacteria Association" have standardized minimum of 10^7 cfu/mL viable probiotic cells to be present in dairy products (Korbekandi *et al.*, 2011; Tamime *et al.*, 2005; Ahmadi *et al.*, 2012 and Mortazavian *et al.*, 2008). In Iran, National standard requires minimums of 10^6 cfu/mL viable probiotic cells in yogurt (Anon, 2008a). It has also stated that probiotic products should be consumed regularly with an approximate amount of 100 g d^{-1} in order to deliver about 10^9 viable cells into the intestine (Korbekandi *et al.*, 2011).

Probiotic bacteria normally lose their viability during fermentation and storage period especially in fermented milks (Mortazavian *et al.*, 2010; Shafiee *et al.*, 2010; Heydari *et al.*, 2011; Mortazavian *et al.*, 2007; Ahmadi *et al.*, 2012; Mortazavian *et al.*, 2006a; Mortazavian *et al.*, 2006b; Mortazavian *et al.*, 2007b and Mortazavian *et al.*, 2011). Therefore, the high-priority attempt of probiotic industry has been increasing the survival and activity of probiotic microorganisms in dairy and non-dairy products, especially those with higher amounts of detrimental factors such as fermented foods. A common trend in fermented milks has been enrichment of milk with nutritious compounds that directly or indirectly increase the viability of probiotics (Korbekandi *et al.*, 2011).

The use of medicinal plants for treatment of different human diseases dates back to the primitive times. The Global Health Organization recently announced that 75-80% of the world's population treats themselves using natural drugs (Mocanu *et al.*, 2010). Artichoke (*Cynara scolymus* L.) is perennial, frost sensitive, thistle like plants with edible flower buds, which sprout from the terminal portion of the main stem and on lateral stems (Lopez-molina *et al.*, 2005). Artichoke is not only popular for its pleasant bitter taste, but also is an interesting and widespread herbal medicine.

Artichoke leaf extract is widely used alone or in association with other herbs for embittering alcoholic and soft drinks and to prepare herbal teas or herbal medicinal products (Bonomi *et al.*, 2001). Artichoke contains very little fat and high levels of minerals (potassium, sodium, phosphorus), vitamin C, fibre, inulin and polyphenols, hydroxycinnamates and flavones (Bianco *et al.*, 1999 and Lattanzio *et al.*, 1994). Inulin and polyphenols possesses hepatoprotective, anticarcinogenic and antioxidant activities (Gebhardt and Fausel, 1997; Jimenez-Escrig *et al.*, 2003). Artichoke has been labelled as 'lifespan essential', since its consumption could decrease the risk of chronic illnesses such as diabetes, cancer and cardiovascular disease as well as cholesterol levels in the blood. It can also treat hepato-biliary dysfunction and digestive complaints such as loss of appetite, qualm and abdominal pain. Enhancement of detoxification reactions of the liver, cholagogue and choleric activities have been also attributed to the ingestion of Artichoke (Holst and Williamson, 2008; Kraft 1997; Kirchhoff *et al.*, 1994; Gebhardt and Fausel, 1997; Gebhardt 1995; Brown, 1998 and Kraft, 2001). Therefore, incorporation of Artichoke extracts into probiotic yogurt could highly enhance its functional properties. Furthermore, It has been hypothesized that some ingredients in mentioned extract might increase the viability of probiotics in products (Mocanu *et al.*, 2011). Considering what was mentioned, the aim of this study was to investigate the effects of artichoke (*Cynara scolymus* L.) leaf extract into yogurt on viability of *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12 at the end of fermentation and during 28 days of refrigerated storage (4°C).

MATERIALS AND METHODS

Preparation of artichoke extract

The artichoke samples were collected from the research stead of Gorgan University of Agricultural Sciences and Natural Resources and leaves were separated from the stem. The leaves washed and dried in an oven (45°C, 24 h) and then were powdered using a laboratory mill (Mardani *et al.*, 2011). Artichoke extract was prepared according to the modified method by Mardani *et al.* (2011). Artichoke leaves were soaked in 30% ethanol solvent. Then, 10 g

powder was added to 100 mL solvent and was mixed for 24 h at ambient temperature using a mechanical stirrer. Then, extract by typical filter paper was separated from solid part. Ethanolic extract was concentrated by rotary vacuum evaporator followed by the powdering of extract by freeze dryer device (Mardani et al., 2011).

Starter culture

Lyophilized pouches of commercial ABY culture (containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, *Lactobacillus delbrueckii* ssp. *Bulgaricus* and *Streptococcus thermophilus*) and ABT culture (containing *L. acidophilus* LA-5, *B. lactis* BB-12 and *S. thermophilus*) were supplied by Chr-Hansen (Horsholm, Denmark). The cultures were maintained according to manufacturer's instructions at -18°C until used.

Preparation of yogurt samples

Four yogurt treatments obtained from two types of formulations: ABY (*Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, and yogurt bacteria) and ABT (*Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, and *Streptococcus thermophilus*) plus artichoke extract (0 or 0.5%) were produced using reconstituted skim milk powder and sterilized potable water. Reconstituted milk samples were heat treated at 85°C-30 min, then was cooled to 37°C. After addition of inoculum and artichoke extract, fermentation was carried out at 37°C until pH reached 4.5±0.02. Biochemical parameters including pH drop and acidity increase were monitored throughout the fermentation period. These parameters were recorded every 30 min. The final samples were cooled down and kept at 4°C for 28 days. Viability of probiotic organisms, pH, total titratable acidity, syneresis, total phenolic content and antioxidant activity were determined at the end of fermentation and within the storage period per 7-day intervals.

Preparation of yogurt water extract for chemical analysis

Yogurt sample (10 g) was mixed with 2.5 mL distilled water and the yogurt pH was adjusted to 4.0 using 0.1 M HCl. The yogurt was then incubated at 45°C for 10 min followed by centrifugation (5000 g, 10 min, 4°C). The supernatant was harvested and the pH was adjusted to 7.0 using NaOH 0.1 M. The neutralized supernatant was re-centrifuged (5000 g, 10 min, 4°C) and the supernatant was harvested and stored in a -20°C freezer until required for analysis (Amirdivani and Baba, 2011).

Determination of total phenolic content of yogurt

The total phenolic content of yogurt was determined by an assay modified from Shetty et al. (1995). Yogurt water extract (1.0 mL) was transferred into a test tube and mixed with 1 mL of 95% ethanol and 5 mL of distilled water. To each sample, 0.5 mL of 50% (v/v) Folin-Ciocalteu reagent was added and mixed. After 5 min, 1 mL of 5% Na₂CO₃ was added and the reaction mixture was allowed to stand for 60 min. The absorbance was read at 725 nm and the values were converted to total phenolics, expressed in milligrams equivalents of gallic acid per gram (GAE/g) sample. Gallic acid was used as standard (Apostolidis et al., 2007).

Determination of antioxidant activity of yogurt

Antioxidant activity of yogurt samples by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) inhibition was determined by an assay modified from Shetty et al. (1995). Yogurt water extract (250 µl) was added into 3 ml of 60 µM DPPH in ethanol. The decrease in absorbance was monitored at 517 nm until a constant reading was obtained. The readings were compared with the controls which contained distilled water (250 µl) instead of yogurt water extract (Apostolidis et al., 2007).

The inhibition percentage was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{extract}}}{A_{\text{control}}} \times 100$$

pH measurement and titratable acidity

pH values of the samples were measured at room temperature using a pH meter (Knick pH-meter 766 calimatic, Germany).

The titratable acidity was determined after mixing 10 mL of sample with 10 mL of distilled water and titrating with 0.1 N NaOH using 0.5% phenolphthalein (Shafiee et al., 2010 and Ahmadi et al., 2012).

Various biochemical parameters were defined and determined as follows:

Mean pH drop rate (mpH-DR) = (final pH value – initial pH value) / incubation time [pH value/min] (Mortazavian et al., 2010 and Mortazavian et al., 2011).

Mean acidity increase rate (mA-IR) = (final acidity value – initial acidity value) / incubation time [Dornic degree/min] (Mortazavian et al., 2010 and Mortazavian et al., 2011).

Time to pH5.5 (t_{5.5}): time from the start of incubation until reaching the pH of 5.5 [min].

Time range of maximum pH drop (t_{max-pH-D}): The 30-min time interval during fermentation in which the greatest pH decline is observed [min-min].

Syneresis measurement of yogurt

25 g of yogurt sample was weighed and placed on Whatman filter paper (No. 41) and on the funnel. Water out of the funnel was expressed after 120 min as syneresis (Tamime et al., 1996).

Microbiological analysis of probiotics in yogurt

MRS-bile agar medium (MRS agar by Merck, Darmstadt, Germany and bile by Sigma-Aldrich, Inc., Reye, USA) was used for the selective enumeration of *L. acidophilus* and bifidobacteria in ABY and ABT culture compositions (Mortazavian et al., 2007 and Sohrabvandi et al., 2012). The plates were incubated aerobically and anaerobically at 37°C for 72 h. Anaerobic conditions were produced using the GasPac system (Merck, Darmstadt, Germany).

Viability proportion index (VPI) of probiotic microorganism at the end of fermentation was calculated as following (Mortazavian et al., 2010; Shafiee et al., 2010 and Mortazavian et al., 2011).

VPI = Final cell population (cfu/mL) / initial cell population (cfu/mL)

Sensory analysis of yogurt

The treatments were analyzed and compared using a scoring method that was based on the Iran National Standard (Anon, 2008b). The sensory parameters were flavor, oral texture and mouthfeel, non-oral texture (pouring, stirring, and scoopability), and appearance (color, syneresis, and homogeneity with respect to the surface and texture). Each of these parameters was scored on a five-point scale: 0 = inconsumable; 1 = unacceptable; 2 = acceptable; 3 = satisfactory; and 4 = excellent. The score for each sensory parameter was multiplied by the relevant coefficient, namely, 6 for flavor, 3.5 for oral texture and feel in the mouth, 2 for appearance, and 1 for non-oral texture.

Statistical analysis

Each treatment was produced four times and each experiment was performed in triplicate. Experiments were set up using a completely randomized design. Data were subjected to analysis of variance and comparison of the means was done using the ANOVA test from SPSS software at significance level of 0.05.

RESULTS AND DISCUSSION

Biochemical, chemical and physical characteristics of yogurts

As is evident in Table 1, ABT-C yogurt (yogurt prepared by ABT culture, containing 0.5% *Cynara scolymus* L.) had the highest mean pH drop rate as well as the highest mean acidity increase rate ($p < 0.05$). In contrast, ABT yogurt (yogurt prepared by ABT culture, containing 0% *Cynara scolymus* L.) had the lowest amounts for both mentioned parameters. ABY yogurt (yogurt prepared by ABY culture, containing 0% *Cynara scolymus* L.) and ABY-C yogurt (yogurt prepared by ABY culture, containing 0.5% *Cynara scolymus* L.) were intermediate in parameters. It seems that the addition of artichoke extract did not significantly affect buffering capacity of the product during fermentation, but stimulate the growth and/or activity of starter cells. The higher mean acidity increase rate in ABT-C yogurt could be mainly attributed to stimulating growth and/or activity of *S. thermophilus*, because ABT yogurt (without *Cynara scolymus* L.) showed the lowest mean pH drop and acidity increase rates. Therefore, addition of *Cynara scolymus* L. significantly stimulated the growth and/or activity of starter cultures especially *S. thermophilus*. These observations were consistent with those reported by Mocanu et al. (2010) for ABT culture composition. They found that during incubation period, for samples containing bilberry extract and mixture of bilberry and liquorice extracts, rate of acidity increase was higher than control sample. In other research, Mocanu et al. (2009) found that adding mixture of sea-buckthorn and liquorice extract to ABY yogurt was indicated the acidity increase rate higher than control sample.

The shortest incubation times were observed for ABT-C that had the highest mean pH drop and mean acidity increase rates. ABY yogurt rendered the longest incubation time, as expected. Time of maximum pH drop (t_{max-pH-D}) in ABY, ABY-C and ABT were 240-270 (min-min), whilst this time for the ABT-C was 180-210 (min-min) (Table 1), indicating that in ABT treatment containing 0.5% artichoke extract, during fermentation, were 60 min sooner in the peak of acidification and activity than others. The data related to the pH of maximum pH drop (pH_{max-pH-D}) also confirmed mentioned observation. For example, ABY-C yogurt showed 0.36 decline in pH (5.55-5.19) within the hours 240-270 during

fermentation, whilst this pH decline value was 0.29 (5.41-5.12) in within the hours 180-210 in ABY-C yogurt (Table 1). According to Table 1, the time to reach pH5.5 (t_{5.5}) for ABY, ABY-C, ABT and ABT-C yogurts were 250, 245, 258 and 168 min, respectively. Amirdivani and Baba (2011) found that adding

O. basilicum, *M. piperita* and *A. graveolens* extracts within yogurt prepared by *S. thermophilus*, *L. bulgaricus*, *L. acidophilus*, and *B. bifidum*, significantly decreased incubation time and shortened the incubation time than control.

Table 1 Biochemical parameters, antioxidant activity, total phenol content and syneresis of treatments at the end of fermentation*

Treatment	Parameters					Incubation time (min)	Syneresis (g/25g)	Total Phenolic Content (mg gallic acid/g sample)	Antioxidant Activity (%inhibition)
	mpH_DR (pH/min)**	mA_IR (° D/min)	t _{5.5} (min)	t _{max-pH-D} (min-min)	pH _{max-pH-D} (pH-pH)				
ABY	0.0040 ^c	0.150 ^c	250 ^{bc}	240-270	5.57-5.25	480 ^c	12.76 ^a	58.56 ^c	6.87 ^c
ABY-C	0.0045 ^b	0.155 ^b	245 ^b	240-270	5.55-5.19	470 ^b	12.42 ^{ab}	87.50 ^a	17.25 ^a
ABT	0.0035 ^d	0.142 ^d	258 ^c	240-270	5.60-5.39	490 ^d	11.14 ^c	51.25 ^d	3.80 ^d
ABT-C	0.0050 ^a	0.160 ^a	168 ^a	180-210	5.41-5.12	460 ^a	10.83 ^d	76.47 ^b	15.50 ^b

*Means in the same column shown with different letters are significantly different (p<0.05).

**mpH-DR = mean pH drop rate, mA-IR = mean acidity increase rate, t_{5.5}=time to pH5.5, t_{max-pH-D} = time range of maximum pH drop, t_{max-A-I} = time range of maximum titrable acidity increase, pH_{max-pH-D} = pH of maximum pHdrop

Syneresis, separation of aqueous phase from continuous phase or gel network, is undesirable in yogurt production. This is common in low-fat yogurt. The use of compounds such as gelatin, pectin, starch and prebiotics has been suggested to reduce syneresis (Harte et al., 2005 and Amaya-Liano et al., 2008). Considering Table 1, adding artichoke extract into yogurt (both, ABT and ABY) decreased syneresis compared to controls. The lowest syneresis was observed for ABT-C yogurt. In contrast highest syneresis related to ABY yogurt. Probably, Inulin in artichoke extract (Adwan et al., 2007) that is a water-structuring agent, acted as a thickener and could form complexes (H-bridge formation) with the protein aggregates in the yogurt. Gustaw et al. (2011) found that addition of 1% inulin, resistant starch and fructooligosaccharide (FOS) within ABT yogurt decreased the syneresis.

Considering Table 1, the greatest total phenolic content (TPC) in yogurts was found in ABY-C (87.50 mg GA eq/g) and the lowest in those without plant extracts. Since ABY and ABT yogurts contains no plant extracts, the TPC values in ABY and ABT yogurts reflected phenolic compounds related to milk components, especially milk proteins (Damin et al., 2009). The amino acid tyrosin for instance has a phenolic side chain suggested to give rise to the reading in TPC (Shah, 2000). It should be noted, that artichoke is a rich source of the polyphenol compounds, with mono- and di-caffeoylquinicacids and flavonoids

(Nichiforescu, 1970; Adzet and Puigmacia, 1985; Dranik et al., 1996 and Wagenbreth, 1996).

Considering Table 1, both herbal yogurts (ABY-C and ABT-C) had higher (p<0.05) antioxidant activity than plain yogurts (ABY and ABT) at the end of fermentation. The Highest antioxidant activity in yogurts was found in ABY-C (17.25%) and the lowest in ABT (3.80%). The higher antioxidant activity in ABY-C and ABT-C yogurts than in plain-yogurts (ABY and ABT) were most likely contributed by individual herbal phytochemical contents and as a result of microbial metabolic activities (Thompson et al., 2007). The antioxidant activity of artichoke has been reported by Gebhardt (1997).The observed effects of cynarin and caffeic acid (within artichoke) have been assigned to the antioxidant activity of these compounds. Also, Brown and Rice-Evans (1998) and Lattanzio et al. (1994) found that luteolin (within artichoke) is a strong antioxidant that protects low density lipoproteins from oxidation.

Viability of probiotic bacteria at the end of fermentation and during refrigerated storage

Table 2 shows viability of probiotic microorganisms as well as the viability proportion indexes (VPI) in different yogurts immediately after fermentation.

Table 2 Viability of probiotic microorganisms and the viability proportion index (VPI) in different treatments at the end of fermentation

Treatment	Initial populati (log cfu/mL)			final population (D ₀) (log cfu/mL)			VPI		
	A*	B	A + B	A	B	A + B	A	B	A + B
ABY	7.57±0.06	7.72±0.08	7.95±0.04	8.18±0.04	8.22±0.07	8.51±0.04	4.11	3.19	3.57
ABY-C	7.57±0.06	7.72±0.08	7.95±0.04	8.42±0.07	8.53±0.06	8.79±0.01	7.14	6.49	6.75
ABT	6.29±0.03	6.48±0.05	6.7±0.09	7.14±0.10	7.24±0.05	7.50±0.06	7.15	5.74	6.29
ABT-C	6.29±0.03	6.48±0.05	6.7±0.09	7.47±0.12	7.54±0.06	7.81±0.09	15.34	11.54	13.02

A* = *L. acidophilus*, B = bifidobacteria, A + B = total probiotics

As represented from this Table, the viability of both probiotic bacteria (*L. acidophilus* LA-5 and *B. lactis* BB-12) were significantly and markedly greater in the treatments containing artichoke extract (ABY-C and ABT-C) than controls (ABY and ABT). For instance according to Table 2, the final population of *L. acidophilus* (at the end of fermentation) in ABY-C was 7.14-fold of initial population, compared to 4.11-fold in ABY. The amounts for ABT-C and ABT were 15.34 and 7.15, respectively. As was evident, the viability of both probiotic bacteria in treatments without *L. delbrueckii* ssp.*bulgaricus* (ABT and ABT-C) was significantly and considerably greater than their related treatments contained mentioned yogurt bacteria (ABY and ABY-C), because it is well-known that *L. delbrueckii* ssp.*bulgaricus* can suppress probiotics (Mortazavian et al., 2006 and Mortazavian et al., 2007).

The positive effects of artichoke on viability of probiotics can be attributed to the reason that artichoke provide higher nutritious and stimulatory media for lactic

acid bacteria and probiotic bacteria, and stimulate their growth and activity. From these substances, exopolysaccharide, adenine, hypoxanthine, free amino acids, and essential vitamins and minerals can be mentioned (Parada et al., 1998; Zielke et al., 1978; Varga et al., 1999; Gibson and Roberfroid, 1995; Kurita, 1979; Shirota et al., 1964; Stengel, 1970 and Webb, 1982). Lutz et al. (2011) reported that artichoke contains proteins, carbohydrates and dietary fiber that could affect viability of probiotics. Also, Llorach et al. (2002) and Fratianni et al. (2007) announced that artichoke contains proteins, minerals, low amounts of lipids, dietary fiber and a high proportion of phenolics. Mocanu et al. (2011) found that adding mixture of rosehip and liquorice extract to ABY and ABT yogurt, increased viability of probiotic bacteria (LA-5 and BB-12) and highest viability of lactic acid bacteria were observed in ABT yogurt containing mixture of rosehip and liquorice extract. Table 3 shows the viability of probiotic microorganisms in different treatments during refrigerated storage.

Table 3 Viability (log cfu/mL) of probiotic microorganisms in different treatments during refrigerated storage

Treatment	D ₇			D ₁₄			D ₂₁			D ₂₈		
	A*	B	A + B	A	B	A + B	A	B	A + B	A	B	A + B
ABY	8.00±0.0	8.02±0.03	8.31±0.01	7.51±0.56	7.60±0.66	8.04±0.46	7.13±0.34	7.14±0.14	7.45±0.24	6.86±0.26	6.84±0.34	7.18±0.24
ABY-C	8.39±0.0	8.41±0.05	8.71±0.03	8.02±0.06	8.01±0.05	8.32±0.03	7.68±0.07	7.69±0.45	8.01±0.28	7.48±0.25	7.46±0.08	7.79±0.12
ABT	6.99±0.0	7.01±0.09	7.30±0.04	6.73±0.29	6.78±0.05	7.07±0.10	6.34±0.83	6.36±0.50	6.81±0.59	6.03±0.06	6.12±0.75	6.48±0.35
ABT-C	7.39±0.4	7.45±0.16	7.76±0.27	7.25±0.29	7.29±0.12	7.59±0.15	6.95±0.10	7.01±0.63	7.36±0.24	6.68±0.14	6.74±0.44	7.05±0.21

A* = *L. acidophilus*, B = bifidobacteria, A + B = total probiotics

Table 4 represents their relative viability proportion index (VPI) during this period. It is notable that the viability of both probiotic bacteria were significantly greater in the treatments containing artichoke extract (ABY-C and ABT-C yogurts) compared to control (ABY and ABT yogurts) during storage period of

28 days. According to Table 4, viability proportion index (VPI) of probiotic bacteria in ABT yogurt was significantly higher than ABY yogurt during refrigerated storage.

Table 4 Viability proportion index (VPI) of probiotic bacteria during 28 d of storage at 4°C, per 7-day intervals (compared to the initial viable cell counts immediately after fermentation or the viable cell counts at the last days of each 7-day storage interval)

Treatment	probiotic	VPI							
		D ₇		D ₁₄		D ₂₁		D ₂₈	
		VPI ₀	VPI ₇	VPI ₀	VPI ₁₄	VPI ₀	VPI ₁₄	VPI ₀	VPI ₂₁
ABY	A*	0.42	0.07	0.16	0.004	0.06	0.004	0.89	
	B**	0.40	0.04	0.10	0.002	0.05	0.0004	0.17	
ABY-C	A	0.80	0.20	0.25	0.01	0.04	0.01	1.30	
	B	0.66	0.05	0.07	0.01	0.11	0.003	0.63	
ABT	A	0.12	0.08	0.63	0.05	0.65	0.01	0.23	
	B	0.13	0.02	0.20	0.01	0.47	0.01	0.61	
ABT-C	A	0.32	0.09	0.29	0.08	0.84	0.09	1.17	
	B	0.21	0.02	0.09	0.03	1.52	0.01	0.38	

A* = *L. acidophilus*, B** = bifidobacteria.

However, in ABT-C and ABY-C yogurts, the rate of probiotic reduction was very slower (ABT-C had the lowest reduction rate in viable count). *A. platensis*-supplemented fermented ABT milk contained significantly higher ($p < 0.05$) levels of lactobacilli than the control during storage time (Kurita et al., 1979). Mocanu et al. (2010) were observed that highest number of probiotic bacteria at the end of refrigerate storage, belongs to ABT yogurt containing mixture of bilberry and liquorice extract compared to ABT yogurt.

Corresponding with Iranian national standard, viability of probiotic bacteria was greater than 10⁶ cfu/mL in all yogurts until the d 28 of storage period (Anon, 2008a).

Sensory characteristics of yogurt samples at the end of fermentation

Table 5 shows the sensory analysis of yogurts using score methodology. As shown, yogurts containing artichoke extract possessed weaker sensory acceptability for all sensory parameters compared to the controls. Artichoke extract in ABT-C yogurt exhibited more unpleasant flavor compared to ABY-C yogurt and had the lowest flavor score. Probably, Unsuitable bitter flavor of ABY-C and ABT-C yogurts was due to presence of sesquiterpene lactones (e.g. cynaropicrin) in artichoke extract (Lohr et al., 2009). Addition of artichoke extract into the yogurt changed the color of this product to yellowish. This characteristic was realized as an in appropriate sensory attribute (appearance) by the panelists. There were not considerable differences among the yogurt samples from non-oral texture points of view. However, differences were remarkable from oral texture standpoint. Yogurts containing artichoke extract had the lowest sensory score for oral texture. Generally, the highest total score was related to ABT yogurt whereas lowest total score belonged to ABT-C yogurt.

Table 5 Sensory analysis of the treatments using score methodology*

Treatments	Parameters**				
	Appearance	Flavor	Oral texture	Non-oral Texture	Total score
ABY	5.1 ^{abc}	16.7 ^{abc}	10.5 ^a	2.8 ^{abcd}	35.1 ^{ab}
ABY-C	2.9 ^d	11.3 ^{acd}	3.9 ^{cd}	2.0 ^d	20.1 ^{cd}
ABT	6.7 ^a	17.3 ^a	10.1 ^{ab}	3.4 ^a	37.6 ^a
ABT-C	4.0 ^{cd}	8.0 ^d	5.1 ^c	2.2 ^{cd}	19.3 ^d

* Means shown with small letters represent significant differences ($p < 0.05$) in the same columns.

**Every data point is the mean of nine replications (nine panellists).

CONCLUSION

Results of this study revealed that adding artichoke extract to ABY and ABT yogurts significantly increased viability of *L. acidophilus* LA-5 and *B. lactis* BB-12 at the end of fermentation as well as during refrigerated storage. Therefore, probiotic herbal yogurts are more functional from probiotic viability points of view.

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