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Original Article

Improvements in Survival of Probiotic Bacteria, Rheology and Sensory Characteristics of Yogurts during Storage

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ABSTRACT

Background and Objectives: Use of aloe vera gel in dairy industries is important for increasing quality of dairy products. In the present study, effects of aloe vera gel with inulin and whey powder on survival of probiotic bacteria, rheological and sensory characteristics of yogurt were investigated.

Materials and Methods: Low-fat (1.5%) probiotic yogurts were prepared from pasteurized milks, 1% of starters and 2% combinations of *Lactobacillus acidophilus* with *Bifidobacterium animalis* at 40 °C and pH 4.4. Probiotic yogurts mixed with aloe vera gels at 0, 5, 10, 15 and 20% were stored seven days at 4 ^{o}C. Since the probiotic yogurt with 10% of aloe vera gel included the best sensory scores compared to other aloe vera gel concentrations after seven days of storage at 4 ^{o}C, this formulation was mixed with inulin at 0.5, 1 and 1.5% (w/w) or whey powder at 5, 10 and 15% (w/w).

Results: Sensory evaluations showed that the probiotic yogurt with 10% of aloe vera gel and 1.5% of inulin included the highest scores of flavor, texture, aroma, color and acceptance within the samples after similar storage conditions. The probiotic yogurt with 10% of aloe vera gel and 1.5% of inulin included the highest survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis*, while controls (with no aloe vera gels) showed the lowest survival rates of *Lactobacillus acidophilus* and *Bifidobacterium animalis*. Apparent viscosity and serum separation of probiotic yogurt with 10% of aloe vera gel and 1.5% of inulin respectively increased three times and decreased to less than 4%, compared to controls and whey samples at similar shear rates and storage times.

The pH decrease of probiotic yogurt with 10% of aloe vera gel and 1.5% of inulin was much slower than those of the control and whey samples during storage, including no serum separations during storage.

Conclusions: Aloe vera gel and inulin improved viability of probiotic bacteria and quality of yogurt samples during storage.

Keywords: Probiotic bacteria, Aloe vera gel, Inulin, Whey powder, Rheological properties, Sensory evaluation

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Introduction

Low growth and death rates of beneficial bacteria (majorly *Lactobacillus acidophilus* and bifidobacteria) in probiotic yogurts (PY) during handling and storage are major nutritional concerns for the consumers. Addition of various substances such as whey protein concentrates, lactulose and plant extracts and proper selection of the starters have decreased losses of probiotic bacteria in yogurts (1, 2, 3, 4, 5). However, some of these additives did not improve consistency or prevent serum separation and could not preserve the natural pleasant flavor and mouthfeel of fresh yogurts during storage. This study was carried out to use combine additions of the following substances to yogurts to solve the highlighted problems in probiotic yogurts during cold storage. The first substance includes aloe vera with elective antimicrobial effects. The substance can inhibit *Staphylococcus aureus* and *Trichphyton mentagrophytes* while it does not affect non-pathogenic and useful microorganisms (6). Aloe vera of less than 1.0% dried matter includes good gastro-protective properties and promotes growth of valuable microorganisms (7). Researchers believe that aloe vera gel (AG) penetrates deeply into cell DNA, prevent cell deformation (by external factors) and improve viability of probiotics (6).

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Another substance, inulin (IN), is an odorless white powder with natural flavor (almost 10% of sucrose sweetness), which is easily solved in water and includes capability to form gels with water. Inulin can prevent colon cancer in rats when fed with probiotic yogurts for 33 weeks (8). In molecular structure, inulin is smaller than other hydrocolloids and hence includes a less water bonding capacity. By increasing inulin concentration in water (> 15%), stable gels or creams are formed. When shearing forces are used on inulin, the substance particles absorb more water, creating desired gel and cream tissues with favorable rheological characteristics (9). Studies have shown that food supplements containing inulin improve growth of useful bacteria, especially bifidobacteria in the large intestine and thus improving function of immune system as well as preventing growth of pathogenic bacteria in the human body (2). Inulin includes sulfur-containing amino acids and potentials to decrease reduction potential of compounds and improve growth of probiotic bacteria (10). Liquid whey from milk coagulation (in cheese making) or a mixture of whey powder with water is relatively inexpensive (compared to whey protein concentrates), easily accessible and highly effective in increasing growth and survival of probiotic bacteria. Whey powder includes excellent digestibility (due to its proteins) and desirable technical properties such as viscosity, foaming capability, emulsification capacity, water absorption capacity and gelation capability. Therefore, survival improvement of probiotic bacteria and production of novel products using raw materials with beneficial nutritional and technical properties such as aloe vera gels seem necessary. Since no information were available on addition of aloe vera gel, inulin and whey powder to low-fat probiotic yogurt, the aim of the current study was to assess combined effects of these ingredients on physiochemical properties, sensory evaluation, rheology and probiotic viability of yogurts during storage.

Materials and Methods

Lactobacillus acidophilus (La-5), *Bifidobacterium animalis* (BI-01) and yogurt starter (CY340) were purchased from Christian Hansen, Denmark and the medium-chain inulin from Sensuous, the Netherlands. Chemicals such as MRS agar and MRS broth culture media, mupirocin and salicin were purchased from Merck, Germany, and sodium hydroxide, phenolphthalein and cysteine hydrochloride from Sigma-Aldrich, USA. Aloe vera plants were provided by the Iranian Jiroft Agricultural Research Center, Jiroft, Iran. Whey powder (pH 5.7), including 3.4% H_2O, 12.9% proteins, 1.16% fats, 75.4% lactose and 8.6% ash, was provided by Pegah Dairy, Kerman, Iran.

Probiotic yogurt preparation

Briefly, 1 g of freeze-dried probiotic culture, containing *L. acidophilus* and *B. animalis*, was mixed well with 100 ml of MRS broth media followed by incubation at 37 ^oC for 24 h. The probiotic culture absorbed water and started to grow in logarithmic stage. On Day 2, 1 ml of the incubated culture was re-diluted with 99 ml of fresh MRS media (1% v/v) and incubated at 37 ^oC for 24 h, followed by refrigeration at 4 ^oC. To purify the probiotic bacteria, nearly 2 ml of the culture media from Day 1 was transferred to 200 ml of the fresh MRS culture media and incubated at 37 ^oC for 18 h. Then, culture was well-stirred and centrifuged (10,000 rpm, 10 min) to separate the liquid phase. The residue was mixed with 0.9% (v/v) physiological serum (saline solution) and centrifuged twice to wash and harvest bacterial cells early at the stationary phase (11). The low-fat milk (1.5% fats, 2.7% proteins and 11.5% total dried matters) was fully homogenized, heated (95 ^oC, 10 min) and cooled down to 40 °C. Milk was mixed with 1% of the prepared starter (CY340) and incubated at 40 °C until the pH reached to 4.4. Then, a suspension of the probiotic bacteria (*L. acidophilus* and *B. animalis*) was mixed with yogurts at 2% (w/w). Then, aloe vera leaves were rinsed and cut into ~20 cm long and their sharp edges were removed. Upper and bottom skins of each strip removed longitudinally to gently release their semi-liquid gels. Aloe vera gel was cut into 2–3 pieces and blended for ~2 min. Prepared aloe vera gel included 98.43% moisture and 1.57% solid contents. Then, four concentrations (0, 5, 10, 15 and 20% w/w) of aloe vera gel were separately well mixed with probiotic yogurts. Samples with various concentrations of aloe vera gel were stored at 4 ^oC until use.

Serum assessments

Samples of probiotic yogurt containing various additives were poured into 250-ml bottles and stored at 4 °C. The following formula used to calculate the serum separation of yogurts during 28 days of storage (12).

Serum separation (%) = (supernatant height) * 100) / (total height of yogurt in bottles)

Sensory evaluation

Sensory evaluation was carried out by 11 panelists (five women and six men, 30–45 year-old, expert members of Kerman Pegah Dairy) after seven days of storage. Samples were randomly presented to each panel member in separate booths at room temperature with air ventilation under fluorescent light illumination suggested by ISO Standards No. 8589 (13) 2 h after breakfast. Samples were assessed for flavor, color, aroma and texture (hardness) using 9-point hedonic scales (9 = liked extremely, 5 = neither liked nor disliked and 1 = disliked extremely) suggested by various researchers (14).

Preparation of probiotic yogurts with various levels of whey and inulin

After sensory evaluation, probiotic bacteria counting and serum separation measurement of the yogurt samples with various levels of aloe vera gel, a probiotic yogurt with 10% of aloe vera gel was selected. Then, the yogurt sample was respectively mixed with two powder samples of whey (5, 10 and 15%) and inulin (0.5, 1 and 1.5%). The nine various concentrations of whey and inulin in probiotic yogurts were fully homogenized and stored at 4 °C. Then, acidity, pH, rheological properties, serum separation, probiotics count and sensory evaluation of the prepared probiotic yogurt samples were assessed within four weeks (28 days) of cold storage.

Assessments of acidity and pH

Based on the titration method No. 920.153 by the Association of Official Analytical Chemists (AOAC) (2000), titratable acidity of each yogurt sample (based on lactic acid) was assessed using 0.1 M sodium hydroxide. Then, pH of each sample was assessed using digital pH-meter (WTW, Wielheim, Germany), calibrated with buffer solutions at two pH values of 4 and 7 (15).

Assessments of rheological properties

Rheological properties of various probiotic yogurt samples were assessed after seven days of cold storage using ultra-rotational rheometer (Brookfield LV-DVIII, Brookfield Engineering Laboratories, Stoughton, MA, USA). Each sample was set at room temperature for a few hours before the rheological assessments until the sample temperature reached to 10 °C ±0.2. A rheometer with two cocylinders was sealed using cap system to prevent sample evaporation during the assessment. Shear rate of 0.01–1000 s^{-1} with time intervals of 3 s was used to assess shear stress, viscosity (as a function of shear rate) and rheological parameters of each yogurt sample. Since the shear rate applied to the oral cavity was nearly 50 s^{-1}, viscosity achieved from the rising curve at the shear rate of 47.6 S^{-1} was reported as the apparent viscosity of each sample (16).

Viability of probiotic bacteria

Number of survived-probiotic bacteria (*L. acidophilus* and *B. animalis*) in each sample was calculated exactly after preparation and every seven days for four weeks of storage at 4 °C. Then, 10 ml of the yogurt sample were mixed with 90 ml of physiological serum and diluting process was continued to 10^{-9}. Then, 1 ml of this concentration was cultured in two various MRS media, one with a mixture of cysteine hydrochloride and mupirocin (appropriate for the growth of *B. animalis*) and the other one with a combination of salisin with 0.15% of bile salts (used for the growth of *L. acidophilus*). Plates of *B. animalis* were wrapped with parafilm and stored in anaerobic jars at room temperature. Furthermore, plates of *L. acidophilus* were incubated under anaerobic conditions at 37 °C for 72 h (17). Then, CFU/ml of the incubated *L. acidophilus* and *B. animalis* was calculated for each yogurt sample.

Statistical analysis

Results were expressed as mean ±SD (standard deviation) and submitted to analysis of variance (ANOVA) and LSD test at $p < 0.05$. All analyses were carried out using SPSS Software v.18 (IBM Analytics, USA).

Results

Effects of aloe vera gel on survival of probiotic bacteria in yogurts

Based on the results from Table 1, number of living cells of probiotic bacteria in all samples decreased significantly over time. In general, 10% concentration of the aloe vera gel showed the highest survival rate of probiotic bacteria at all storage times. By increasing concentration of aloe vera gel to more than 10%, survival rate of the probiotic bacteria decreased (Table 1).

Table 1. Effects of aloe vera gel on bacterial viability (CFU/ml × 10^6) of the probiotic yogurts during storage at 4 °C

AG (%)	Culture	1 day of storage	7 day of storage	14 day of storage	21 day of storage	28 day of storage
0	*Lactobacillus acidophilus*	17000 ± 3000^d	46 ± 7^h	$1^j\leq$	$1^h\leq$	$1^d\leq$
0	*Bifidiobacterium animalis*	56000 ± 1000^a	340 ± 30^f	6.2 ± 0.4^g	$1^h\leq$	$\leq1^d$
5	*Lactobacillus acidophilus*	16000 ± 1000^d	3600 ± 100^d	47 ± 5^d	7.3 ± 0.4^d	3.8 ± 0.1^b
5	*Bifidiobacterium animalis*	31000 ± 6000^c	5800 ± 700^c	4200 ± 600^b	14 ± 4^c	2.7 ± 0.4^c
10	*Lactobacillus acidophilus*	17000 ± 9000^d	46000 ± 3000^a	1600 ± 200^c	310 ± 70^a	13 ± 3^a
10	*Bifidiobacterium animalis*	46000 ± 6000^b	36000 ± 3000^b	28000 ± 1000^a	63 ± 1^b	11 ± 2^a
15	*Lactobacillus acidophilus*	15000 ± 2000^d	21 ± 4^i	17 ± 9^f	3.1 ± 0.2^f	$\leq1^d$
15	*Bifidiobacterium animalis*	51000 ± 6000^a	580 ± 10^e	34 ± 3^e	5.3 ± 0.4^e	$\leq1^d$
20	*Lactobacillus acidophilus*	16000 ± 2000^d	21 ± 40^i	2.7 ± 0.9^h	۱$^h\leq$	$1^d\leq$
20	*Bifidiobacterium animalis*	54000 ± 6000^a	58 ± 1^g	1.4 ± 0.3^i	1.1 ± 0.4^g	$1^d\leq$

[1]The numbers with the different subscriptions in each column are significant (P<0.05)

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Effects of aloe vera gel on serum separation and sensory evaluation of probiotic yogurts

By increasing concentration of the aloe vera gel in probiotic yogurts, rate of the serum separation decreased considerably during storage (Table 2). Since no similar studies were available to compare the current results with them, other gel stabilizers such as pectin, gelatin and Arabic gum were used for the comparison.

Table 3 shows that the increasing levels of aloe vera gel in probiotic yogurts included no negative effects on sensory attributes (apparent color, aroma and flavor) of the probiotic yogurts, compared to the control samples. However, they significantly ($p < 0.05$) enhanced texture scores (uniformity and hardness) of the yogurts and consequently their overall acceptability. However, increasing aloe vera gel to more than 10% produced a gelatinous sticky texture in probiotic yogurts, which was not pleasant and hence decreased their overall acceptance. Therefore, probiotic yogurts with 10% of aloe vera gel were selected as the bases for improvement by mixing with various levels of inulin or whey powder.

Effects of inulin and whey powder on survival of probiotic bacteria in yogurts with 10% of aloe vera gel

Table 4 shows numbers of the survived probiotic bacteria in various treatments of the probiotic yogurts during storage. While the highest living cell counts were achieved in all yogurt samples on Day 1 (immediately after fermentation and preparation), the counts decreased significantly during storage. By increasing inulin levels up to 1.5% in probiotic yogurts with 10% of aloe vera gel, number of the probiotic bacteria increased three times (200%), compared to the control samples up to 28 days of storage (Table 4).

Table 2. Effects of aloe vera gel on serum separation (%) of the probiotic yogurts during storage at 4 °C

AG (w/w %)	1 Day	7 Day	14 Day	21 Day	28 Day
0	0^{g**}	37^{b}	39.6^{ab}	41.4^{ab}	42.4^{a}
5	0^{g}	26.2^{d}	32.3^{c}	36.7^{b}	39.0^{ab}
10	0^{g}	19^{e}	25.6^{d}	27.7^{d}	32.1^{c}
15	0^{g}	12^{fg}	12.3^{fg}	14.7^{f}	15.2^{f}
20	0^{g}	9.66^{g}	10.1^{g}	10.7^{g}	13.4^{f}

**Numbers with different superscripts (a, b and c) in each column are significantly different (p <0.05) from each other

Table 3. Effects of aloe vera gel on sensory properties of the probiotic yogurts after one week of storage at 4 °C

AG (% w/w)	Color	Aroma	Flavor	Texture	Total Acceptability
0	$7.58±2.32^{a}$	$8.22±1.84^{a}$	$8.56±1.84^{a}$	$6.56±2.01^{b}$	$8.83±2.34^{a}$*
5	$7.56±2.32^{a}$	$8.21±1.84^{a}$	$8.88±2.32^{a}$	$5.89±2.04^{c}$	$8.78±2.33^{a}$
10	$7.54±2.32^{a}$	$8.56±1.92^{a}$	$8.78±0.87^{a}$	$7.78±2.11^{a}$	$8.89±2.33^{a}$
15	$7.78±1.42^{a}$	$8.44±1.92^{a}$	$8.56±2.32^{a}$	$5.87±2.01^{c}$	$7.89±2.24^{b}$
20	$7.76±1.42^{a}$	$8.22±1.84^{a}$	$6.45±1.40^{b}$	$5.00±2.02^{d}$	$7.22±2.11^{c}$

[1]The numbers with the different subscriptions in each column are significant (P<0.05) .

Table 4. Effects of inulin and whey powder on viability of the probiotic bacteria (CFU/ml) in probiotic yogurts during storage at 4 °C

Treatments	1Day	7Day	14Day	21Day	28Day
PY10%AG	$1.7±0.3×10^{10d}$	$3.1±0.2×10^{8c}$	$7.3±0.1×10^{6d}$	$10^{6e}<$	$10^{6c}<$
PY10%AG + 0.5% Inulin	$1.9±0.1×10^{10d}$	$1.1±0.5×10^{9b}$	$5.6±0.3×10^{6e}$	$1.4±0.1×10^{6d}$	$10^{6c}<$
PY10%AG + 1.0% Inulin	$3.2±0.3×10^{10b}$	$8.2±0.1×10^{9a}$	$5.2±0.4×10^{7b}$	$4.4±0.1×10^{6b}$	$1.3±0.1×10^{6b}$
PY10%AG + 1.5% Inulin	$4.4±0.2×10^{10a}$	$7.7±0.2×10^{9a}$	$4.2±0.4×10^{7c}$	$6.3±0.3×10^{6a}$	$3.1±0.4×10^{6a}$
PY10%AG + 5% Whey	$1.9±0.1×10^{10d}$	$2.1±0.4×10^{8d}$	$3.1±0.2×10^{7d}$	$10^{6e}<$	$10^{6c}<$
PY10%AG + 10% Whey	$1.1±0.2×10^{10d}$	$3.4±0.4×10^{8c}$	$5.3±0.4×10^{7b}$	$2.1±0.1×10^{6c}$	$1.1±0.1×10^{6b}$
PY10%AG + 15% Whey	$2.6±0.3×10^{10c}$	$3.9±0.2×10^{8c}$	$6.1±0.2×10^{7a}$	$2.5±0.4×10^{6c}$	$1.4±0.2×10^{6b}$

*Numbers with different superscripts (a, b and c) in each column are significantly different (p <0.01) from each other.

Although mixing aloe vera probiotic yogurts with whey powder (up to 15%) improved number of the survived probiotic bacteria up to 40% (compared to the control samples) after 28 days of storage, however, they showed lower survival than samples containing inulin (Table 4).

Effects of inulin and whey powder on pH and acidity of probiotic yogurt with 10% of aloe vera gel

Results showed that pH of the samples decreased with increasing the storage time. At Day 28 of storage, the lowest pH and the highest acidity belonged to the treatments with various concentrations of whey. The pH and acidity of inulin-containing samples did not show significant differences, compared to the control samples (Tables 5 and 6).

Effects of inulin and whey powder on rheological properties of probiotic yogurt with 10% of aloe vera gel

Figures 1 and 2 show the effects of shear rate on apparent viscosity and shear stress of the probiotic yogurts with 10% of aloe vera gel mixed with various levels of inulin and whey powder. Results clearly showed that all probiotic yogurt samples well followed the shear-diluting potential (quasi-plastic law), clearly describing relationships between the shear rate and apparent viscosity. When the shear rate of probiotic yogurts mixed with 10% of aloe vera gel and various levels (0.5, 1.0 and1.5 %) of inulin and whey powder (5, 10 and 15 %) increased from 0.01 to 100 (s^{-1}), the aparent viscosity decreased (figure 1). Table 7 shows that probiotic yogurts with 10% of aloe vera gel and 1.5% of inulin (PY10%AG1.5%IN) had the highest viscosity (~20 cp) and the lowest flow index (0.54), compared to those the other probiotic yogurt samples.

Table 5. Effects of inulin and whey powder on pH of the probiotic yogurts during storage at 4 ^{o}C

Treatments	1Day	7Day	14Day	21Day	28Day
PY10%AG	4.3^{a}	4.12^{ab}	4.01^{ab}	3.95^{b}	3.93^{b}
PY10%AG + 0.5% Inulin	4.35^{a}	4.14^{ab}	4.02^{ab}	3.89^{b}	3.91^{b}
PY10%AG + 1.0% Inulin	4.34^{a}	4.15^{ab}	4.1^{ab}	3.88^{b}	3.95^{b}
PY10%AG + 1.5% Inulin	4. 4^{a}	4. 1^{ab}	4.13^{ab}	4.0^{ab}	3.89^{b}
PY10%AG + 5% Whey	4.42^{a}	4.04^{ab}	3.89^{b}	3.78^{b}	3.69^{c}
PY10%AG + 10% Whey	4. 4^{a}	4.00^{ab}	3.77^{bc}	3.78^{bc}	3.64^{c}
PY10%AG + 15% Whey	4.44^{a}	4.00^{ab}	3.69^{c}	3.60^{c}	3.55^{c}

*Numbers with different superscripts in each column aare significantly different (p <0.01) from each other.

Table 6. Effects of inulin and whey powder on acidity of the probiotic yogurts during storage at 4 ^{o}C

Treatments	1Day	7Day	14Day	21Day	28Day
PY10%AG	0.78^{bc}	0.78^{bc}	0.81^{b}	0.81^{b}	0.84^{b}
PY10%AG + 0.5% Inulin	0.77^{bc}	0.79^{bc}	0.80^{b}	0.81^{b}	0.85^{b}
PY10%AG + 1.0% Inulin	0.76^{bc}	0.77^{bc}	0.82^{b}	0.83^{b}	0.83^{b}
PY10%AG + 1.5% Inulin	0.78^{bc}	0.79^{bc}	0.83^{b}	0.83^{b}	0.84^{b}
PY10%AG + 5% Whey	0.76^{bc}	0.79^{bc}	0.81^{b}	0.84^{b}	0.89^{ab}
PY10%AG + 10% Whey	0.83^{b}	0.99^{ab}	1.09^{a}	1.22^{a}	1.16^{a}
PY10%AG + 15% Whey	0.84^{b}	1.10^{ab}	1.19^{a}	1.27^{a}	1.36^{a}

*Numbers with different superscripts in each column are significantly different (p <0.01) from each other.

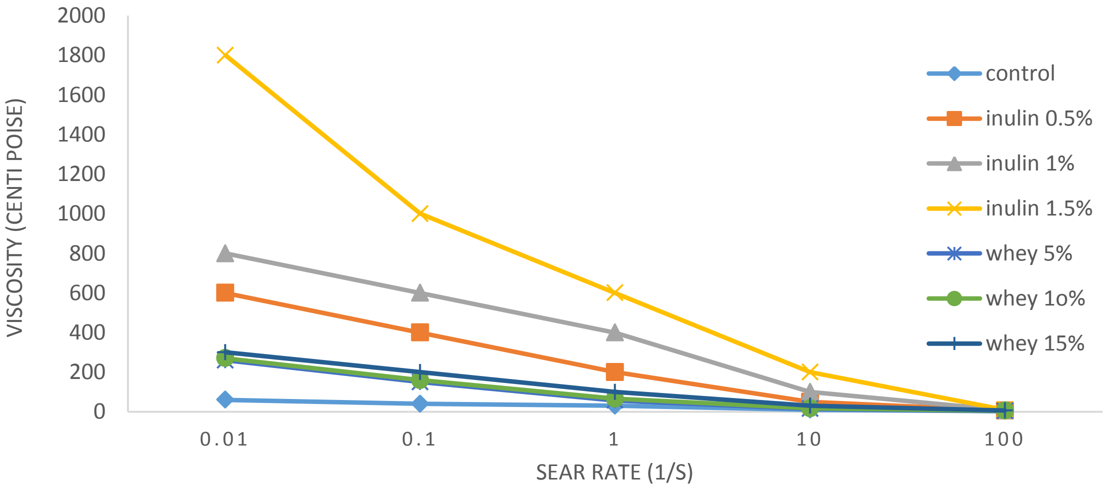

Figure 1. Effects of shear rate (s^{-1}) on apparent viscosity of probiotic yogurt with inulin and whey powder at the first week of storage at 4 ^{o}C

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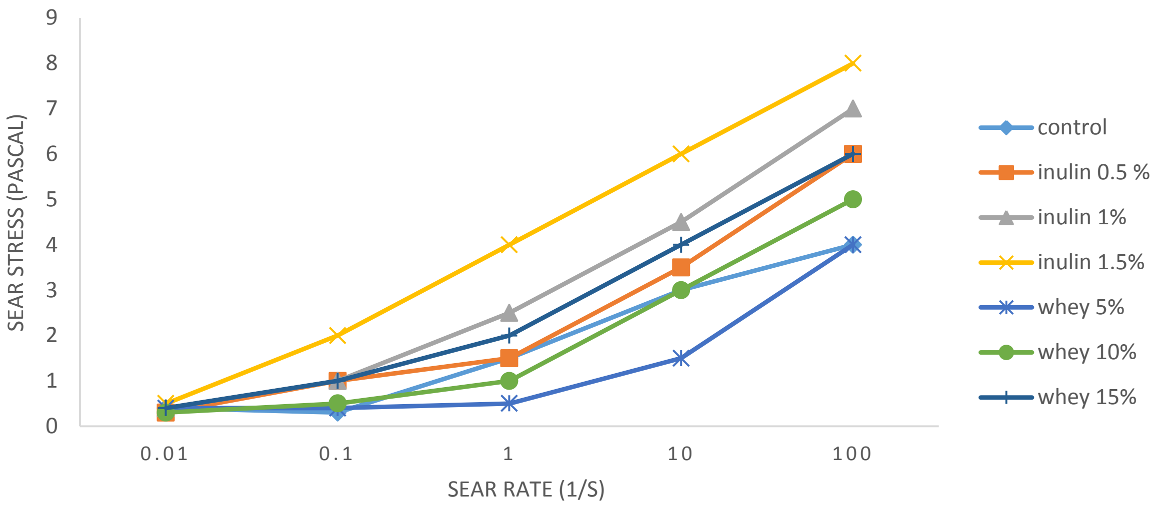

Figure 2. Effects of shear rate (s^{-1}) on shear stress of probiotic yogurt with inulin and whey powder at the first week of storage at 4 °C

Table 7. Effects of inulin and whey powder on rheological properties of the probiotic yogurts after one week storage at 4 °C

Treatment	Viscosity(cp)	Index of flow(n)	Index of consistency(k)	R^2
PY10%AG	11.09^{cd}	0.73	86.80	0.98
PY10%AG + 0.5% Inulin	13.3^{c}	0.71	87.30	0.99
PY10%AG + 1.0% Inulin	16.14^{b}	0.54	88.53	0.94
PY10%AG + 1.5% Inulin	19.51^{a}	0.54	95.49	0.94
PY10%AG + 5% Whey	7.38^{e}	0.86	98.12	0.98
PY10%AG + 10% Whey	10.2^{d}	0.86	100.85	0.98
PY10%AG + 15% Whey	10.31^{d}	0.81	118.84	0.99

*Numbers with different superscripts in each column are significantly different (p <0.01) from each other.

Effects of inulin and whey powder on serum separation of probiotic yogurt with 10% of aloe vera gel

Biphasication is a major problem in acidic dairy products, including deposition of milk proteins during storage. Although adding 10% of aloe vera gel significantly decreased serum separation of the probiotic yogurts (p<0.05) (Table 2), adding up to 1.5% of inulin to probiotic yogurts decreased serum separation up to 5% after 28 days of cold storage (Table 8). Table 8 shows that adding 1.5% inulin to PY10%AG included a stronger potential to preserve the yogurt texture (with much less serum separation), compared to yogurts prepared with whey powder due to viscosity enhancement by inulin.

Effects of inulin and whey powder on sensory properties of probiotic yogurt with 10% of aloe vera gel

Results of sensory evaluation showed that the treatments did not include significant effects on aroma, color and overall acceptance of the yogurt samples. The highest and the lowest taste and texture (hardness) scores belonged to PY10%AG1.5%IN and control, respectively (Table 9).

Table 8. Effects of inulin and whey powder on serum separation (%) of the probiotic yogurts during storage at 4 °C

Treatment	1Day	7Day	14Day	21Day	28Day
PY10%AG	0^{k}	31^{c}	35.2^{ab}	35.5^{ab}	38.4^{a}
PY10%AG + 0.5% Inulin	0^{k}	7.5^{i}	8.1^{i}	8.6^{i}	8.8^{i}
PY10%AG + 1.0% Inulin	0^{k}	6.7^{ij}	7^{b}	7.3^{j}	7.9^{i}
PY10%AG + 1.5% Inulin	0^{k}	0^{k}	0^{k}	0^{k}	4.2^{j}
PY10%AG + 5% Whey	0^{k}	22.3^{e}	26.5^{d}	27.6^{d}	28.9^{d}
PY10%AG + 10% Whey	0^{k}	17.6^{f}	19.5^{f}	24.7^{e}	25.9^{e}
PY10%AG + 15% Whey	0^{k}	12.8^{g}	14.2^{fg}	15.7^{f}	18.36^{f}

*Numbers with different superscripts in each column are significantly different (p <0.01) from each other.

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Table 9. Effects of inulin and whey powder on sensory properties of the probiotic yogurts during storage at 4 °C

Treatments	Flavor	Texture	Aroma	Color	Total Acceptability
PY10%AG	5.86±0.87^{c}	6.56 ±2.01^{b}	8.12 ±1.82^{a}	7.52 ±2.33^{a}	8.62 ±0.78^{b}
PY10%AG + 0.5% Inulin	8.42±0.87^{ab}	7.11 ±2.11^{ab}	8.11 ±1.82^{a}	7.56 ±2.32^{a}	8.65 ±0.78^{b}
PY10%AG + 1.0% Inulin	8.43±0.85^{ab}	7.12 ±2.11^{ab}	8.44 ±1.82^{a}	7.54 ±2.32^{a}	8.79 ±2.33^{ab}
PY10%AG + 1.5% Inulin	8.62±0.85^{a}	7.81 ±2.11^{a}	8.34 ±1.84^{a}	7.78 ±2.32^{a}	8.89 ±2.33^{ab}
PY10%AG + 5% Whey	8.41±0.87^{ab}	6.85 ±2.01^{b}	8.21 ±1.84^{a}	7.76 ±2.32^{a}	8.62 ±0.89^{b}
PY10%AG + 10% Whey	8.46±0.89^{ab}	7.22 ±2.02^{ab}	8.32 ±1.84^{a}	7.12 ±1.42^{ab}	8.56 ±0.89^{b}
PY10%AG + 15% Whey	8.47±0.89^{ab}	7.14 ±2.02^{ab}	8.41±1.82^{a}	7.14 ±1.42^{ab}	8.65 ±0.89^{b}

*Numbers with different superscripts in each column are significantly different (p <0.01) from each other.

Discussion

By increasing concentration of the aloe vera gel to more than 10%, survival rate of the probiotic bacteria decreased. Although aloe vera gel with nutrients such as vitamins, minerals, amino acids and natural sugars provides good conditions for the growth of *L. acidophilus* and *B. animalis* in probiotic yogurts, high concentrations of aloe vera gel may inhibit the bacterial growth because of the gel antimicrobial and anti-inflammatory agents (18). The antimicrobial (majorly dihydroxy anthraquinone) and anti-fungi compounds (saponin, salicylic acid and aloin) of the aloe vera gel (especially at high concentrations) may prevent growth of the bacteria and eventually lead to death of the microorganisms (Gram-positive and Gram-negative bacteria) as well as molds and yeasts (19, 20, 21, 22). Lawrence et al. (2009) studied antimicrobial activity of aloe vera gel against four Gram-positive and Gram-negative bacteria (23). Compounds with maximum antibacterial activity isolated from aloe vera gel extracts included as *p*–coumaric acid, ascorbic acid, pyrocatechol and cinnamic acid (24). These include reasons for high decreases in *L. acidophilus* and *B. animalis* during seven days of storage when the aloe vera gel concentration in probiotic yogurts increased to 15 and 20% possibly due to the high concentrations of antimicrobial compounds. These gel stabilizers cover casein molecules and prevent their agglomeration, sedimentation and consequent separation from the serum phase in dairy products, resulting in a homogenous phase in yogurts (25, 26). Serum separation is due to the increases in yogurt acidification as well as summation of the liquid phase, decreases of solid contents and emerging sediments. Dezyani et al (2017) reported that by increasing the concentration of aloe vera gel during storage, the serum secretion decreased (27).

In normal yogurt samples (with no probiotic cultures), biphasication enhanced due to the decreases in living cells and increases in lactic acid contents during storage. However, this study showed that the presence of *L. acidophilus* and *B. animalis* in probiotic yogurts controlled and retarded the acidification process during storage. Previous studies have verified that the probiotic bacteria include potentials to lower the acidification rate of yogurts during storage (28). By increasing the inulin level up to 1.5% in probiotic yogurts with 10% of aloe vera gel in the present study, the number of probiotic bacteria increased three times (200%), compared to the control samples even after 28 days of storage. Inulin with a concentration of 40 mg/g stimulated growth of *B. animalis* even in non-fat fermented milks and increased concentration of the probiotic bacteria (from 7.5 to 9.1 CFU/ml) during seven days of cold (4 °C) storage (2). Whey proteins included protective effects on survival of probiotic microorganisms during storage (10). Additionally, the whey concentrate (particularly unsalted) included positive effects on texture and syneresis of the yogurt samples. Dairy beverages processed with various concentrations of unheated whey (0, 20, 25, 50, 65, and 80) provide good nutritional media for the growth of probiotics bacteria because of using relatively natural casein (as a source of protein) instead of denatured casein in heated and condensed whey (29). However, yogurt samples containing 10% of aloe vera gel mixed with inulin provided better environments for the survival of probiotic bacteria, compared to that the yogurt samples mixed with whey powder did.

While yogurt acidification (in addition to fermentation) was an uncontrollable activity and occurred during storage even at 2–4 °C, inulin could retard pH reduction in probiotic yogurts much better than that the whey powder could. This was due to the fact that inulin controlled acidification activity of the yogurt starter bacteria and therefore titratable acidity of the low-fat probiotic samples did not change significantly (30). Nevertheless, whey powder was a good nutritional source for the probiotics (*L. acidophilus* and *B. animalis*). However, high concentrations of this compound increased acid production because the compound included high levels of lactose and water-soluble proteins such as α-lactalbumin and β-lactoglobulin (5). Apparent viscosity of the probiotic yogurts with 10% of aloe vera gel and inulin or whey powder decreased with increases in shear rate. This behavior was the reason for naming these types of viscoelastic fluids. The molecules were arranged irregularly and aligned partially at lower shear rates, resulting in a high viscosity. Increased shear rate led to breakage of irregular molecules and increased their alignment and consequently decreased apparent viscosity or internal friction for the molecules movement (31). In fact, the high viscosity of PY10%AG1.5%IN at low shearing rates (0.01 1/s) was due to the secondary gelled structure (in addition to protein network) formed by inulin in the yogurt samples (32, 33, 34). Although all the probiotic yogurt samples (containing inulin or whey powder) showed non-Newtonian behaviors with flow index (n < 1), the PY10%AG1.5%IN included the highest consistency within various samples (Fig. 1). However, at high shear rates, these particles were broken and consequently viscosity decreased. Although various researchers added inulin to plain (non-fat or low-fat) yogurts to increase viscosity (33, 35), these results showed that adding inulin to probiotic yogurts significantly enhanced their viscosity because inulin included a high hydrophilic property and absorbed (restrained) large volumes of water. Although the total solid content of milks included linear effects on apparent viscosity of the final yogurt products (36), water absorbing capacity of the whey powder was much less than that of inulin at similar conditions, most possibly because the powder protein was denatured and could not absorb the yogurt water. This was the reason that whey powder did not include potentials to increase the yogurt viscosity, compared to the control samples with no significant differences at $\alpha = 0.05$.

In this study, addition of up to 1.5% inulin decreased serum separation up to 5% after 28 days of cold storage. Microcrystals resulted from the inulin decomposition reacted with other components of the yogurt samples to enhance thier stability (9, 37). Inulin increases water holding capacity as well as consistency of the yogurt samples (30). Addition of up to 15% of whey powder to probiotic yogurts decreased up to 50% of the serum separation rate of the yogurts after 28 days of storage, compared to the control samples. This may be due to the formation of more complex serum proteins with casein, more salts and more hydrophilicity of serum proteins than casein (10). Results of sensory evaluation showed that the treatments did not include significant effects on aroma, color and overall acceptance of the yogurt samples. The highest and the lowest taste and texture scores belonged to PY10%AG1.5%IN and control, respectively. Researchers could achieve similar results when trained expert panels recognized creamy mouthfeel in normal yogurts mixed with 4% of inulin (34).

CONCLUSION

In conclusion, this study has shown that addition of aloe vera gel, inulin and whey powder prevents serum separation and improves viability of probiotic bacteria (majorly *L. acidophilus* and *B. animalis*) during cold storage (4 ^{o}C). Sensory evaluations by trained panelists have verified that combination of aloe vera gel with inulin improves texture and creamy mouthfeel significantly.

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