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Chemical Properties and Sensory Evaluation of Probiotic Yoghurt Manufactured with Aqueous Extract of *Aloe vera*

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ABSTRACT

In recent years, consumer demand for a new range of dairy products, including yoghurts, which have functional and designed sensory properties have increased. In the present research physicochemical, microbiological and sensory attributes of yogurts manufactured from cow milk with aqueous extract of *Aloe vera* and *Lactobacillus casei* before and after cold storage for different periods of time (1, 3, 5, 7 and 10 days) were investigated. Titrable acidity (TA) of examined yoghurts during storage period at 4°C increased and their pH decreased significantly (P<0.05). The percentages of Water Holding Capacity (WHC) and Syneresis of yoghurt samples through the 10 days storage period were significantly decreased and increased, respectively (P<0.05). Viability of *L. casei* was significantly higher in probiotic yoghurt samples than others with *Aloe vera* extract after the end storage time. Sensory evaluation of examined yoghurts showed that *Aloe vera* extract had no effect on sensory quality of probiotic yoghurt samples. It was concluded that probiotic yoghurt with 2.5% *Aloe vera* extract with low syneresis and high WHC had better physicochemical, microbiological and sensory properties in comparison with the other probiotic yoghurt samples.

Key words: *Aloe vera*, Aqueous extraxt, Yoghurt, *L. casei*, Organoleptic quality, Chemical properties.

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1. INTRODUCTION

Yoghurt, one of the most popular fermented dairy products, is widely consumed all over the world. Yoghurt products are more nutritious than all other fermented milk products because of their high level of milk solid containing in addition to nutriments developed during the fermentation by lactic acid bacteria (LAB) (1). Different forms of yoghurt are now available in the markets for consumers including stirred, set, frozen, probiotic, liquid and herbal yoghurt. For preservation of yoghurt products, inherent quality during storage, in particular, physicochemical, sensory characteristics and appropriate packaging are prominent (2). The most popular flavors used for yoghurts are fruit juices with pulps. Among non-traditional additives also pulps, vegetable powders and natural herbal extracts obtained from raw vegetables and herbs have been used in the production of fermented milk products including yoghurt (3). Vegetables are important sources of nutrients and vitamins; however, they are low in calories and in contrast rich in minerals, dietary fiber and other nutrients as well as many bioactive compounds, such as antioxidants(carotenoids, tocopherols, ascorbic acid, phenolic substances) (4). Biological antioxidants are compounds which are able in low concentration to delay or prevent the oxidative damage of various biomolecules lead to various diseases including liver disease, cancer, Alzheimer's disease, aging, arthritis, diabetes, inflammation, Parkinson's disease, atherosclerosis and AIDS (5). Increased fruit and vegetable consumption is an effective strategy to increase antioxidant intake and also help to prevent developing chronic diseases, especially cancer and cardiovascular disease (6). The aim of this research was to examine the effect of *Aloe vera* aqueous extract and probiotic bacteria on physicochemical (pH, titrable acidity (TA), water holding capacity (WHC), synersis) and sensory characteristics of yoghurt during cold storage.

2. MATERIALS AND METHODS

2.1. Extraction of Aloe Vera Gel

Aloe Vera plant purchased from Qazvin market, Iran. The fully expanded leaves of *Aloe vera* were selected, washed with distilled water and were sterilized using 70% ethanol and then by 0.1% $HgCl_2$. The parenchymatous covering of the leaves were removed and the *Aloe vera* gel drained out. Slurry was formed with the aim of mortar and pestle (7).

2.2. Preparation of aqueous extract

Fifty grams of crushed Aloe vera was transferred into the extraction container then distillated water was added. The Container was placed onheater source, stirred constantly until the first signs of boiling were seen. After boiling for 15 minutes, the solution was filtered (Whatman filter paper No. 1) and then poured in sterile dark glass containers and kept in the refrigerator (8).

2.3. Starter and probiotic bacteria

Freeze dried yoghurt inoculants (Christian Hansen Co., R 704, Denmark) containing *Lactobacillus delbrueckii,* ssp. *bulgaricus* and *Streptococcus salivarius ssp. thermophilus* (1:1) were used as starter. A commercial lyophilized culture of *L. casei* ATCC 3939 obtained from the Organization of Iranian Industrial Research was also used as probiotic bacteria . Subcultivation and preparation of the probiotic bacteria were conducted according to the standard method (9).

2.4. Preparation and inoculation of yoghurt

Raw cow milk was subjected to a thermal processing at 90°C for 20 min, then cooling to 40 – 45°C. *Aloe Vera* gel aqueous extract was added to the milk in different concentrations (5 and 10 %). As starter culture, yoghurt (*L. bulgaricus* and *S. thermophilus*) (1.5%) was added to the milk, and then mixed. Finally *L. casei* (10^8-10^9 CFU/ml) was added to the mixture and then packed in sterilized glass capped cups with capacity of 250 mL and incubated at 40°C for three hr till jelly was formed (in pH 4.5). Freshly yoghurt was cooled and finally stored at 4°C for 10 days (cooling storage period) (10).

2.5. Physiochemical Analysis.

2.5.1. pH and TA

The pH values of yoghurt samples were measured with the aid of a digital pH meter (Nick, 776Jena, Germany). The TA was determined by the method desribed by Iwalokun and Shittu (2007), which is in accordance with IDF standard (11). Five grams of yoghurt sample was titrated with 0.1 N NaOH solution using 1% phenolphthalein as indicator. The TA was calculated in grams of lactic acid in 100 g of yoghurt using the following equation: TA = ($V \times 0.9$)/m. Where V is the volume (in mL) of 0.1 N NaOH solution consumed, m is the mass (in grams) of test portion of yoghurt sample and 0.9 is the correction factor for lactic acid (12).

2.5.2. Determination of syneresis

According to Guzman-Gonzalez et al. (13), Susceptibility of yoghurt to syneresis was determined by centrifuging 20 g of yogurt sample at 500 rpm for 5 min and weighing the supernatant. Measuring the volume of supernatant recovered, the percentage of syneresis was calculated based on the following formula:

$$\%\text{Syneresis}=\frac{\text{Volume of Supernatent}}{\text{Weight of Sample}}\times 100 \quad (1)$$

2.5.3. WHC of yoghurt samples

WHC of yoghurt was determined using the procedure described by Guzman- Gonzalez et al. (14). An amount of 20 g of yoghurt (Y) was centrifuged for 30 min at 1250 *g* and 20°C (h = 4.8 cm). The whey expelled (WE) was removed and weighed. The water-holding capacity (WHC) was determined as follow: $\text{WHC}=\frac{100\times(Y-WE)}{Y}$ (2)

2.6. L. casei survival

Survival of *L. casei* during storage time of yoghurt samples (on days 1, 7, 14, 21 and 28) was estimated according to the method described by Phillips et al. (15). 1 g of yoghurt sample was homogenized in a stomacher with 9 mL of sterile peptone water (Merck, Darmstadt, Germany) (0.1%) and 10-fold (10^2–10^8) serial dilutions were prepared in aseptic condition. The enumeration of plates was carried out using spread plates with a 100 ppm inoculum on reinforced clostridial agar (RCA) with bromocresol green and vancomycin (RCABV) medium. The pH of the RCA agar base was adjusted to 5.5 before autoclaving and then bromocresol green stock 0.2% w/v (prepared as previously described) added (2% v/v). Vancomycin stock solution (2% w/v) was prepared with distilled water and filter sterilized through a 0.45-mm membrane. Vancomycin was added to the molten agar at the concentration of 0.5 mL/L. The plates were incubated in gas jars using the GasPak System, (Thermo Fisher Scientific Inc., Watham, MA) in anaerobic condition for 48 h at 37 °C prior observation. Plates containing 25–250 colonies were enumerated and reported as colony forming units (CFU/g) of the product (15). All plate counts were carried out in triplicates.

2.7. Sensory Analysis

After adding the aqueous extract of Aloe Vera Gel and probiotic bacteria to the yoghurt samples, the sensory features of the examined samples were evaluated with the aim of an acceptance test. Yoghurt samples with various amounts of aqueous extract were equally divided into seven parts (each part 20 grams) and they were placed in clear containers then coded with a three-digit random number system. The sensory evaluation was implemented by a panel of seven judges including the scientific staff and students of the Department of Food Hygiene, Qazvin University of medical sciences, experienced in the sensory

evaluation of food and drinks. Each panelist has evaluated the samples by ranking them using a 9-point scale, where 9= like extremely and 1= dislike extremely, for various properties such as (appearance) color, odor and flavor (16).

2.8. Statistical analysis

All experiments were carried out in triplicate and each sample was analyzed in duplicate. The statistical analysis was conducted using SPSS software ver. 18.0 (Chicago, IL, USA). Analysis of variance (ANOVA) was performed. Significant differences (p<0.05) between means were determined by Duncan`s multiple range test.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analysis

According to Table 1 and Table 2 TA of yoghurt samples during storage period at 4°C were increased and their pH was decreased.

Table 1. Changes in the percentage of TA of the yoghurt samples during storage period at 4°C

Yoghurt samples	Storage period (days)				
	1^{NS}	3^{NS}	5^{NS}	7^{NS}	10^{NS}
A	0.65±0.01	0.69±0.02	0.75±0.09	0.83±.09	0.89±0.10
B	0.73±0.10	0.84±0.02	0.92±0.03	0.96±0.07	0.99±0.13
C	0.64±0.06	0.86±0.13	0.95±0.03	0.99±0.01	1.12±0.05
D	0.70±0.13	0.80±0.11	0.88±0.09	0.92±0.01	0.95±0.06
E	0.67±0.19	0.75±0.14	0.83±0.08	0.90±0.03	0.93±0.02
F	0.65±0.07	0.73±0.04	0.80±0.11	0.86±0.19	0.90±0.15

The mean values followed by the same letter in the column are no significantly different (P<0.05) by Duncan`s multiple comparison.
^{NS} Not Significant
Yoghurt samples including A: yoghurt with no additive; B: probiotic yoghurt without extract; C: probiotic yoghurt with 2.5% Extract; D: probiotic yoghurt with 5% Extract; E: yoghurt with 2.5% Extract and F: yoghurt with 5% Extract.

Table 2. Changes in pH of the yoghurt samples during storage period at 4°C

Yoghurt samples	Storage period (days)				
	1^{NS}	3^{NS}	5^{NS}	7^{NS}	10^{NS}
A	4.52±0.21	4.44±0.28	4.39±0.18	4.32±0.20	4.25±0.25
B	4.58±0.23	4.39±0.36	4.38±0.18	4.29±0.18	4.12±0.07
C	4.65±0.27	4.41±0.07	4.30±0.16	4.19±0.02	4.00±0.09
D	4.61±0.29	4.45±0.02	4.32±0.13	4.21±0.07	4.05±0.08
E	4.65±0.05	4.47±0.02	4.33±0.07	4.30±0.20	4.08±0.13
F	4.56±0.22	4.49±0.11	4.35±0.12	4.32±0.33	4.19±0.25

The mean values followed by the same letter in the column are no significantly different (P<0.05) by Duncan`s multiple comparison.
^{NS} Not Significant
Yoghurt samples including A: yoghurt with no additive; B: probiotic yoghurt without extract; C: probiotic yoghurt with 2.5% Extract; D: probiotic yoghurt with 5% Extract; E: yoghurt with 2.5% Extract and F: yoghurt with 5% Extract.

There was no any significant difference between probiotic, non-probiotic and extract contained yoghurt samples. When yogurt was produced, lactic acid production will begin, leading to enhance TA and decline in pH value (17). Decrease in the pH creates a suitable medium for better survival and growth of probiotic bacteria (18). The

decrease of pH is because of production of lactic acid by which utilization of residual lactose, small amount of CO_2 and formic acid by lactic acid and probiotic activity. Post-acidification during the storage period in yoghurt samples is due to β-galactosidase being active at 0-5 °C (13). Researchers reported that the declination in pH value during the storage time is due to residual enzymes released by lactic acid bacteria during their fermentation (14). The percentage of WHC of yoghurt samples through the storage period at 4 °C were decreased, while the percentage of Syneresis of yoghurt samples was increased. These changes are significantly different between probiotic, non-probiotic and extract contained samples (Table 3, Table 4).

Table 3. Changes in the percentage of WHC of the yoghurt samples during storage period at 4°C

Yoghurt samples	Storage period (days)				
	1	3	5	7	10
A	63±2.42^{a}	58±3.85^{b}	56±2.95^{a}	53±3.33^{b}	50±2.85^{b}
B	64±3.13^{a}	62±1.90^{ab}	59±3.86^{a}	57±2.11^{ab}	53±2.50^{ab}
C	70±2.05^{a}	67±3.60^{a}	65±2.10^{a}	63±2.15^{a}	60±3.01^{a}
D	68±3.15^{a}	65±3.30^{ab}	61±3.63^{a}	59±4.01^{ab}	55±3.13^{ab}
E	65±2.15^{a}	63±2.58^{ab}	57±3.68^{a}	55±3.14^{ab}	52±3.25^{b}
F	63±2.19^{a}	62±3.46^{ab}	59±3.92^{a}	56±2.96^{ab}	54±2.55^{ab}

The mean values followed by the same letter in the column are no significantly different (P<0.05) by Duncan`s multiple comparison.
Yoghurt samples including A: yoghurt with no additive; B: probiotic yoghurt without extract; C: probiotic yoghurt with 2.5% Extract; D: probiotic yoghurt with 5% Extract; E: yoghurt with 2.5% Extract and F: yoghurt with 5% Extract.

Table 4. Changes in the percentage of Syneresis of the yoghurt samples during storage period at 4°C

Yoghurt samples	Storage period (days)				
	1	3	5	7	10
A	48.00±2.50^{a}	49.00±1.80^{a}	53.66±2.80^{a}	57.33±1.55^{a}	60.33±1.48^{a}
B	42.00±2.25^{ab}	43.66±3.00^{ab}	45.35±1.48^{bc}	47.20±2.35^{b}	49.00±1.89^{b}
C	37.33±2.01^{b}	39.25±2.23^{b}	40.55±2.85^{c}	41.00±1.95^{c}	42.25±2.00^{c}
D	40.25±1.89^{b}	41.00±1.87^{b}	45.66±1.98^{bc}	49.65±2.12^{b}	51.65±1.87^{b}
E	41.35±2.18^{b}	45.00±2.58^{ab}	48.00±1.45^{ab}	51.00±2.18^{b}	53.00±2.01^{b}
F	40.25±3.00^{b}	43.00±2.14^{ab}	46.25±1.68^{bc}	48.25±1.89^{b}	51.25±2.45^{b}

The mean values followed by the same letter in the column are no significantly different (P<0.05) by Duncan`s multiple comparison.
Yoghurt samples including A: yoghurt with no additive; B: probiotic yoghurt without extract; C: probiotic yoghurt with 2.5% Extract; D: probiotic yoghurt with 5% Extract; E: yoghurt with 2.5% Extract and F: yoghurt with 5% Extract.

WHC was minimum in yoghurt samples without any additive and was maximum in probiotic yoghurt samples with 2.5% extract, however, syneresis in yoghurt samples without any additive was maximum, and it was minimum in probiotic yoghurt with 2.5% extract. The obtained results are in accordance with the results of the previous works where they showed that the percentage of syneresis was directly related to the TA and inversely to pH value changes (13, 14).

3.2. L.casei counting and sensory evaluation

Probiotic viability of yoghurt samples after storage time at 4°C is shown in Figure 1.

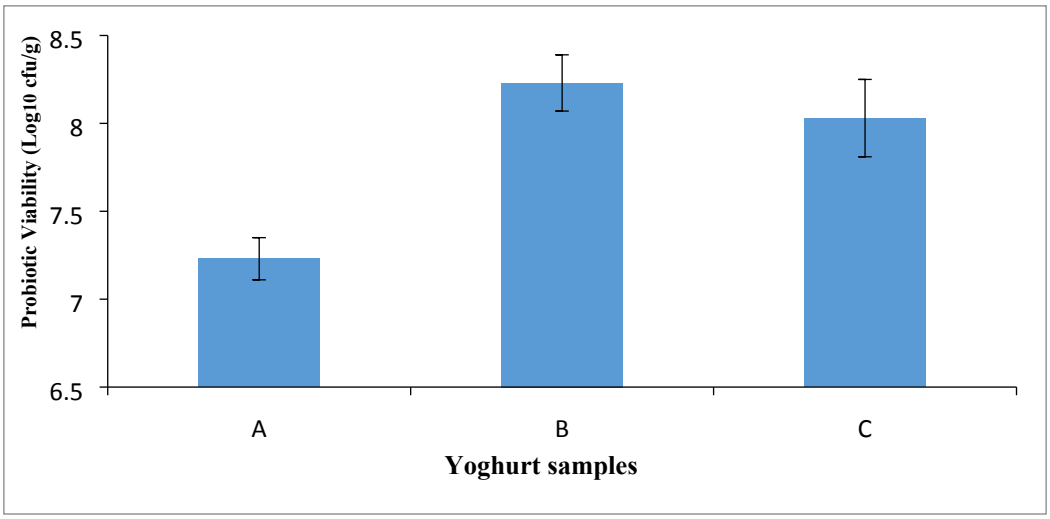

Figure 1. Probiotic viability in yoghurt samples after storage period at at 4°C (A: probiotic yoghurt without extract; B: probiotic yoghurt with 2.5% Extract and C: probiotic yoghurt with 5% Extract)

Microbial viability was significantly more in probiotic yoghurt samples with *Aloe vera* extract after storage time at 4 °C than other samples. These results are in accordance with the results reported by the other researchers who found that probiotic organisms are acid tolerance and they will survive in high numbers then remain viable in fermented dairy products during the cold storage period. This is also beneficial to utilize these organisms on industrial scale to manufacture functional products such as dairy and non-dairy probiotic products (17). Sensory evaluation of yoghurt samples after storage time at 4 °C is presented in Figure 2.

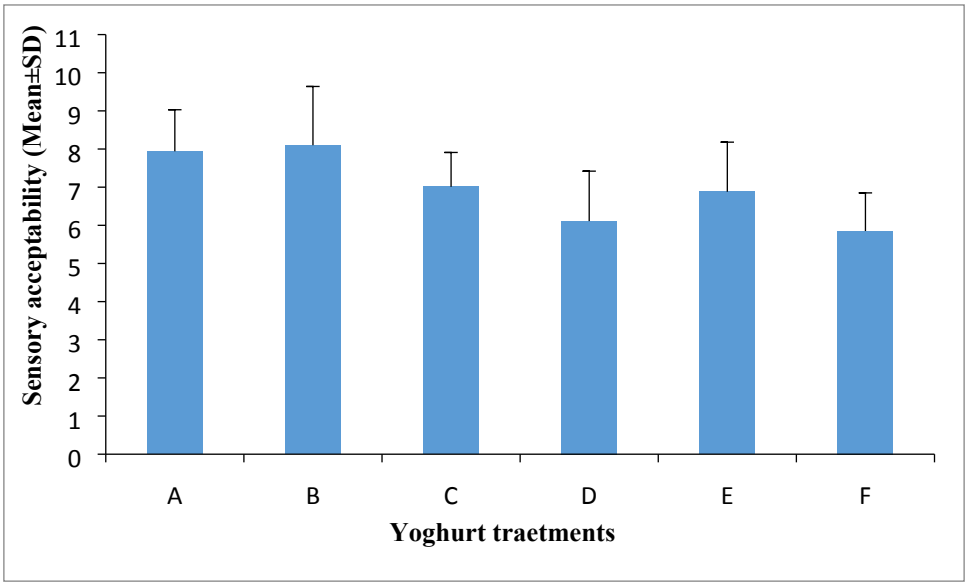

Figure 2. Sensory evaluation of yoghurt samples after storage period at at 4° (A: yoghurt with no additive; B: probiotic yoghurt without extract; C: probiotic yoghurt with 2.5% Extract; D: probiotic yoghurt with 5% Extract; E: yoghurt with 2.5% Extract and F: yoghurt with 5% Extract)

The obtained results showed significant differences in sensory features among probiotic, non-probiotic and *Aloe vera* extract contained yoghurt samples. Yoghurt samples without any additive as control samples were compared with other samples to evaluate the sensory characteristics. Probiotic yoghurt samples without extract were similar to original sensory properties of yoghurt. Adding *Aloe vera* extract, sensory properties of probiotic yoghurt samples differed significantly from other samples. Extra addition of *Aloe vera* extract to yoghurt samples leads to more changes in sensorial quality of the samples. In conclusion, adding any vegetable or herbal extract in yoghurt cause to change in sensory properties of yoghurt that leads to be ranked low by consumers; consequently, it would be better to be optimized in industrial scale (16).

4. CONCLUSION

TA of yoghurt samples during the storage at 4°C increased and pH decreased. In addition, WHC % and Syneresis % of yoghurt samples through the storage period decreased and increased, respectively. Rate of syneresis percentage is related to the TA directly and inversely to pH value changes. Probiotic viability is significantly more in probiotic yoghurt samples with *Aloe vera* extract after storage time. Sensory evaluation of samples presented a negative effect of *Aloe vera* extract on quality of probiotic

yoghurt samples. As a result, it is suggested by this research that probiotic yoghurt with 2.5% *Aloe vera* extract include lower syneresis, higher WHC and the best sensory evaluation in compared with other probiotic yoghurt samples with *Aloe vera* extract.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration among all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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