

Chemical Characteristics, and Effect of Inulin Extracted from Artichoke (*Cynara scolymus* L.) Root on Biochemical Properties of Synbiotic Yogurt at the End of Fermentation

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ABSTRACT: *The aim of this study was to produce synbiotic yogurt using different inulin levels (0, 1 and 2%) and probiotic bacteria. Inulin in two forms commercial and extracted from artichoke root was prepared, and chemical and sensory evaluation were performed. The results of inulin analysis showed that pH, dry matter, the degree of polymerization, purity, appearance, and taste for the inulin extracted from the artichoke root were 6.39, 92.8%, 30.5, 88.5%, white powder and neutral, whereas these parameters for commercial inulin were 6.14, 97.8%, 23, 92.3%, white powder and neutral, respectively. In the following, the production of synbiotic yogurt was carried out at 42°C until pH reached 4.5±0.02. The results of biochemical, microbiological and sensory characteristics at the end of fermentation showed that probiotic yogurt containing 2% (w/w) commercial inulin (ABY-Ch (2%)) had a faster acidity increase, shorter incubation time and greater final titrable acidity than other yogurt samples. Although inulin extracted from artichoke increased the viability of probiotic bacteria in yogurt, but ABY-Ch (2%) yogurt had the ever greatest viability of probiotics. the highest lightness (L*) is related to ABY-Ch (1%) and ABY-Ch (2%) yogurt samples, and ABY-C (2%) yogurt (yogurt containing 2% (w/w) artichoke root inulin) exhibited more unpleasant flavor compared to ABY yogurt (control) and had the lowest flavor score.*

KEYWORDS: *Chemical evaluation; Degree of polymerization; Inulin; lightness; pH; Synbiotic yogurt.*

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INTRODUCTION

Nowadays throughout the world, turning to functional food consumption and production considerably has been increased. These foods are of higher health benefits and have been identified as containing compounds with biological activity that can promote health and reduce the risk of disease. These foods consist of antioxidants, dietary fiber [1], vitamin and mineral fortification, cholesterol reduction, phytochemicals, probiotics, prebiotics and synbiotic [2].

The word probiotic derived from the Greek language meaning resuscitative and microorganisms that succeeding oral administration causes to improve the properties of microflora in host intestinal tract and have beneficial effects on consumers' health [3]. There are health benefits associated with probiotic bacteria including reducing cholesterol level, producing some digestive enzymes and vitamins, reducing gut pH [1], having anticarcinogenic effects and antagonistic activity against enteric pathogens [4]. The most widely probiotic species used in dairy products particularly in yogurts are *Lactobacillus* and *Bifidobacterium*. *Lactobacillus* species as *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus rhamnosus*. *Bifidobacterium* species as *Bifidobacterium animalis*, *Bifidobacterium bifidum* and *Bifidobacterium longum* [5]. One of the important challenges associated with probiotic products is losses in the viability of probiotic bacteria owing to difficult conditions in food production as well as gastrointestinal unfavorable conditions. According to FAO, the standard probiotic product has 10^6 - 10^7 cfu.g⁻¹ active and live probiotic microorganism [6]. Therefore, the key factor for the effective use of probiotics properties is to maintain the viability and activity of probiotics bacteria during storage stage and to survive passage through the acidic conditions of the stomach, enzymes and bile salts in the small intestine. Accordingly, It seems necessary that the use of prebiotic components stimulates the growth of probiotics in the gut and besides it aids to maintain product stability [7].

Prebiotics are non-digestible ingredients by the upper gastrointestinal tract that causes to stimulate the growth and activity of probiotic bacteria in the colon and has a positive effect on improving host health [8]. Nutrition scientists feel that inulin is a soluble dietary fiber, nevertheless, researches suggested that it has prebiotic and bifidogenic properties that cause inulin to be named

as a functional food [9, 10]. One of the main reasons for paying attention to inulin is that based on findings inulin has a positive impact on the composition of the intestinal flora. On the other hand, some studies illustrate the beneficial effects of prebiotics on mineral absorption, blood lipid composition, and prevention of colon cancer. In addition, inulin is a low-calorie fiber that can be used in producing fat reducer foods [11-16]. Attributable to the high percentage of inulin (As a prebiotic) to dry material within artichoke (*Cynara scolymus* L.) root, this medicinal plant is one of the most important sources of inulin [17].

Synbiotic is a blend of probiotics and prebiotics [18]. Yogurt and dairy drinks are commonly used to produce synbiotic products [19]. Yogurt, due to exclusive sensory attributes and health benefits, is the most acceptable dairy product. At present, probiotics are widely and approximately used in preparing fermentable foods (especially yogurt), 65% of the functional foods are probiotics. One of the benefits of probiotic bacteria in yogurt is their role in enhancing the bioavailability of calcium, zinc, iron, manganese, copper, phosphorus, protein digestibility and synthesis of vitamins [20].

Based on what mentioned above, the simultaneous presence of prebiotics and probiotic bacteria within yogurt (synbiotic yogurt) offers a synergistic effect. Therefore, this study was to investigate both chemical properties of inulin extracted from artichoke (*Cynara scolymus* L.) root and characteristics of biochemical, microbiological and sensory of probiotic yogurt which is produced with artichoke root inulin and commercial inulin at the end of fermentation.

EXPERIMENTAL SECTION

Materials

Artichoke roots were collected from the farm of Gorgan University of Agricultural Sciences and Natural Resources (Gorgan, Iran). Commercial inulin (Chicory) was provided from Benco company (Italy). Lyophilized pouches of commercial ABY culture (containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, *Lactobacillus delbrueckii* spp. *Bulgaricus* and *Streptococcus thermophilus*) from Biochem company (Italy) and milk powder from Golestan Pegah factory (Iran) were purchased. The chemical reagents including calcium hydroxide, phosphoric acid, phenol, sulfuric acid and 3, 5-Dinitrosalicylic acid were bought from Sigma Aldrich Chemie GmbH (Germany).

Methods

Extraction of inulin from artichoke root

The roots after the harvest, in order to remove impurities, washed with cold water and after drying of water from the surface were kept at 4°C. The roots after the peeling were crushed in a blender with hot water (1:9) and the suspension obtained were heated for 35 min at 95°C. Then, extract obtained was filtrated and its pH for removing of particles and colloidal substances such as pectin, protein and cell wall by using calcium hydroxide 5% to about 11 was reached. The extract was placed for 30 min at a 50°C and fluff like sediment was removed by using filter paper. Then, to remove excess calcium and other organic matter, pH of the extract with phosphoric acid 10% solution to 5.8 was reached and for 2 hours at 60°C was held until sediment is formed. After the filtration of sediment obtained by using filter paper, purified extract be bleached with adding 20 g activated carbon for 1 kg root and intense agitation at 60°C for 30 min was performed and in the following activated carbon with filter paper was separated. An extract obtained was concentrated by using under vacuum evaporator device to Brix 42. In order to precipitation of carbohydrates and inulin from concentrated extract, ethanol 96% with a ratio (8:1) to it was added. Formed suspension for complete sedimentation for 2 days at 40°C was placed and then its ethanol was separated. Sediment obtained for 4 days at 50°C was kept, and then dried sediment was fragmented and its final weight relative to the primary root weight was calculated [21].

Analysis of inulin powder extracted artichoke root

In order to the determination of inulin quality, parameters of total carbohydrate, reducing carbohydrate, the degree of polymerization (DP), pH, dry matter and ash were measured.

Total carbohydrate

The phenol-sulfuric acid was used to measure the total carbohydrate content in the samples. The first, 1 mL phenol 5% was added to 1 mL inulin extracted from artichoke root in concentration 1% (w/v). Then 5 mL sulfuric acid 98% was added to the sample and absorption was measured using a spectrophotometer device at wavelength 490 nm, after the 20 min. In this method, glucose was used as a standard, and after drawing the standard curve, total carbohydrate within the sample was determined [22].

Reducing carbohydrate

The measurement of reducing carbohydrate within the sample was performed with 3, 5-Dinitrosalicylic acid indicator and spectrophotometer device at a wavelength of 575 nm. In this test, glucose was considered as a standard [23].

The degree of polymerization (DP)

The average of the degree of polymerization (DP) was determined from the division of total carbohydrate (wt%) to the reducing carbohydrate (wt%). This factor is very important in measuring of inulin quality [24].

pH

For the determination of pH, the solution 10% inulin in water was prepared and the pH of the solution was measured using an electronic pH meter [25].

Dry matter

A little sample was dried in temperature 100°C and then dry matter percent was determined with the following formula (1) [26].

$$\text{Dry matter(\%)} = \frac{\text{Sample mass}}{\text{Initial sample mass}} \times 100 \quad (1)$$

Ash

A little sample was burned in the electric furnace at a temperature 550°C and after reaching the constant weight, ash percent was calculated based on the sample initial weight and the obtained ash according to the following formula (2) [27].

$$\text{Ash(\%)} = \frac{\text{Ash mass}}{\text{Sample mass}} \times 100 \quad (2)$$

Production of synbiotic yogurt and analysis of quality parameters

Production of synbiotic yogurt consists of two stages. Firstly, Preparation of starter cultures and probiotic bacteria and then Preparation of yogurt.

Preparation of starter cultures and probiotic bacteria

Lyophilized pouches of commercial ABY-1 culture (containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus*)

was supplied by Biochem company. The cultures were maintained according to the manufacturer's instructions at -18°C until used. For each experimental stage, a 10-unit pouch of ABY-1 starter culture was dissolved in 1000 mL sterilized milk and then an inoculum of 9 mL was used to inoculate 3000 mL of reconstituted skimmed milk (12% (w/v)).

Preparation of yogurt

Five yogurt treatments obtained from ABY-1 (*Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, and yogurt bacteria) plus standard inulin and artichoke root inulin (0, 1, 2 %) were produced using milk powder and sterilized potable water (Reconstituted milk at 12% (w/v)). Reconstituted milk samples were heat treated at 85°C -30 min, then was cooled to 42°C . After addition of inoculum and inulin, fermentation was carried out at 42°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 . The viability of probiotic organisms, pH and total titratable acidity were determined at the end of fermentation [28].

pH and titratable acidity

From the beginning to the end of the incubation period, every 30 minutes, 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 [29,30].

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) [31,32].

Mean acidity increase rate (mA-IR) = (final acidity value – initial acidity value) / incubation time (Dornic degree/min) [31,32].

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 ($t_{\text{max-pH-D}}$): The 30-min time interval during fermentation in which the greatest pH decline is observed (min-min).

The viability of probiotic bacteria in yogurt

MRS-bile agar medium (MRS agar by Merck, Darmstadt, Germany and bile by Sigma-Aldrich, Inc., Reyde, USA) was used for the selective enumeration of *L. acidophilus* and bifidobacteria in ABY culture composition [33]. 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 [30,31].

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

Colorimetric test of yogurt

The color parameters consist of L^* (lightness-darkness), a^* (green-red axis) and b^* (yellow-blue axis) values of the yogurt samples were determined by the modified method described by Yam and Papadakis [34] at 8°C . High-resolution photos of the samples were taken by a digital camera. The light source was fluorescent. The photos were analyzed by Adobe Photoshop for Windows® ver 8. Colorimetric values were determined in the Lab mode of the software [34].

Sensory analysis of yogurt

Sensory evaluation of yogurt samples by ten panelists trained was performed in the Food and Drug Laboratory of Golestan University of Medical Sciences. The treatments were analyzed and compared using a scoring method that was based on the Iran National Standard [35]. The sensory parameters were flavor, oral texture and mouthfeel, non-oral texture (pouring, stirring, and scoop ability), 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

Table 1: Chemical and sensory characteristics of artichoke root inulin and commercial inulin.

Parameters	Type of Inulin	
	Commercial inulin	Artichoke root inulin
pH	6.14	6.39
Ash (%)	0.2	5.42
Dry Matter (%)	97.8	92.8
Total Carbohydrate (%)	96.5	91.5
Reducing Carbohydrate (%)	4.2	3
Degree of Polymerization	23	30.5
Inulin Purity (%)	92.3	88.5
Appearance	White	White
Taste	Neutral	Neutral

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 and Duncan's test from SPSS software at a significant level of 0.05.

RESULTS AND DISCUSSION

Chemical and sensory characteristics of artichoke root inulin and commercial inulin

In Table 1, the chemical and sensory characteristics of artichoke, root inulin are compared with commercial inulin. Artichoke root inulin has a bland, neutral taste and is not sweet. The color of inulin obtained of artichoke root and commercial inulin was white. Thus, it mixed easily with other food ingredients without changing color and flavour. These results in accordance with results reported by *Lopez-molina et al.* [25] about artichoke bracts inulin. The pH of artichoke root inulin was 6.39, in contrast commercial inulin was 6.14. also, the amounts of ash, dry matter, total carbohydrate and reducing carbohydrate in artichoke root inulin were 5.42, 92.8, 91.5 and 3%, while, these parameters in commercial inulin were 0.2, 97.8, 96.5 and 4.2%, respectively. Inulin extracted from various plant sources differ in their degree of polymerization (DP). This characteristic represented prebiotic effects, digestibility, caloric value, sweetening power, health-promoting potential and water binding capacity [36–38]. In this research, DP of artichoke root inulin was 30.5, while commercial inulin was 23. The high DP of artichoke root inulin could be used as a fat replacement and in combination with gelling agents such as gelatin, as well as It makes better the stability of foams and

emulsions, for ,example ice creams and sauces [17]. Also, inulin purity for artichoke root inulin was 88.5%, whereas this parameter for commercial inulin was 92.3%.

Biochemical characteristics of yogurt samples during the fermentation stage

Fig. 1 shows changes in pH drop and acidity increase during the fermentation period in different treatments. As shown in this Figure, for all treatments, three distinguished phases could be observed according to the breakpoints in graph charts: lag and pre-log phases, log phase, and late log and stationary phases. This result was in correspondence with those reported by *Heydari et al.* [39] and *Ahmadi et al.* [29].

For all treatments of synbiotic yogurts, the minimum decrease rate of pH and the minimum increase rates of acidity were observed within the initial steps of fermentation which could be attributed to the being in the late lag/early log phases of bacterial growth as well as the relatively high buffering capacity of milk [30,32].

As is evident in Table 2, ABY-Ch (1%) and ABY-Ch (2%) yogurt samples (yogurt samples prepared by ABY culture, containing 1 and 2% commercial inulin) had the highest mean pH drop rate (mpH-DR) in fermentation stage and other samples had lower mean pH drop rate (mpH-DR). as well as the highest mean acidity increase rate (mA_IR) ($p < 0.05$) in ABY-Ch (2%) sample was observed. In contrast, ABY yogurt (yogurt prepared by ABY culture, containing 0% inulin) had the lowest amounts mean acidity increase rate (mA_IR) in during the fermentation stage. The higher mean acidity increase

Table 2: Biochemical parameters of treatments at the end of fermentation.

Yogurt	Parameters							
	mpH_DR (pH/min)	mA_IR (° D/min)	t _{5.5} (min)	t _{max-A-I} (min-min)	t _{max-pH-D} (min-min)	pH _{max-pH-D} (pH-pH)	Inconation time (min)	Final acidity (°D)
ABY	0.0006 ^b	0.20 ^e	147 ^c	180-210	150-180	5.6-5.3	300 ^d	85 ^d
ABY-C (1%)	0.0006 ^b	0.22 ^d	147 ^c	150-180	150-180	5.54-5.21	285 ^c	89 ^c
ABY-C (2%)	0.0006 ^b	0.24 ^c	120 ^b	150-180	120-150	5.5-5.19	274 ^b	93 ^b
ABY-Ch (1%)	0.0007 ^a	0.25 ^b	120 ^b	150-180	90-120	5.85-5.47	270 ^b	94 ^b
ABY-Ch (2%)	0.0007 ^a	0.26 ^a	118 ^a	120-150	90-120	5.81-5.42	260 ^a	96 ^a

**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 pH drop

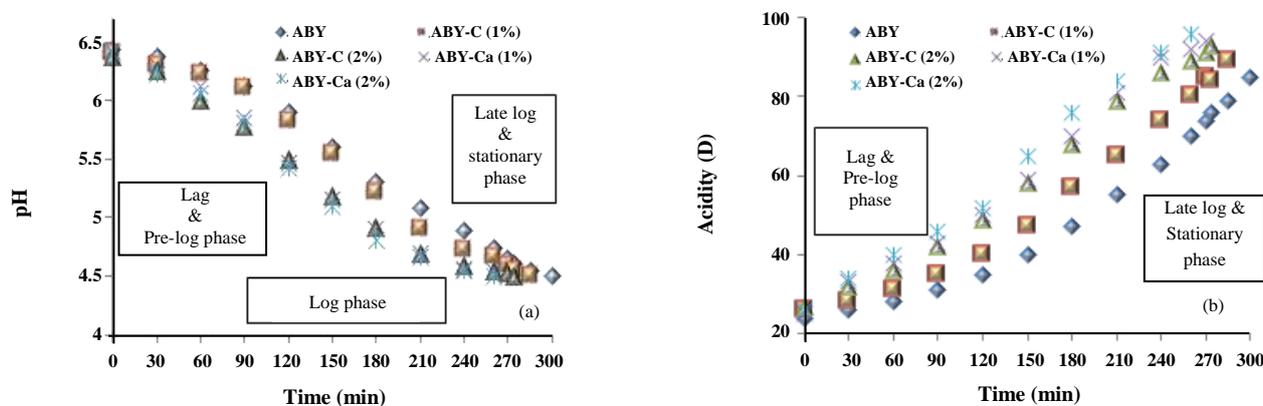


Fig. 1: Changes in pH drop (a) and acidity increase (b) during the fermentation period in different treatments of yogurt; ABY represent the treatment containing 0% inulin; ABY-C represents the treatments containing 1 and 2% inulin extracted from artichoke (*Cynara scolymus L*) root; ABY-Ch represents the treatments containing 1 and 2% commercial inulin.

rate in ABY-Ch (2%) yogurt could be mainly attributed to stimulate growth and/or activity of *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus*, because ABY yogurt (without Inulin) showed the lowest mean pH drop and acidity increase rates. Therefore, the addition of inulin significantly stimulated the growth and/or activity of starter cultures. These observations were consistent with those reported by Stijepic et al [40] for probiotic yogurt with 1.5% inulin and Mocanu et al [41] for ABT yogurt containing bilberry extract and a mixture of bilberry and liquorice extracts. In other research, Gabriel-Danut et al. [42] found that adding a mixture of sea-buckthorn and liquorice extract to ABY yogurt was indicated the acidity increase rate higher than the control sample.

The shortest incubation time was observed for ABY-Ch (2%) (260 min), in contrast, the long incubation time belonged to the ABY sample (300 min). Also the highest

final acidity was observed in ABY-Ch (2%) (96°D). According to Table 1, time of maximum pH drop (t_{max-pH-D}) in ABY and ABY-C (1%) were 150-180 (min-min), whilst this time for the ABY-C (2%) was 120-150 (min-min). Also, this parameter for ABY-Ch (1%) and ABY-Ch (2%) samples have been in the interval minutes 90-120 (min-min). These results indicate that treatments containing 1 and 2% commercial inulin (ABY-Ch (1%) and ABY-Ch (2%)) during the fermentation period were 30 min sooner in the peak of acidification and activity than ABY sample, and also ABY-C (1%) sample was 60 min sooner in the peak of acidification and activity. The data related to the pH of maximum pH drop (pH_{max-pH-D}) are in accordance with the data related to the time of maximum pH drop (t_{max-pH-D}) in treatments. For example, ABY yogurt showed a 0.3 decline in pH (5.3-5.6) within the minutes 150-180 during fermentation, whilst this pH

decline value was 0.39 (5.42-5.81) in within the minutes 90-120 in ABY-Ch (2%) yogurt (Table 1). This result was shown that the most decline pH in during the fermentation period in ABY-Ch (2%) sample started of pH5.81, whereas in ABY sample started of pH5.6.

According to Table 1, the time to reach pH5.5 ($t_{5.5}$) for ABY, ABY-C (1%), ABY-C (2%), ABY-Ch (1%) and ABY-Ch (2%) yogurts were minutes 147, 147, 120, 120 and 118, respectively. Comparing the data related to this parameter with those related to $pH_{\max-pH-D}$ and $t_{\max-pH-D}$ reveals that the sharpest pH decline in ABY, ABY-C (1%) treatments was within minutes 150-180, and also ABY-C (2%), ABY-Ch (1%) and ABY-Ch (2%) treatments were within minutes 120-150, 90-120 and 90-120 of fermentation, respectively. On the other words, in ABY and ABY-C (1%) yogurt samples, the fastest pH decline period (the minutes 150-180 during fermentation) was after pH5.50, whilst in ABY-C (2%), ABY-Ch (1%) and ABY-Ch (2%) treatments, this period (120-150, 90-120 and 90-120, respectively) includes pH5.50. Amirdivani and Baba [43] 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. These observations were consistent with that reported by Ehsani et al [28] for probiotic yogurt containing 0.5% (w/w) Artichoke leaf extract. also, Stijepic et al [40] revealed that probiotic yogurt containing 1.5% inulin had the shortest time of fermentation (250 min) compared to simple probiotic yogurt.

The viability of probiotic bacteria within yogurt during the fermentation stage

Table 3 shows the viability of probiotic bacteria as well as the Viability Proportion Indexes (VPI) in different yogurts immediately after fermentation. As represented from this Table, the viability of both probiotic bacteria (*L. acidophilus* LA-5 and *B. lactis* BB-12) was significantly and markedly greater in the treatments containing inulin (ABY-C (1%), ABY-C (2%), ABY-Ch (1%) and ABY-Ch (2%)) than control (ABY) ($p < 0.05$).

According to Table 2, the viability proportion index (VPI), especially for *L. acidophilus* (LA-5) in ABY-Ch (2%) and ABY-C (2%) (8.03 and 7.04) is significantly greater than other treatments, also this index (VPI) for

B. lactis (BB-12) in ABY-Ch (1%) and ABY-Ch (2%) treatments was reported 6.56 and 8.18 respectively, that was greater than other samples. The final population of *L. acidophilus* (at the end of fermentation) in ABY-C (1%), ABY-C (2%), ABY-Ch (1%) and ABY-Ch (2%) was 8.27, 8.37, 8.33 and 8.42 $cfu.mL^{-1}$, compared to 8.22 $cfu.mL^{-1}$ in ABY yogurt. Also, the final population of *B. lactis* (at the end of fermentation) in ABY-C (1%), ABY-C (2%), ABY-Ch (1%) and ABY-Ch (2%) was 8.36, 8.42, 8.46 and 8.56 $cfu.mL^{-1}$, compared to 8.26 cfu/mL in ABY yogurt. As was evident, the viability of both probiotic bacteria in treatments prepared with commercial inulin (ABY-Ch (1%) and ABY-Ch (2%)) was significantly and considerably greater than treatments prepared with artichoke root inulin (ABY-C (1%) and ABY-C (2%)). The positive effects of commercial inulin and artichoke root inulin on the viability of probiotics can be attributed to the reason that inulin provides nutritious and stimulatory media for lactic acid bacteria and probiotic bacteria, and stimulate their growth and activity. Also, higher viability of probiotic bacteria in samples containing commercial inulin was due to lower DP than artichoke root inulin [24,44,45]. Aghajani et al [46] found that adding inulin (1.5%) to probiotic yogurt, increased viability of probiotic bacteria (LA-5 and BB-12). Also, Ehsani et al [28] reported that the addition of artichoke leaf extract within ABY yogurt caused the viability of probiotic bacteria (LA-5 and BB-12) at the end of fermentation was increased.

Colorimetric test of yogurt samples at the end of the fermentation stage

The product color has an important role in consumer selection. The white visage of milk is derived from its physical structure, affecting lightness (L^*), also the milk color affected by plasma carotenoids, for instance, lutein (a^*) and β -carotene (b^*).

Table 4 shows colorimetric parameters of yogurt samples at the end of fermentation. As represented from this table, the highest lightness (L^*) is related to ABY-Ch (1%) and ABY-Ch (2%) samples. Probably inulin polymer interacted with casein micelle, that with the fat globules are responsible for the dispersion of incident light [47]. The results of this research were similar with that reported by Balthazar et al [48] for ovine probiotic yogurt containing 2 and 6% (w/w) inulin.

Table 3: Viability of probiotic microorganisms and the viability proportion index (VPI) in different treatments at the end of fermentation*.

Yogurt	Initial population (log cfu/mL)			Final population (Day ₀) (log cfu/mL)			VPI		
	A**	B***	A+B****	A	B	A+B	A	B	A+B
ABY	7.52	7.64	7.89	8.22 ^e	8.26 ^e	8.54 ^d	5	4.14	4.51
ABY-C (1%)	7.52	7.64	7.89	8.27 ^d	8.36 ^d	8.62 ^c	5.68	5.19	5.40
ABY-C (2%)	7.52	7.64	7.89	8.37 ^c	8.42 ^c	8.70 ^b	7.04	6.02	6.46
ABY-Ch (1%)	7.52	7.64	7.89	8.33 ^b	8.46 ^b	8.70 ^b	6.45	6.56	6.52
ABY-Ch (2%)	7.52	7.64	7.89	8.42 ^a	8.56 ^a	8.80 ^a	8.03	8.18	6.11

*Means shown with different English letters represent significant differences ($p < 0.05$) in the same columns (among the treatments).

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

Table 4: Colorimetric Parameters of yogurt samples at the end of fermentation.

Treatment	Colorimetric Parameters		
	L^*	a^*	b^*
ABY	70.33	0.83	1.67
ABY-C (1%)	71.17	0.83	4.33
ABY-C (2%)	72.33	0.00	8.00
ABY-Ch (1%)	75.67	0.33	3.33
ABY-Ch (2%)	74.67	0.67	2.33

L^* = lightness-darkness, a^* = green-red axis, b^* = yellow-blue axis

By examining the a^* factor it can be seen that approximately all samples are reluctant to green color. According to the table, inulin did not affect this color parameter. These results agreed with that reported by Balthazar et al. [48].

Also, b^* parameter was higher for probiotic yogurt samples containing commercial inulin and artichoke root inulin than simple probiotic yogurt (ABY). These results indicated that probably acidic hydrolysis happens in probiotic yogurt containing inulin because of lactic acid presence at a temperature above 40°C [49]. As is evident in Table 4, ABY-C (2%) yogurt had the most tend to be yellow color. This result was in accordance with those reported by Mazloomi et al. [50] and Balthazar et al. [48].

Sensory properties of synbiotic yogurt samples at the end of the fermentation stage

Fig. 2 shows the sensory analysis of yogurt samples using score methodology. As shown, probiotic yogurt samples containing artichoke root inulin possessed weaker sensory acceptability for all sensory parameters

compared to the control and samples containing commercial inulin. ABY-C (2%) yogurt exhibited more unpleasant flavor compared to ABY yogurt and had the lowest flavor score. In this parameter, the highest score belonged to ABY and ABY-Ch (2%) yogurt samples ($p < 0.05$). There were not significant differences among the ABY, ABY-Ch (1%) and ABY-Ch (2%) samples from non-oral texture points of view. Of course, the lowest non-oral texture score belonged to ABY-C (2%) yogurt. ABY, ABY-Ch (1%) and ABY-Ch (2%) had the highest score for appearance parameter ($p < 0.05$). However, differences were remarkable from an oral texture standpoint. Yogurt samples containing artichoke root inulin had the lowest sensory score for oral texture. Generally, the highest total score was related to ABY and ABY-Ch (2%) yogurt samples. The results obtained by Aryana and Mc Grew [51] showed that yogurt sample with 1.5% inulin had better texture compared to a simple yogurt. Also, from the point of view sensory properties of probiotic yogurt containing commercial inulin, the results of this research were in accordance with results reported

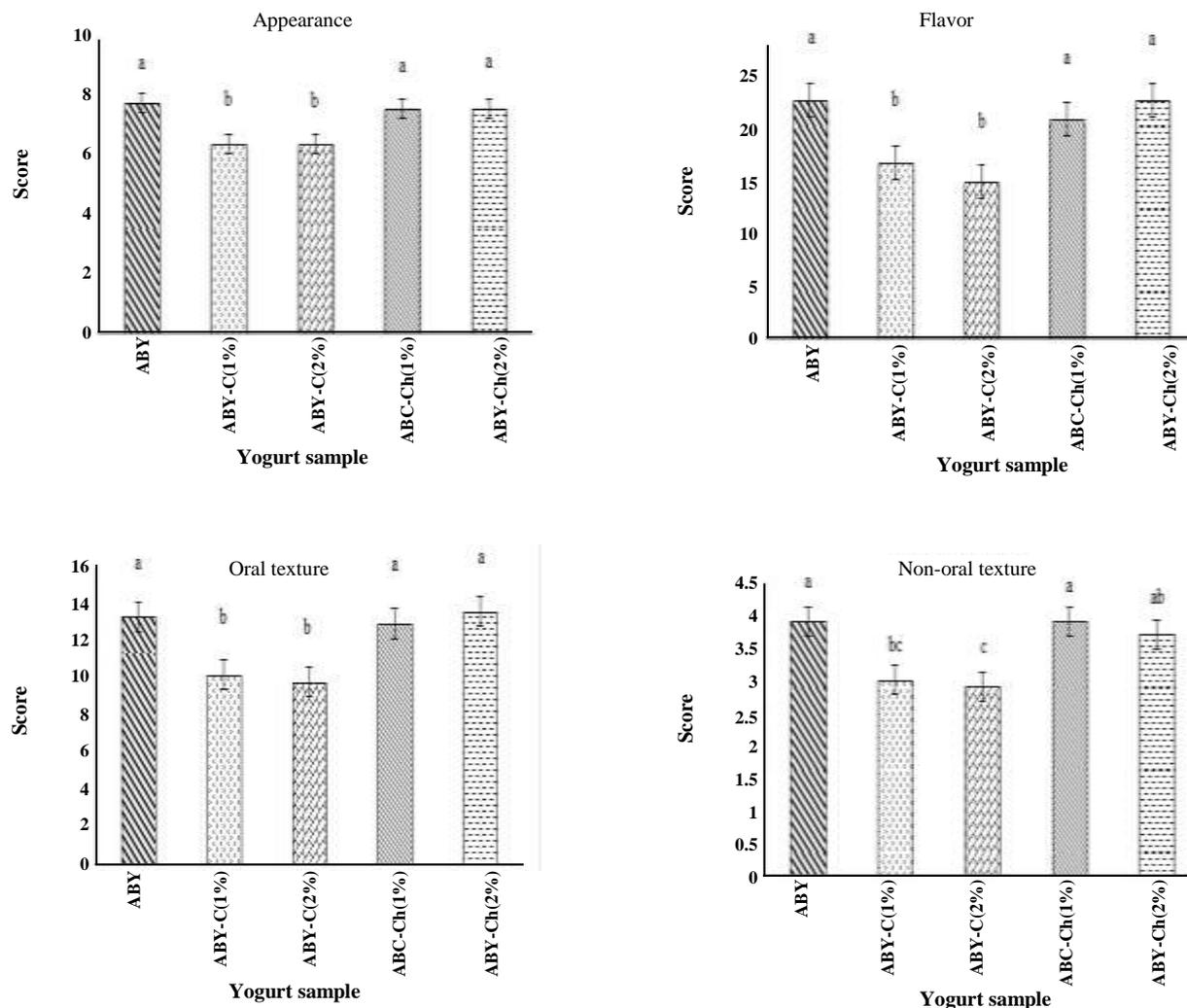


Fig. 2: Sensory analysis of the yogurt samples using score methodology. ABY represent the treatment containing 0% inulin; ABY-C represent the treatments containing 1 and 2% inulin extracted from artichoke (*Cynara scolymus L*) root; ABY-Ch represent the treatments containing 1 and 2% commercial inulin.

by Mazloomi *et al.* [50] and Balthazar *et al.* [48]. However, the addition of artichoke root inulin within the yogurt samples changed the color of this product to the pale cream. Probably, this characteristic was realized as an inappropriate sensory attribute by the panelists.

CONCLUSIONS

The results obtained from the chemical characteristics of inulin extracted artichoke root showed that the high DP of artichoke root inulin could be used as fat replacement and texture modifier, Although adding inulin extracted artichoke root and commercial inulin (prebiotic effect) to ABY yogurt significantly increased viability of *L. acidophilus* LA-5 and *B. lactis*

BB-12 at the end of fermentation. Also, yogurt samples inulin extracted artichoke root and commercial inulin had faster pH drop and acidity increase as well as. However, yogurt samples containing inulin extracted artichoke root had less sensory acceptability in flavor, appearance, oral texture and non-oral texture. Probably, this defect can be attributed to the white partial cream color of synbiotic yogurt samples, that should be taken into consideration in future researches. This research could be done through (a) investigation of new methods for extracting inulin, (b) the bleaching of inulin in the several times of extraction and (c) using different treatment of heating, time and optimization of extraction condition.

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REFERENCES

- [1] Grajek W., Olejnik A., Sip A., **Probiotics, Prebiotics and Antioxidants as Functional Foods**, *ACTA Biochemica Polonica-English Edition*, **52**(3): 665–671 (2005).
- [2] Gibson G.R., Williams C.M., **Functional Foods**, *IFIS Publishing*, 393 p (2005).
- [3] Shortt C., **The Probiotic Century: Historical and Current Perspectives**, *Trends in Food Science and Technology*, **10**(12): 411–417 (1999).
- [4] Mitall B.K., Garg S.K., **Anticarcinogenic, Hypocholesterolemic, and Antagonistic Activities of Lactobacillus Acidophilus**, *Critical Reviews in Microbiology*, **21**(3): 175–214 (1995).
- [5] Saxelin M., Tynkkynen S., Mattila-Sandholm T., de Vos W.M., **Probiotic and other Functional Microbes: from Markets to Mechanisms**, *Current Opinion in Biotechnology*, **16**(2): 204–211 (2005).
- [6] Bergamini C.V., Hynes E.R., Quiberoni A., Suárez V.B., Zalazar C.A., **Probiotic Bacteria as Adjunct Starters: Influence of the Addition Methodology on their Survival in a Semi-Hard Argentinean Cheese**, *Food Research International*, **38**(5): 597–604 (2005).
- [7] Desmond C., Ross R.P., O'callaghan E., Fitzgerald G., Stanton C., **Improved Survival of Lactobacillus Paracasei NFBC 338 in Spray Dried Powders Containing Gum Acacia**, *Journal of Applied Microbiology*, **93**(6):1003–1011 (2002).
- [8] Roberfroid M.B., Cumps J., Devogelaer J.P., **Dietary Chicory Inulin Increases Whole-Body Bone Mineral Density in Growing Male Rats**, *The Journal of Nutrition*, **132**(12): 3599–3602 (2002).
- [9] Gibson G.R., **Fibre and Effects on Probiotics (the Prebiotic Concept)**, *Clinical Nutrition Supplements*, **1**(2): 25–31 (2004).
- [10] Flamm G., Glinsmann W., Kritchevsky D., Prosky L., Roberfroid M., **Inulin and Oligofructose as Dietary Fiber: a Review of the Evidence**, *Critical Reviews in Food Science and Nutrition*, **41**(5): 353–362 (2001).
- [11] Hellwege E.M., Raap M., Gritscher D., Willmitzer L., Heyer A.G., **Differences in Chain Length Distribution of Inulin from Cynara Scolymus and Helianthus Tuberosus are Reflected in a Transient Plant Expression System Using the Respective 1-FFT cDNAs**, *Federation of European Biochemical Societies letters*, **427**(1): 25–28 (1998).
- [12] Pollock C.J., **Tansley Review no. 5 Fructans and the Metabolism of Sucrose in Vascular Plants**, *New Phytologist*, **104**(1): 1–24. (1986).
- [13] Van Loo J., Cummings J., Delzenne N., Englyst H., Franck A., Hopkins M., **Functional Food Properties of Non-Digestible Oligosaccharides: a Consensus Report from the ENDO Project (DGXII AIRII-CT94-1095)**, *British Journal of Nutrition*, **81**(2): 121–132 (1998).
- [14] Frehner M., Keller F., Wiemken A., **Localization of Fructan Metabolism in the Vacuoles Isolated from Protoplasts of Jerusalem Artichoke Tubers (Helianthus tuberosus L.)**, *Journal of Plant Physiology*, **116**(3): 197–208 (2012).
- [15] Roberfroid M.B., Delzenne N.M., **Dietary Fructans**, *Annual Review of Nutrition*. **18**(1): 117–143 (1998).
- [16] Hellwege E.M., Czaplá S., Jahnke A., Willmitzer L., Heyer A.G., **Transgenic Potato (Solanum tuberosum) Tubers Synthesize the Full Spectrum of Inulin Molecules Naturally Occurring in Globe Artichoke (Cynara scolymus) Roots**, *Proceedings of the National Academy of Sciences*, **97**(15): 8699–8704 (2009).
- [17] Franck A., **Technological Functionality of Inulin and Oligofructose**, *British Journal of Nutrition*, **87**(S2): S287–S291 (2002).
- [18] Gibson G.R., Roberfroid MB., **Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics**, *The Journal of Nutrition*, **125**(6): 1401–1412 (1995).
- [19] Singh R.S., Singh R.P., **Production of Fructooligosaccharides from Inulin by Endoinulinases and Their Prebiotic Potential**, *Food Technology and Biotechnology*, **48**(4): 435–450 (2010).

- [20] Agrawal R., [Probiotics: an Emerging food Supplement with Health Benefits](#), *Food Biotechnology*, **19**(3): 227–246 (2005).
- [21] Paseephol T., Small D., Sherkat F., [Process Optimisation for Fractionating Jerusalem Artichoke Fructans with Ethanol Using Response Surface Methodology](#), *Food Chemistry*, **104**(1): 73–80 (2007).
- [22] Southgate D.A.T., ["Determination of Food Carbohydrates"](#), Elsevier, 232 p (1991).
- [23] Miller G.L., [Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar](#), *Analytical Chemistry*, **31**(3): 426–428 (1959).
- [24] Paseephol T., Sherkat F., [Probiotic Stability of Yoghurts Containing Jerusalem Artichoke Inulins During Refrigerated Storage](#), *Journal of Functional Foods*, **1**(3): 311–318 (2009).
- [25] López-Molina D., Navarro-Martínez M.D., Rojas-Melgarejo F., Hiner A.N.P., Chazarra S., Rodríguez-López J.N., [Molecular Properties and Prebiotic Effect of Inulin Obtained from Artichoke \(*Cynara scolymus* L.\)](#), *Phytochemistry*, **66**(12): 1476–1484 (2005).
- [26] AOAC., Method 990.20., ["Determination of Solids by Direct Forced Air Oven Drying Method"](#), 17th ed Washington DC, (2000).
- [27] AOAC., Method 945.46., ["Determination of Ash by Gravimetric Method"](#), 17th ed, Washington DC, (2000).
- [28] Ehsani J., Mortazavian A.M., Khomeiri M., Ghasen Nejad A., [Effects of Artichoke \(*Cynara scolymus* L.\) Extract Addition on Microbiological and Physico-Chemical Properties of Probiotic Yogurt](#), *Journal of Microbiology, Biotechnology and Food Sciences*, **4**(6): 536–541 (2015).
- [29] Ahmadi E., Mortazavian A.M., Fazeli M.R., Ezzatpanah H., Mohammadi R., [The Effects of Inoculant Variables on the Physicochemical and Organoleptic Properties of Doogh](#), *International Journal of Dairy Technology*, **65**(2): 274–281 (2012).
- [30] Shafiee G., Mortazavian A.M., Mohammadifar M.A., Koushki M.R., Mohammadi A., Mohammadi R., [Combined Effects of Dry Matter Content, Incubation Temperature and Final pH of Fermentation on Biochemical and Microbiological Characteristics of Probiotic Fermented Milk](#), *African Journal of Microbiology Research*, **4**(12): 1265–1274 (2010).
- [31] Mortazavian A.M., Khosrokhavar R., Rastegar H., Mortazaei G.R., [Effects of Dry Matter Standardization order on Biochemical and Microbiological Characteristics of Freshly Made Probiotic Doogh \(Iranian Fermented Milk Drink\)](#), *Italian Journal of Food Science*, **22**(1): 98–104 (2010).
- [32] Mortazavian A.M., Ghorbanipour S., Mohammadifar M.A., Mohammadi M., [Biochemical Properties and Viable Probiotic Population of Yogurt at Different Bacterial Inoculation Rates and Incubation Temperatures](#), *The Philippine Agricultural Scientist*, **94**(2): 111–116 (2011).
- [33] Mortazavian A.M., Ehsani M.R., Mousavi S.M., Rezaei K., Sohrabvandi S., Reinheimer J.A., [Effect of Refrigerated Storage Temperature on the Viability of Probiotic Micro-Organisms in Yogurt](#), *International Journal of Dairy Technology*, **60**(2): 123–127 (2007).
- [34] Yam K.L., Papadakis S.E., [A Simple Digital Imaging Method for Measuring and Analyzing Color of Food Surfaces](#), *Journal of Food Engineering*, **61**(1): 137–142 (2004).
- [35] Anon, ["Iranian National Standard for Yogurt \(Specifications and Test Methods\)"](#), No. 695, (2008).
- [36] Van Loo J., Hermans J., [Inulin Products with Improved Nutritional Properties](#), *European Patent Application, EP, 1125507:A1*, (2000).
- [37] Leeuwen M.B., Slaghek T.M., Wit D.de., Kuzee H.C., Raaijmakers H.W.C., [Process for the Preparation of Purified Inulin](#), *WO, 97/23511* (1997).
- [38] De Gennaro S., Birch G.G., Parke S.A., Stancher B., [Studies on the Physicochemical Properties of Inulin and Inulin Oligomers](#), *Food Chemistry*, **68**(2): 179–183 (2000).
- [39] Heydari S., Mortazavian A.M., Ehsani M.R., Mohammadifar M.A., Ezzatpanah H., [Biochemical, Microbiological and Sensory Characteristics of Probiotic Yogurt Containing Various Prebiotic Compounds](#), *Italian Journal of Food Science*, **23**(2): 153–163 (2011).
- [40] Stijepić M., Glušac J., Đurđević-Milošević D., Pešić-Mikulec D., [Physicochemical Characteristics of Soy Probiotic Yoghurt with Inulin Addition During the Refrigerated Storage](#), *Rom Biotechnol Lett*, **18**(2): 8077–8085 (2013).

- [41] Mocanu D., Rotaru G., Botez E., Andronoiu D., Nistor O., **Probiotic Yogurt with Medicinal Plants Extract: Physical-Chemical, Microbiological and Rheological Characteristics**, *Journal of Agroalimentary Processes and Technologies*, **16**(4): 469-476 (2010).
- [42] Gabriel-Danut M., Gabriela R., Elisabeta B., Aida V., Liliana G., Doina A., **Research Concerning the Production of a Probiotic Dairy Product with Added Medicinal Plant Extracts**, *The Annals of the University of Dunarea de Jos of Galati Fascicle VI Food Technology*, **32**: 37-43 (2009).
- [43] Amirdivani S., Baba A.S., **Changes in Yogurt Fermentation Characteristics, and Antioxidant Potential and in Vitro Inhibition of Angiotensin-1 Converting Enzyme Upon the Inclusion of Peppermint, Dill and Basil**, *LWT-Food Science and Technology*, **44**(6): 1458–1464 (2011).
- [44] Roberfroid M.B., Van Loo J.A.E., Gibson G.R., **The Bifidogenic Nature of Chicory Inulin and Its Hydrolysis Products**, *The Journal of Nutrition*, **128**(1): 11–19 (1998).
- [45] Voragen A.G.J., **Technological Aspects of Functional Food-Related Carbohydrates**, *Trends in Food Science and Technology*, **9**(8): 328–335 (1998).
- [46] Aghajani A.R., Pourahmad R., Adeli H.R.M., **Evaluation of Physicochemical Changes and Survival of Probiotic Bacteria in Synbiotic Yoghurt**, *Journal of Food Biosciences and Technology*, **2**: 13–22 (2012).
- [47] Nozière P., Graulet B., Lucas A., Martin B., Grolier P., Doreau M., **Carotenoids for Ruminants: From Forages to Dairy Products**, *Animal Feed Science and Technology*, **131**(3):418–450 (2006).
- [48] Balthazar C.F., Gaze L.V., Azevedo da Silva H.L., Pereira C.S., Franco R.M., Conte-Júnior C.A., **Sensory Evaluation of Ovine Milk Yoghurt with Inulin Addition**, *International Journal of Dairy Technology*, **68**(2): 281–290 (2015).
- [49] Schreiner G.E., **Determination of Inulin by Means of Resorcinol**, *Experimental Biology and Medicine*, **74**(1): 117–120 (1950).
- [50] Mazloomi S.M., Shekarforoush S.S., Ebrahimnejad H., Sajedianfard J., **Effect of Adding Inulin on Microbial and Physicochemical Properties of Low Fat Probiotic Yogurt**, *Iranian Journal of Veterinary Research*, **12**(2): 93–98 (2011).
- [51] Aryana K.J., McGrew P., **Quality Attributes of Yogurt with Lactobacillus Casei and Various Prebiotics**, *LWT-Food Science and Technology*, **40**(10): 1808–1814 (2007).