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Enhanced Probiotic Activity, Physicochemical and Sensory Properties of Set-Yoghurt Incorporated with *Asparagus officinalis* Inulin

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Abstract

Inulin extracted from *Asparagus Officinalis* roots was evaluated for its effect on physicochemical, microbiological and sensory quality attributes of set-yoghurt during refrigerated storage of 21 days. Set-yoghurt was developed with1%, 2%, 3% (w/w) inulin levels andan authenticated control was carried out without inulin. Commercial starter cultures containing *Bifidobacterium bifidum, Lactobacillus bulgaricus* ssp. *Bulgaricus* and *Streptococcus thermophiles* were used as probiotic bacteria. The yield of inulin powder from *A. officinalis* roots were 15.28% (w/w). Inulin incorporated yoghurt showed higher *(p>0.05)* viable cell counts throughout the entirestorage period and the highest viable cell counts were observed in yoghurt with 3% inulin. Average viable cell counts of *B.bifidum* (~8.90 log cfu/mL) and *L.Bulgaricus* ssp. *Bulgaricus* (~8.96 log cfu/mL) had more or less similar counts while *S.Tthermophilus* (~9.11 log cfu/mL) had the highest *(p>0.05)* cell counts. Different inulin levels had no effect (*p>0.05*) on acidification of set-yoghurts. Set-yoghurt produced with2% nulin resulted low syneresis, improved sensory attributes with proven prebiotic effect. Inulin powder extracted from *A. officinalis* root tubers could be effectively utilized as a prebiotic source in set-yoghurt.

Keywords: *Asparagus officinalis*; Inulin; Prebiotic Starter Cultures; Set-Yoghurt; Sensory Attributes

Introduction

Global functional food market is rapidly growing with the increasing demand of health-conscious consumers for functional food which delivers additional health benefits beyond the basic nutritional value. Being widely consumed food; dairy products have been extensively used in the food industry for the development of functional food. Functionalization of dairy products mainly including fermented milk, yoghurt and cheese is most commonly practiced using probiotics, prebiotics or bioactive extracts of plant food [1]. Yoghurt is a fermented dairy product, produced by controlled fermentation of lactic acid in milk using starter cultures with fermenting bacteria such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* [2,3]. Yoghurt has become highly popular among consumers due to the proven health benefits such as enhanced gut immune function, improved lactose tolerance, prevention of many diseases including acute infectious diarrhea, osteoporosis, colon cancers and various health aspectsassociated with probiotics [4].

Prebiotics are defined as non-digestible dietary components that beneficially affect the host by selectively stimulating the growth or activity of one or limited number of beneficial bacteria, specifically *Bifidobacterium* and some *Lactobacillus* spp. in the gastro intestinal tract [5,6]. The combination of both prebiotic and probiotics creates symbiotic effect and this combination always promotes the survival of probiotic organisms. Among prebiotics,inulin is one of the popular therapeutic nutritional preparations which are extensively used in the food industry to be incorporated in functional food products. Inulin is a fructan-type polysaccharide; formed of fructose chains which can be vary from 2 to 60 monomers in length [7]. Prebiotic effect of inulin has been proven through many *in vitro* and *in vivo* studies and it is widely used in European market for the development of new improved food products including yoghurts, drinks, bakery products and table spreads [8]. Inulin could act as a soluble alimentary fiber which resists digestion in the upper intestinal tract and reaches the large intestine practically intact, where it is fermented by bacteria [9].

Incorporation of inulin in food products promote the probiotic activity and viability of probiotics, without adversely affecting the flavor of food products. Inulin significantly increase the growth of *Bifidobacteria* usually noted as 'Bifidogenic effect' where it facilitates protection from enteric infections, suppression of putrefactive and pathogenic bacteria, production of vitamins, activation of intestinal function, assistance of digestion and absorption and stimulation of the immune response [8,10,11]. The consumption of inulin provides several other health benefits including lowering of blood cholesterol and triacylglycerol [12], anticarcinogenic properties, decreasing the incidence of colon cancer [13], relieving constipation [14], facilitating digestion of high-protein diets and retarding fat absorption [5]. According to the current scenario, the industrial inulin extraction is solely limited to two plant species grown in temperate countries namely *Chicoriumintybus* and *Helianthus tuberosus*, commonly known as Chicory and Jerusalem artichoke respectively. Those plants are not found or limitedly available in tropical regions and the commercial inulin is exported to most countries at high cost to be incorporated in food products [15]. There are several other plant species containing higher levels of inulin which can be successfully employed for the extraction of inulin and imparts characteristic prebiotic effect [16]. In order to compensate the high cost of inulin, various plant species have been used for the inulin extraction and most of the investigated plant materials so far are costly or limitedly available [17].

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Asparagus species contain fairly high amounts of inulin in their tubers and has higher worldwide annual production than Chicory and Jerusalem artichoke [15-17]. *Asparagus falcatus* and *Asparagus racemosus* has shown 17.74% and 11.83% availability of inulin the tubers on fresh weight basis. However, *A. officinalis* have been barely studied and there are limited reliable studies on applications of asparagus inulin in food systems and their prebiotic properties in probiotic dairy products. Therefore, the aim of the current study was to analyze the inulin content in *A. officinalis* tubers and determine the prebiotic potential of *A. officinalis* inulin by incorporating them in set-type yoghurt. Further, the effect of *A. officinalis* inulin on probiotic viability during the shelf life of yoghurt and sensory properties of *A. officinalis* incorporated yoghurt were evaluated.

Materials and Methods

Extractionof Inulin

Harvested roots of *A. officinalis* were thoroughly washed with tap water to remove soil and cleaned roots were cut into small pieces of around 1 cm followed by blanching immediately in boiling water acidified with ascorbic acid (0.1% w/w) for 1 to 2 minutes. Precut and blanched roots were transferred into warring blender and blended with 2 L of potable hot water (70°C). Slurry was mixed with additional 2 L of potable water and allowed to stand for 50 minutes at 80 ± 5°C in a water jacketed pan. Slurry was filtered through 4 layers of muslin cloth and the residue was weighed and re-extracted following the same procedure. Root filtrate was treated with Ion Exchange Resins (IER) to remove excess ash. Strong cation exchange resin (H+) (Amberlite FPC 22RF H) followed by weak anion exchange resin (OH−) (Dowex 66) were used as IERs and then concentrated the root extracts at 60°C to a brix value of approximately 8°C using a rotavapor (BUCHI-model R-114; SIBATA Scientific Technology Ltd., Japan) [18]. Concentrated sample was freeze dried at the conditions of -50°C and 20Pa until received a clear powder.

Chemical Characterization of Inulin

Proximate analysis including moisture content, protein, fat and insoluble fiber content was analyzed according to AOAC [19] protocols. Solubility analysis was conducted according to the procedure described by Wada et al.[20] and total water soluble sugar content was analyzed by phenol sulfuric methodas described byDubois et al.[21]. Approximately 0.1 g of inulin powder was weighed and diluted in a 100 mL volumetric flask using distilled water. Afterwards 0.1g of glucose sample was diluted in 100 mL volumetric flask and used as the stock solution. Dilution series was prepared using stock solution for prepare the calibration curve. Two micro liters of the sample was added to test tubes followed by adding 1 mL of the 5% phenol and 5 mL of the 95% concentrated sulfuric acid. Samples were vortex for few seconds and incubate at 30°Cin a water bath. The absorbance was measured at 490 nm using UV-visible spectrophotometer (UV-1601, Shimadzu Co., Japan). The pH of the samples was measured using electric pH meter (SensION, E-08328, Spain). Solubility of the extracted powder was measured by adding 1 g of inulin to 10 mL of distilled water at 25°C, stirring until complete dissolution and saturation.

Preparation of Inulin Incorporated Set Yoghurt

Commercial starter cultures purchased from Chr. Hansen Company (Hoersholm, Denmark) were used for the yoghurt production according to the instructions given by the manufacturer. Sterilized milk was inoculated with thermophilic yoghurt culture (YF-L903, Chr. Hansen, Denmark) containing Streptococcus *thermophilus*, *Lactobacillus bulgaricus*ssp. *bulgaricus* and *Bifidobacterium bifidum* culture (ABY-10, Chr. Hansen, Denmark) at a rate of 1% (w/v).

Cow milk was standardized up to 2.5% (w/v) fat and subjected to preheating at 55-60°C. Inulin was added prior to the homogenization at four different levels as 2%, 3%, 4% and 5% with a control without inulin. Homogenization was done at 55°C for 15 minutes under 10-20 Mpa. Then the pasteurization was done at 80-85°C for 30 minutes and cooled down to 42°C and inoculated with starter bacteria. The cultures were used to inoculate milk samples as per the instructions given by the manufacturer. Incubation was done at 42±2°C temperatures until the pH reached 4.5 and then stored in a refrigerator at 4°C. For the preparation of inulin incorporated yoghurt, inulin was added before the homogenization.

Microbiological Analysis

The viability of the thermophilic starter bacteria and *Bifidobacteria* were determined using the spread plate method. De Man Rogosa and Sharpeagar (MRS agar), M17 agar and *Bifidobacterium* Selective Media (BSM) were used to enumerate the counts of *Lactobacillus bulagaricus, Streptococcus thermophilus* and *Bifidobacterium bifidum* respectively [22].

Measurement of pH, Titratable Acidity and Syneresis

The yoghurt samples were analyzed for different parameters during the storage on 1,7, 14 and 21 day. The pH of the yoghurt during storage was measuredusing a pH meter (SensION, E-08328, Spain) at room temperature. For the determination of titratable acidity, 10 g of yoghurt was suspended in 20 mL of de-ionized water and titrated against 0.1N NaOH using phenolphthalein as the indicator [23]. Syneresis of the yoghurt samples was determined according to the procedure reported by Prasanna et al. [24].

Sensory Evaluation

Sensory analysis of inulin incorporated yoghurt was carried out using 30 untrained panelists. The panelists were asked to evaluate the products for flavor, texture, appearance, color and overall acceptability of the inulin incorporated yoghurt according to the five-point hedonic scale going from like very much (5) to dislike very much (1).

Statistical Analysis

Viable cell counts of each starter bacteria, pH, titratable acidity and syneresis throughout the shelf life were analyzed by using one-way ANOVA with MINITAB (version18) software. Sensory evaluation data were analyzed by Friedman nonparametric test using MINITAB software package with 95% of confidence interval.

Results and Discussion

Physicochemical Properties of Extracted Inulin

The percentage yield of inulin powder from *A. officinalis* roots were 15.28% (w/w dry basis). The yield is comparatively lower than the values reported for *A. falcatus*and higher than that of *A. racemosus* whereas studies conducted with chicory which is mostly used in commercial extraction of inulin has showed relatively lower yield [18,25]. The variations could be attributed to the difference of plant species and different technical conditions of the extraction methods employed [15,26,27]. Physicochemical properties of extracted inulin are shown in Table 1. Solubility of the *A. officinalis* inulin was much greater than the previously reported solubility values of commercial chicory inulin and inulin extracted from *A. falcatus*. Smaller particle size and presence of more hydrophilic sites in extracted inulin lead to higher solubility values. Moisture content of *A. officinalis* inulin was comparatively higher than the values reported oncommercial inulin, *A. falcatus* and *Taraxacum* *javanicum* inulin powders by spray drying [15] and lower than the values reported for inulin produced from Jerusalem artichoke tubers in bench and pilot plant- scale [28]. The freeze-drying technique practiced in current study instead of spray drying may have resulted higher moisture content. Freeze drying was used for solidification of inulin from the inulin extract since it is performed at low temperature, thus the degradation of inulin can be minimized [29].

Parameter	Value
Moisture content	(4.52 ± 0.04)g/100 g
Crude protein	(0.57 ± 0.01)g/100 g
Crude fat	(2.15 ± 0.02)g/100 g
Insoluble fiber	(0.24 ± 0.01)g/100 g
Total water-soluble sugars	(86.7 ± 1.70)g/100 g
Yield of inulin powder	15.28% w/w
pH	(5.12 ± 0.07)
Color	40.23L 4.93a 21.65b
Solubility	(158.5 ± 1.24) g/L

Table 1. Physicochemical properties of inulin powder extracted from *A. officinalis* root tubers.

The pH of *A. officinalis* inulin was 5.12 ± 0.07 which is relatively lower than the pH values (5.53 ± 0.05) previously reported for commercial chicory inulin while it is more or less similar to the pH value of inulin extracted with *A. falcatus* [18]. Total water-soluble sugar content of the extracted inulin was 86.7% while crude protein, crude fat and insoluble fiber content were 0.57%, 2.15% and 0.24% respectively. Similar trends were observed by *Mudannayake et al.*[18] who reported the properties of *A. Falcatus*inulin except for protein content which was much higher (2.13%) than the values reported for *A. officinalis* in the current study. The color of the extracted inulin was relatively yellowish brown in color which was indicated by the lower L and greater b values. These changes in color among inulin samples might be interrelated with the presence of phenolic compounds in the extracted inulin [28].

Prebiotic Effect of Inulin

Inulin is a well-defined group of prebiotics which is extensively used in the functional food products and the prebiotic effect of commercial inulin on human digestion has been already proven in literature [30,31]. In the current study, inulin from *A. officinalis* roots was assessed for fermentability by *B. bifidum*, *L. bulgaricus* subspp. *bulgaricus* and *S. thermophilus* since certain prebiotics are not suitable with certain genus or bacterial species [32,33]. The results revealed that the growth of all three probiotic species was increased in the presence of *A. officinalis* inulin. *B. bifidum* showed a better growth in inulin incorporated yoghurt while the highest viable *B. bifidum* counts were observed with 3% inulin Figure 1. Inulin incorporated yoghurts showed a significant difference in *B. bifidum* counts compared to that of controlat 21 day of storage period.Similarly, the optimum growth of *Bifidobacterium animalis*(Bb-12) has been reported at 3% inulin concentrationin cultured skim milk incorporated with the inulin from *A. falcatus* and *T. javanicum* [34]. Furthermore, it has been reported that, Bb-12 counts in skim milk containing 3% *T. javanicum* inulin were comparable to the counts of skim milk incorporated with 5% commercial chicory inulin.

S.thermophilus and *L.bulgaricus* subspp. *Bulgaricus* also showed the highest viable counts in yoghurt incorporated with 3% inulin as illustrated in Figure 2 and 3 respectively. The final concentration of *L. bulgaricus* was significantly *($p<0.05$)* affected by the addition of inulin at last week of storage period. Similarly, Aryana et al.[5] and Sadekand Murad [35] has reported that addition of inulin in yoghurt help to promote the growth of *Lactobacillus* spp. Several studies have reported some contradictory results that none of the tested *Lactobacillus* strains could utilize inulin [34,36,37]. The highest viable counts were observed in *S. thermophilus* followed by the *B. bifidum* and *L. bulgaricus* while the viability of *S. thermophilus* between yoghurt incorporated with 2% and 3% inulin showed significant difference (*$p<0.05$*) compared to *Bifidobacteria* and *Lactobacillus* spp. Lower survival rate of *L. bulgaricus* could be due to the post acidification process while higher *B. bifidum* count relate with the synergistic effect of both *Streptococcus* spp. and *Lactobacillus* spp. [38]. Further, lactic acid bacteria are capable of producing exopolysaccharides (EPS) during their growth which have positive effect on growth since increased levels of inulin lead to production of higher amounts of EPS [39]. *A. officinalis*inulin has increased the probiotic survival rate although the strain specific differences were observed. Therefore, it can be suggested that *A. officinalis* inulin has the ability to act as a prebiotic source in co-cultures of *B. bifidum*, *L. bulgaricus* sub spp. *bulgaricus* and *S. thermophilus* utilizing *A.officinalis* inulin as a carbon source [8]. All the viable counts were above 7–9 log cfu/mL which is the minimum dose able to assure the therapeutic effects of probiotics [40,41].

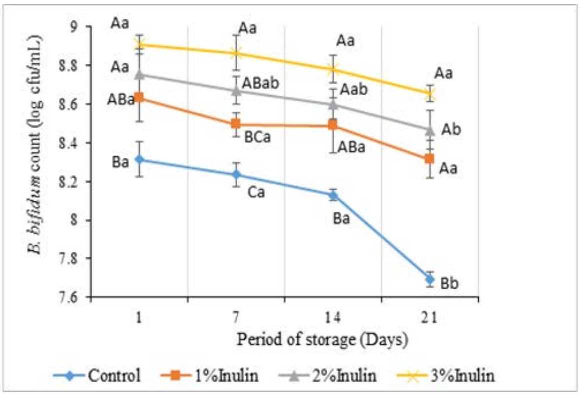

Figure 1. Variation of *Bifidobacterium bifidum* viability with different inulin incorporation levels in set yoghurt during the period of storage. Vertical lines represent standard deviations. ABC Means with different uppercase are significantly different (p<0.05) between each treatment of set-yoghurt for a particular day of storage. abc Means with different lower case are differ significantly (p<0.05) between each day, for each treatment of set-yoghurt during storage.

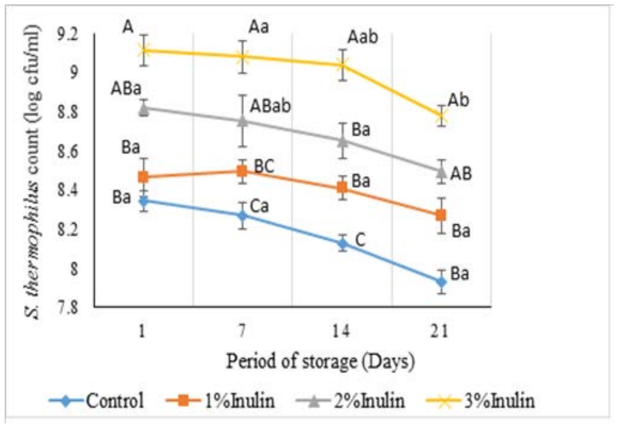

Figure 2. Variation of *Streptococcus thermophiles* viability with different inulin incorporation levels in set yoghurt during the period of storage. Vertical lines represent standard deviations. ABC Means with different uppercase are significantly different (p<0.05) between each treatment of set-yoghurt for a particular day of storage. abc Means with different lower case are differ significantly (p<0.05) between each day, for each treatment of set-yoghurt during storage.

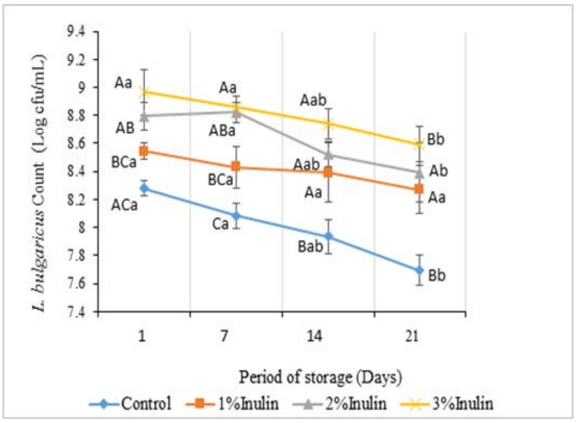

Figure 3. Variation of *Lactobacillus bulgaricus* viability with different inulin incorporation levels in set yoghurt during the period of storage. Vertical lines represent standard deviations. ABC Means with different uppercase are significantly different (p<0.05) between each treatment of set-yoghurt for a particular day of storage. abc Means with different lower case are differ significantly (p<0.05) between each day, for each treatment of set-yoghurt during storage.

Changes of pH and Titratable Acidity in Inulin Incorporated Set-Yoghurt

There were no differences *(p >0.05)* in pH among the treatments throughout the period of storage Table 2. The pH values of set-yoghurts ranged from 4.52 to 4.35 after 1 day of storage and 4.24 to 4.16 after 21 days. A significant *(p<0.05)* decrease of pH during storage was observed in all the treatments. Similar results were reported by Guven et al. [42] and Mazloumiet al. [43] who reported that there was no significant difference in pH among set-yoghurts prepared with inulin. Moreover, they observed a continuous reduction of pH of all set-yoghurt during storage. The reduction of pH takes place with the organic acid production due to the microbial fermentation of carbohydrates by starter culture bacteria [44]. At the end of storage, pH of the inulin incorporated set yoghurt was significantly higher *(p<0.05)* than the control while pH was reduced in treatments with the increasing levels of inulin. Titratable acidity of the samples was not affected by different inulin levels and increases were found to be significant during storage *(p<0.05)*. This was also observed by Crispín-Isidro et al. [45] and Dabija et al. [46] who reported that acidity wasn't vary independently of inulin incorporation levels due to a buffering effect of the protein.

Syneresis of Yoghurt during Storage

Separation of the liquid phase in milk gels is termed as syneresis; the most common quality defect of yoghurt. The values of syneresis of the different yoghurts are shown in figure 4. A significant difference *(p<0.05)* was observed in syneresis among the treatments throughout the shelf life. The lowest and the highest syneresis was observed in the yoghurt produced with 2% and 3% inulinlevels respectively while the control and 1% inulin incorporated yoghurt had more or less similar syneresis values during the shelf life. Addition of fiber like inulin decrease the syneresis since fiber have the ability to absorb the whey released by the yoghurt gel preventing its free movement and interact with the milk constituents providing stability to protein network [47,48]. The results are in line with the work done by Staffolo et al. [49] and Rinaldoni et al.[50] who reported that yoghurt incorporated with increased levels of commercial inulin havenegative impact on the gel formation process of the yoghurt after a certain level of incorporation.

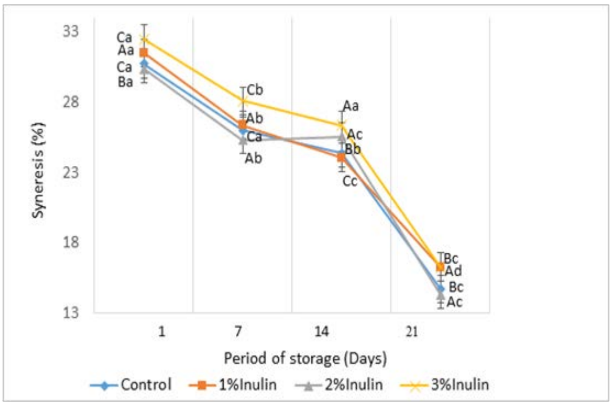

Figure 4. Variation of *Lactobacillus bulgaricus* viability with different inulin incorporation levels in set yoghurt during the period of storage. Vertical lines represent standard deviations. ABC Means with different uppercase are significantly different (*p*<0.05) between each treatment of set-yoghurt for a particular day of storage. abc Means with different lower case are differ significantly (*p*<0.05) between each day, for each treatment of set-yoghurt during storage.

Sensory Analysis of Inulin Incorporated Yoghurt

The results of the sensory evaluation of the yoghurts are shown in figure 5. The highest *(p<0.05)* preference for the odor, color, texture, taste and overall acceptability was observed in yoghurt incorporated with 2% inulin while control had the lowest acceptability for the evaluated properties. Inulin enhanced (*p<0.05*) the sensory attributes of set yoghurt without interfering the overall sensory attributes up to a certain extent. Higher sensory acceptance of set yoghurt incorporated with 2% inulin could be attributed the improved growth of the *B. bifidum*, *L. bulgaricus* subsp. *bulgaricus* and *S. thermophilus* leading to higher production of flavor compounds and better textural properties accompanied with the lowest syneresis.

Parameter	Treatment	Storage period (Days)			
		1	7	14	21
pH	Control	4.35 ± 0.01^{Ca}	4.33 ± 0.01^{BCa}	4.26 ± 0.02^{Bb}	4.13 ± 0.01^{Bc}
	1% Inulin	4.52 ± 0.01^{Aa}	4.46 ± 0.01^{Ab}	4.32 ± 0.02^{Ac}	4.24 ± 0.01^{Ad}
	2% Inulin	4.46 ± 0.01^{Ba}	4.36 ± 0.02^{Bb}	4.26 ± 0.01^{Bc}	4.21 ± 0.02^{Ac}
	3% Inulin	4.38 ± 0.01^{Ca}	4.30 ± 0.01^{Cb}	4.19 ± 0.01^{Cc}	4.15 ± 0.01^{Bc}
Titratable acidity	Control	1.104 ± 0.008^{Ad}	1.142 ± 0.010^{Ac}	1.237 ± 0.004^{Ab}	1.336 ± 0.004^{Aa}
	1% Inulin	1.038 ± 0.017^{Bc}	1.084 ± 0.009^{Bbc}	1.073 ± 0.002^{Db}	1.265 ± 0.184^{Ba}
	2% Inulin	1.046 ± 0.003^{Bd}	1.104 ± 0.008^{Bc}	1.143 ± 0.011^{Cb}	1.254 ± 0.002^{Ba}
	3% Inulin	1.056 ± 0.004^{Bd}	1.133 ± 0.010^{Ac}	1.215 ± 0.007^{Bb}	1.313 ± 0.010^{Aa}

Table 2. Titratable acidity and pH values of set yoghurt produced with different levels of *A. officinalis* inulin during storage. ABC Means in the same column for each treatment differ significantly (*p* < .05) for a particular day of storage. abc Means in the same row without common letter differ significantly (*p* < .05) for each type of set-yoghurt. Data are expressed as mean ± standard deviation

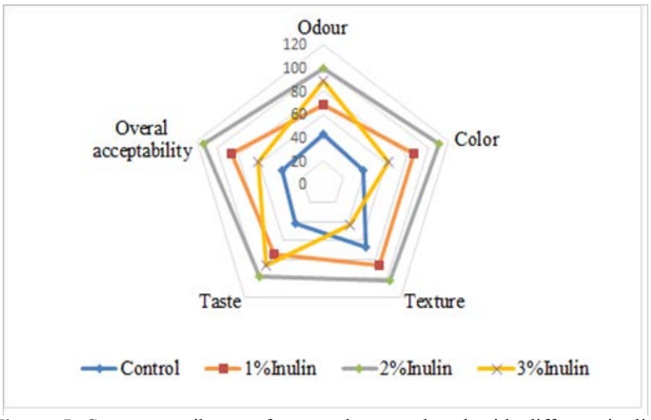

Figure 5. Sensory attributes of set yoghurt produced with different inulin incorporation levels.

The results imply that incorporation of inulin in low concentrations positively affect sensory and rheological properties of fermented dairy products. The results are consistent with *Rinaldoni et al.* [50] who has reported highest acceptability for soy yoghurt included with 50g/L than 70g/L in sensorial analysis for soy yoghurt with different inulin levels. Previous studies showed that set-yoghurt prepared with 2% or 3% inulin enhanced the flavor scores of the low fat yoghurt with a smooth mouth feel due to its fat replacement ability [51,52].

Conclusions

In conclusion, set yogurt containing *A. officinalis* inulin not only retains its nutritional properties, but better physicochemical and sensory properties. *A. officinalis* inulin effectively acts as a source of prebiotic for *B. bifidum*, *L. bulgaricus* subspp. *bulgaricus* and *S. thermophilus.* Viable counts and survival rate of the selected probiotic organisms at the end of the shelf life were increased with the increased levels of inulin. Therefore, *A. officinalis* inulin can be effectively used as a functional ingredient in development of fermented dairy products like yoghurt while 2% incorporation level act as the best incorporation level to be incorporated in set-yoghurt.

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Conflict of Interest

The authors have declared no conflicts of interest for this article.

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