

Some Properties of Bio-Yogurt Enriched with Cellulose Fiber

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How to cite this paper: Güler-Akın, M.B., Goncu, B. and Serdar Akın, M. (2018) Some Properties of Bio-Yogurt Enriched with Cellulose Fiber. *Advances in Microbiology*, 8, 54-64.

<https://doi.org/10.4236/aim.2018.81005>

Received: December 25, 2017

Accepted: January 28, 2018

Published: January 31, 2018

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Abstract

In this study, bio-yogurts, which contain *L. acidophilus* and *Bifidobacterium animalis* subsp. *lactis*, were produced with cellulose fiber (CF) addition at a various level (0%, 0.5%, 1% and 2%) and stored at 4°C ± 1°C for 20 days. Bio-yogurts were analysed 1, 10 and 20 days after production. The storage period significantly influenced overall properties of the samples. Addition of CF has positively affected the physical and textural properties of yoghurt, such as serum separation, water holding capacity, viscosity, firmness, adhesiveness, cohesiveness, springiness, gumminess and chewiness ($p < 0.05$). It also slightly stimulated the development of probiotic bacteria ($p < 0.05$). Bio-yogurt, which contains 0.5% CF in sensory aspect, is the most appreciated.

Keywords

Bio-Yogurt, Cellulose Fiber, Prebiotic Effect, Yogurt Quality

1. Introduction

Due to their attributed health benefits, probiotic bacteria (such as *L. acidophilus* and/or *Bifidobacteria*) have been increasingly included in yogurts and fermented milks during the past three decades. The addition of probiotic bacteria is made not only because of certain claimed health-promoting effects in the intestinal tract but also because of the sensory aspects as well as the expanding variety of products that can be formulated with them [1].

In order to produce therapeutic benefits, a suggested minimum level for probiotic bacteria in fermented milk is from 10^6 to 10^7 cfu·mL⁻¹ [2]. However, these organisms often show poor viability in market preparations [3] [4] [5]. Therefore, manufacturers were interested in developing a process that can provide high densities of the probiotic strains in the product. Prebiotics, such as oligo-

saccharides are added to food mainly to allow the preferential growth of probiotic organisms [6].

Dietary fiber (DF) is a remnant of the edible part of plant; it is analogous carbohydrates that are resistant to digestion and absorption in the human small intestine and undergo complete or partial fermentation in the human large intestine. DF includes oligosaccharides, lignin, resistant starch, tannins and associated plant substances [7] [8].

Cellulose, the earth's most abundant natural material, is a long-chain carbohydrate polymer of repeating glucose units. Cellulose has β -glycosidic linkages, which cannot degrade human carbohydrate digestive enzymes and is widely accepted as a dietary fiber [9]. Many dietary fibers have been shown to offer potential health benefits to the gastrointestinal tract [10]. CF is indigestible in the upper gastrointestinal tract (GIT) but partially fermentable in the colon [11] and so may potentially have limited use as a prebiotic in functional food.

The aim of this study was to determine the prebiotic effect of CF on the *L. acidophilus* and *Bifidobacterium BB-12* and the effect of addition of CF on the overall quality of bio-yogurt.

2. Materials and Methods

2.1. Materials

Cow's milk (morning milking) used in the manufacture of bio-yogurt was collected two times during March 2017 in Şanlıurfa in southeastern Turkey. The milks were inoculated with ABT3 culture consisting of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* (BB12), which was obtained from Peyma-Chr. Hansen (Turkey).

2.2. Preparation of Culture

Reconstituted skim milk, with 12% dry matter, was prepared and sterilized at $105^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 3 min. Then it was cooled to 40°C and inoculated with ABT3 culture, using a 0.05% (w/v) inoculum. The cultures were grown for 4 h at 37°C . The milk was kept cooled at 4°C until for 8 h, before using yogurt production.

2.3. Production of Bio-Yogurt

Manufacture of yogurts was achieved according to Tamime and Robinson [12]. Two different trials were performed for the manufacture of bio-yogurt. On each trial, the large flocs were removed from raw cow's milk by using a cloth filter and divided into four equal portions (each 5 litres). The first (A) batch was control, the second (B), third (C) and fourth (D) batches were fortified with CF at a rate of 0.5%, 1% and 2%, respectively. After addition of skimmed milk powder (3% w/w) each milk was heat-treated at 90°C for 10 min, cooled to 45°C , inoculated with ABT3 culture at a rate of 5% and dispensed into plastic cups (200 ml). All batches were incubated at 40°C until pH 4.6. Then they were transferred to a cold store ($4^{\circ}\text{C} \pm 1^{\circ}\text{C}$) immediately.

2.4. Analytical Methods

The pH of the milk and bio-yogurt was measured using a digital pH-meter and titratable acidity was determined according to the Soxhlet-Henkel method [13]. The protein, moisture and ash contents of milk, yogurt and bio-yogurt were estimated from the crude nitrogen content of the samples determined by the Kjeldahl, oven-drying and gravimetric methods, respectively [14]. The total fat contents of milk and bio-yogurt samples were determined by the Gerber method [13]. Whey separation and water holding capacity of the samples were determined by the method described by Kessler and Kammerlahner [15] and Goncu *et al.* [16], respectively. The viscosity of bio-yoghurts were determined at 4°C using a digital Brookfield Viscometer, Model DV-II (Brookfield Engineering Laboratories, Stoughton, MA, USA) [17].

The textural properties of bio-yogurt were evaluated by the textural profile analysis (TPA) method [18]. TPA was performed on cheese samples using a double compression test (TA-XT2i Texture Analyzer; Stable Micro Systems, NY, USA) on 75 g packed samples. As yogurt presents a pseudoplastic behavior and exhibits partial thixotropy, firmness was measured as the force required to break the structure formed after the cessation of stirring and during the cold storage of the yoghurt. Conditions were as follows: a cylindrical probe (5 mm in diameter) was inserted to a depth of 15 mm into the sample at a speed of 1 mm·s⁻¹. A 1-byte penetration test was applied using a 25-kg load cell. Data collection and calculation were carried out using the Texture Expert Exceed Version 2 V3. The results were expressed as the average of three measurements.

Bio-yogurt samples (10 g) were decimally diluted in 100 mL sterile peptone water (0.1%) and 1 mL aliquot dilutions were poured onto plates of the various selective and differential agars in triplicate. M17 agar was used for the enumeration of *S. thermophilus* [19]. *L. acidophilus* and *Bifidobacterium* BB-12 were incubated by using MRS with sorbitol agar and MRS-NNLP agar [20], respectively. All plates were incubated at 37°C for 72 h. M17 was incubated aerobically, whereas all other media plates were incubated anaerobically. Anaerobic conditions were created using Anaerocult A sachets (Merck). Plates containing 20 - 200 colonies were counted and the results are expressed as colony-forming units per gram (cfu·g⁻¹) of sample.

The samples were organoleptically assessed by ten panelists using a sensory rating scale of 1 - 10 for flavour and taste, and 1 - 5 for consistency and appearance as described by Bodyfelt *et al.* [21]. The panel of assessors was an external panel of non-smokers who were very familiar with fermented dairy products and were selected on the basis of sensory acuity and consistency.

The experiment was designed according to a 4 × 3 × 2 (yogurt type × storage period × replication) factorial design. Statistical analysis of data via one-way analysis of variance (ANOVA) was performed to check the significance of differences at $p < 0.05$ using SPSS Version 5.0 (SPSS Inc. Chicago, IL, USA). Statistically different groups were determined by the Tukey test [22].

3. Results and Discussions

3.1. Bacterial Counts

Viable bacterial counts of bio-yogurt samples during storage are shown in **Table 1**. *S. thermophilus* counts were higher in the samples with CF the control. This could be due to the stimulated growth of Streptococcus species by CF. *S. thermophilus* counts slightly increased with increasing the fiber levels ($p < 0.05$). Sendra et al. [23] and Akin and Akin [24] reported that *S. thermophilus* counts increased in the yogurt supplemented with orange fiber and apple fiber, respectively. The counts of *S. thermophilus* increased slowly during the storage period.

L. acidophilus and *Bifidobacterium* BB-12 counts of the samples fortified CF were slightly higher than control. *L. acidophilus* and *Bifidobacterium* BB-12 counts slowly increased with increasing the fiber levels up to at a rate of 1%, then they reduced. The result of this could be attributed to lower water activity (a_w) of the samples enriched with CF. As known, cellulose had very higroscopic [9] [25] [26]. So the probiotic bacteria couldn't find enough water for growing. During the storage period *L. acidophilus* and *Bifidobacterium* BB-12 counts decreased about 1 and 1.8 log cycle, respectively. It could be attributed to acidity of bio-yoghurts. The most important factors affecting the viability of *L. acidophilus* and *Bifidobacterium* BB-12 are acidity [27]. Acidity of the samples increased during the storage period. At the end of storage, although the viable counts of probiotic bacteria dropped in all bio-yogurts, the counts of *L. acidophilus* were found to be above the threshold for therapeutic minimum ($10^6 - 10^7$ cfu·g⁻¹) in all samples. On the other hand, after 10th day of storage the counts of *Bifidobacterium* BB-12 were found to be under the threshold for therapeutic minimum ($10^6 - 10^7$ cfu·g⁻¹) in all samples.

Table 1. The changes of viable bacteria counts of yogurt and bio-yogurt incubated at different temperature during storage period (log cfu·g⁻¹)**.

Organisms	Day of Storage	A*	B	C	D
<i>S. thermophilus</i>	1	8.77 ± 0.007 ^{d3}	8.93 ± 0.015 ^{c3}	8.98 ± 0.146 ^{b3}	9.07 ± 0.173 ^{a2}
	10	8.99 ± 0.001 ^{b2}	9.02 ± 0.016 ^{b2}	9.07 ± 0.024 ^{a2}	9.11 ± 0.015 ^{a1}
	20	9.09 ± 0.056 ^{a1}	9.12 ± 0.158 ^{a1}	9.13 ± 0.034 ^{a1}	9.13 ± 0.121 ^{a1}
<i>L. acidophilus</i>	1	6.97 ± 0.018 ^{c2}	7.05 ± 0.029 ^{a2}	7.08 ± 0.040 ^{a2}	7.02 ± 0.021 ^{b2}
	10	7.30 ± 0.011 ^{c1}	7.42 ± 0.005 ^{b1}	7.52 ± 0.157 ^{a1}	7.25 ± 0.211 ^{d1}
	20	6.19 ± 0.111 ^{b3}	6.31 ± 0.116 ^{a3}	6.34 ± 0.147 ^{a3}	6.12 ± 0.122 ^{c3}
<i>Bifidobacterium</i> BB-12	1	6.99 ± 0.007 ^{c1}	7.45 ± 0.06 ^{a1}	7.13 ± 0.018 ^{b1}	6.95 ± 0.047 ^{c1}
	10	5.56 ± 0.443 ^{c2}	5.94 ± 0.258 ^{a2}	5.66 ± 0.066 ^{b2}	5.34 ± 0.035 ^{d2}
	20	5.26 ± 0.159 ^{c3}	5.67 ± 0.147 ^{a3}	5.45 ± 0.031 ^{b3}	5.25 ± 0.027 ^{c3}

A: control, **B:** 0.5% CF, **C:** 1% CF, **D:** 2% CF added bio-yogurts. **Different letters in the same line indicate significant differences among the samples depending on CF level and different numbers in the same column indicate significant differences among the samples depending on storage time ($p < 0.05$).

3.2. Physico-Chemical Characteristics

The chemical composition of milk used for the production of bio-yogurt (data not shown) fell within the following averages: titratable acidity 0.162% (± 0.06) lactic acid, pH 6.7 (± 0.05), total solids 12.95% (± 0.095), fat 3.45% (± 0.1), protein 3.47% (± 0.115), and ash 0.84% (± 0.005).

Some properties of bio-yogurt are shown in **Table 2**. Addition of CF influenced the pH, titratable acidity, whey separation, water holding capacity and viscosity of bio-yogurt significantly.

The mean level of acidity of the samples supplemented with CF was higher than the control. It could be related the stimulation of bacteria in starter culture by cellulose. Nsor-Atindana *et al.* [9] reported that microcrystalline cellulose has minimal colonic fermentation. Addition of CF decreased the pH, while the lactic acid increased. As expected, the storage time significantly affected the level of acidity in the samples. Titratable acidity contents increased, while the pH decreased during storage.

A negative correlation was determined between whey separation and CF. This result could be related to the water binding capacity of CF. As known cellulose is

Table 2. The changes of some physico-chemical properties of bio-yogurt enriched cellulose fiber (CF) during storage period**.

Properties	Day of Storage	A*	B	C	D
pH	1	4.66 \pm 0.035 ^{a1}	4.63 \pm 0.070 ^{a1}	4.60 \pm 0.135 ^{b1}	4.53 \pm 0.075 ^{c1}
	10	4.52 \pm 0.055 ^{a2}	4.50 \pm 0.035 ^{a2}	4.48 \pm 0.035 ^{a2}	4.47 \pm 0.035 ^{b2}
	20	4.48 \pm 0.090 ^{a2}	4.47 \pm 0.015 ^{b2}	4.47 \pm 0.070 ^{b2}	4.31 \pm 0.025 ^{c3}
Titratable acidity (%Lactic acid)	1	0.38 \pm 0.006 ^{c2}	0.41 \pm 0.006 ^{b2}	0.41 \pm 0.006 ^{b2}	0.42 \pm 0.002 ^{a2}
	10	0.44 \pm 0.025 ^{c1}	0.44 \pm 0.043 ^{c1}	0.47 \pm 0.002 ^{b1}	0.49 \pm 0.018 ^{a1}
	20	0.45 \pm 0.030 ^{b1}	0.45 \pm 0.060 ^{b1}	0.48 \pm 0.050 ^{a1}	0.50 \pm 0.080 ^{a1}
Whey separation (%)	1	24.29 \pm 0.050 ^{a1}	20.82 \pm 0.215 ^{b1}	18.85 \pm 0.640 ^{c1}	17.90 \pm 0.330 ^{d1}
	10	19.88 \pm 0.615 ^{a2}	18.86 \pm 0.590 ^{b2}	18.56 \pm 0.295 ^{b2}	16.84 \pm 0.535 ^{c2}
	20	19.43 \pm 0.420 ^{a3}	18.14 \pm 0.535 ^{b3}	17.91 \pm 0.680 ^{b3}	16.71 \pm 0.685 ^{c2}
Water holding capacity (%)	1	50.88 \pm 0.870 ^{c3}	60.04 \pm 2.075 ^{b3}	62.62 \pm 3.395 ^{a2}	62.66 \pm 0.680 ^{a3}
	10	53.28 \pm 3.005 ^{c2}	62.82 \pm 0.495 ^{b2}	63.25 \pm 0.445 ^{a2}	64.17 \pm 3.430 ^{a2}
	20	57.00 \pm 1.405 ^{c1}	66.90 \pm 1.780 ^{b1}	67.96 \pm 0.350 ^{b1}	70.98 \pm 0.535 ^{a1}
Viscosity (cP)	1	2995 \pm 139 ^{d3}	4770 \pm 126 ^{c3}	5479 \pm 59 \pm b ³	5798 \pm 134 ^{a3}
	10	4926 \pm 320 ^{d2}	5631 \pm 217 ^{c2}	6876 \pm 588 ^{b2}	7652 \pm 142 ^{a2}
	20	5171 \pm 265 ^{d1}	6042 \pm 178 ^{c1}	7095 \pm 455 ^{b1}	7947 \pm 267 ^{a1}

*A: control, B: 0.5% CF, C: 1% CF, D: 2% CF added bio-yogurts. **Different letters in the same line indicate significant differences among the samples depending on CF level and different numbers in the same column indicate significant differences among the samples depending on storage time ($p < 0.05$).

used as a hygroscopic agent in many foods. Reserches reported that microcrystalline cellulose improving reological and textural properties various food [9] [26] [28]. Syneresis in the samples decreased during storage. This could be due to metbolic activity of yogurt starter cultures, and to decrease in net pressure in protein matrix, which cause to diminish in syneresis [29]. La Torre, Tamime and Muir [30] obtained similar results in set-type yogurt and bio-yogurt.

Bio-yoghurts with CF showed a significantly higher level water holding capacity ($p < 0.01$) than control. As known fiber may act as a stabilizer due to its capacity for binding water [31] [32]. Water holding capacity of the samples increased as the fiber rate increased ($p < 0.05$).

The viscosity of the bio-yogurt samples fortified with CF was higher than control sample. The increased viscosity of the CF-enriched bio-yogurt seems to be caused by the contribution of insoluble fibers to the increase of total solids [33], affecting the three dimensional conformation of the hydrated biopolymers. Viscosity values significantly increased with increasing the fiber levels. This can be explained by the interactions of the CF and liquid components of bio-yoghurt. CF, being very hygroscopic, would bind water [9] [26] [28]. Viscosity and water holding capacity of bio-yoghurts increased continuously throughout storage period in a similar way for all the samples ($p < 0.01$).

3.3. Textural Properties

Addition of CF into the bio-yoghurts, as well as the storage period, affected the textural properties of the samples ($p < 0.01$). Firmness, cohesiveness, springiness, gumminess and chewiness values of bio-yoghurts increased as CF level increased, but adhesiveness decreased (**Table 3**). This can be explained by increasing dry matter of bio-yoghurts enriched with CF. Increasing in dry matter caused to firmer and stronger gel. Zisu and Shah [34] reported that cheeses with the lower moisture content showed greater cohesiveness. As with cohesiveness, the relationship between moisture and hardness and their effects on the protein microstructure existed for springiness and are responsible for the loss in the ability of the cheese to recover to its original state [34]. As a result while firmness, cohesiveness, springiness, gumminess and chewiness values are increasing, adhesiveness value reduced in bio-yoghurts. On the other hand, highly water binding capacity of CF may cause to be firmer gel network. Do Espiroto Santo *et al.* [35] also reported that addition of passion fruit peel powder increased firmness and consistency of skim yoghurt. However, Garcia-Perez *et al.* [36] found that the addition of orange fiber below 1% concentration reduce the firmness of skim yoghurt.

All texture parameters (except cohesiveness and springiness) significantly increased during cold storage. It could be related to depending on the decrease in pH, the protein-protein interactions and therefore, slow protein rearrangements in the acid casein gels continue during cold storage [17].

Table 3. The changes of textural properties of bio-yogurt enriched cellulose fiber (CF) during storage period**.

Properties	Day of Storage	A*	B	C	D
Firmness	1	201.26 ± 9.179 ⁸³	216.91 ± 7.139 ^{f3}	238.76 ± 11.981 ^{e3}	251.45 ± 9.487 ^{d3}
	10	243.41 ± 11.80 ^{e2}	247.33 ± 12.217 ^{d2}	277.19 ± 12.69 ^{c2}	287.21 ± 10.54 ^{b2}
	20	274.99 ± 14.203 ^{c1}	293.97 ± 8.26 ^{b1}	318.04 ± 11.50 ^{a1}	323.94 ± 13.46 ^{a1}
Adhesiveness	1	-116.07 ± 0.946 ^{h3}	-97.76 ± 0.647 ⁱ³	-94.26 ± 1.759 ^{j3}	-92.30 ± 2.281 ^{k3}
	10	-142.60 ± 2.331 ^{e2}	-124.81 ± 3.108 ^{f2}	-119.62 ± 1.591 ^{g2}	-111.69 ± 1.957 ^{h2}
	20	-213.21 ± 2.352 ^{a1}	-201.04 ± 0.765 ^{b1}	-164.82 ± 1.786 ^{c1}	-145.40 ± 2.712 ^{d1}
Cohesiveness	1	0.34 ± 0.006 ^{b1}	0.35 ± 0.006 ^{b1}	0.37 ± 0.033 ^{a1}	0.38 ± 0.003 ^{a1}
	10	0.33 ± 0.0014 ^{b2}	0.34 ± 0.001 ^{b2}	0.35 ± 0.009 ^{b1}	0.36 ± 0.009 ^{a1}
	20	0.32 ± 0.006 ^{c2}	0.34 ± 0.038 ^{b2}	0.34 ± 0.001 ^{b2}	0.36 ± 0.007 ^{a1}
Springiness	1	93.64 ± 0.279 ^{f2}	95.36 ± 0.449 ^{d2}	96.41 ± 0.150 ^{b1}	96.93 ± 0.324 ^{a1}
	10	93.91 ± 0.692 ^{e2}	96.03 ± 0.524 ^{c1}	96.56 ± 0.299 ^{b1}	96.97 ± 0.868 ^{a1}
	20	92.96 ± 0.749 ⁸³	94.01 ± 0.449 ^{e2}	96.16 ± 0.197 ^{b3}	96.22 ± 0.240 ^{c3}
Gumminess	1	82.85 ± 1.401 ^{j3}	83.97 ± 2.315 ⁱ³	84.22 ± 0.365 ⁱ³	88.59 ± 2.0 ⁸³
	10	85.19 ± 0.697 ⁱ²	87.52 ± 0.584 ^{h2}	88.82 ± 1.394 ^{f2}	95.43 ± 1.035 ^{d2}
	20	91.83 ± 0.994 ^{e1}	102.04 ± 1.641 ^{c1}	105.22 ± 0.118 ^{b1}	109.82 ± 0.425 ^{a1}
Chewiness	1	69.88 ± 0.402 ^{j3}	75.72 ± 1.787 ⁱ³	81.18 ± 0.832 ⁸³	88.15 ± 0.115 ^{e3}
	10	74.30 ± 1.099 ⁱ²	79.95 ± 0.709 ^{h2}	87.69 ± 1.164 ^{f2}	92.12 ± 0.406 ^{c2}
	20	88.49 ± 1.030 ^{e1}	90.31 ± 1.136 ^{d1}	95.98 ± 1.402 ^{b1}	100.51 ± 1.313 ^{a1}

*A: control, B: 0.5% CF, C: 1% CF, D: 2% CF added bio-yogurts. **Different letters in the same line indicate significant differences among the samples depending on CF level and different numbers in the same column indicate significant differences among the samples depending on storage time ($p < 0.05$).

3.4. Sensory Evaluations

The results of organoleptic evaluation indicated that the bio-yogurt enriched with 0.5% CF received highest scores (Figures 1-3). This could be related to better consistency and acidity of these samples. The higher fiber concentration (1% and 2%) caused the lower sensory scores. It could be related to insolubility of the CF. All sensory scores of the samples decreased during storage. This could be associated with acidity and flavor of the bio-yoghurts. At the beginning of storage, samples had intensive flavour and low acidity. But, after 20 days they were more acidic, so they had received lower organoleptic scores.

4. Conclusions

Addition of CF had significant effects on the physico-chemical, textural, viable bacterial counts and sensory characteristics of bio-yogurt. Whey separation and pH reduced, while the level of titratable acidity, water holding capacity and viscosity increased in the bio-yogurts supplemented with CF. During the storage, whilst the whey separation and pH values declined gradually, the titratable acidity water holding capacity and viscosity increased at the same time.

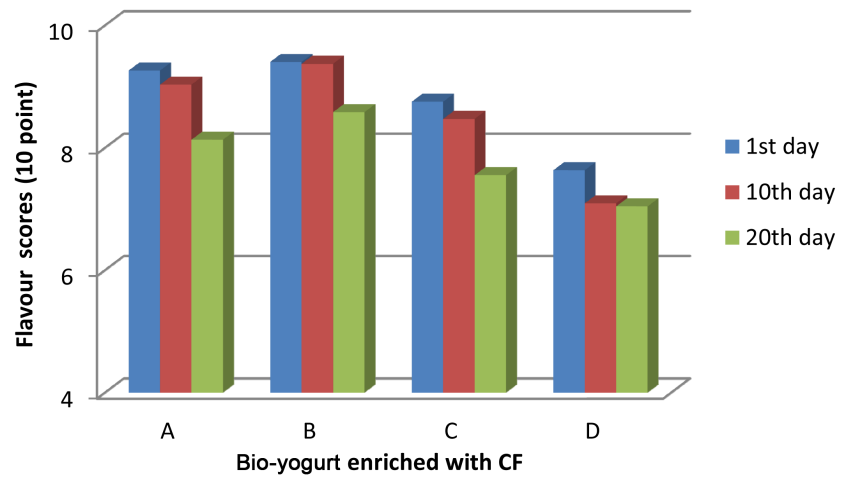


Figure 1. The changes of flavour scores of bio-yogurt* enriched cellulose fiber (CF) during storage period. **A: control, B: 0.5% CF, C: 1% CF, D: 2% CF added bio-yogurts.

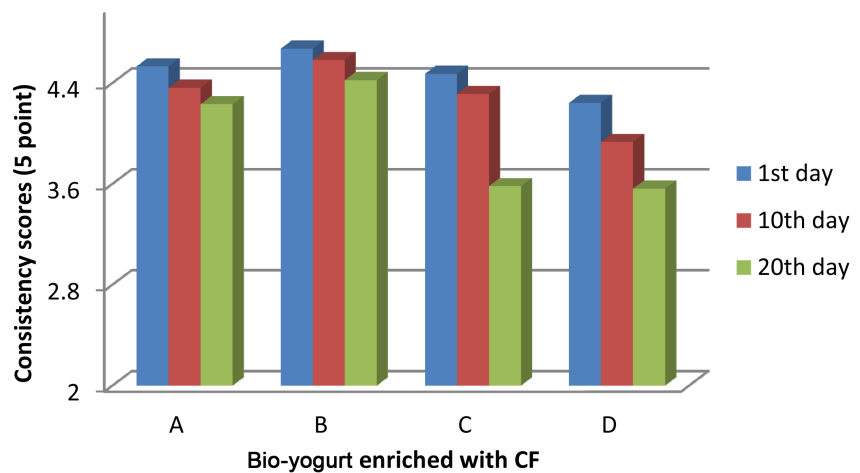


Figure 2. The changes of consistency scores of bio-yogurt* enriched cellulose fiber (CF) during storage period. **A: control, B: 0.5% CF, C: 1% CF, D: 2% CF added bio-yogurts.

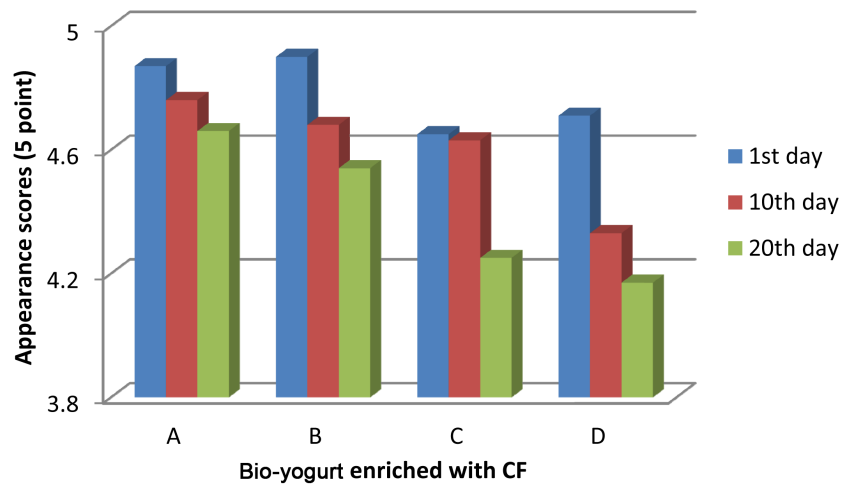


Figure 3. The changes of appearance scores of bio-yogurt* enriched cellulose fiber (CF) during storage period. **A: control, B: 0.5% CF, C: 1% CF, D: 2% CF added bio-yogurts.

The counts of probiotic bacteria were adversely affected by addition of CF. Addition of CF at a rate of 0.5% led to an improvement in viability of *L. acidophilus* and *Bifidobacterium* BB-12. Usage of CF at a rate of 1% and 2% had negatively affected the viability of *L. acidophilus* and *Bifidobacterium* BB-12. On the contrary, *S. thermophilus* counts increased with increasing CF level. During the storage, the viable counts of both probiotics and *S. thermophilus* dropped in all samples.

Addition of CF at a rate of 0.5% led to an improvement of sensory scores of bio-yogurts. Sensory scores declined with increasing CF level. Consequently, addition of CF at a rate of 0.5% could be satisfactorily used to produce bio-yogurt.

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