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Rheological Properties and Sensory Characteristics of Set-Type Soy Yogurt

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The study examined chemical composition and rheological and sensory properties of probiotic soy yogurt during 28 day storage at 4 °C. Soymilk supplemented with 2% (w/v) inulin or 1% (w/v) each of raffinose and glucose was used as a base for soy yogurt manufacture. Viability of probiotic organisms and their metabolic activity measured as production of organic acids and aldehyde content responsible for beany flavor, as well as rheological and sensory properties of soy yogurt, were examined. Inulin or raffinose/glucose supplementation in soymilk increased the bacterial population by one log cycle and the amount of lactic acid. Probiotic bacteria metabolized more aldehyde than yogurt culture and substantially reduced the beaniness in soy yogurt as determined by sensory evaluation. The probiotic soy yogurts showed more viscous and pseudoplastic properties than the control soy yogurts, but the sensory evaluation results showed preference for the control soy yogurts which were slightly less viscous. Control soy yogurt provided better mouth feel than probiotic soy yogurts.

KEYWORDS: Set-type soy yogurt; rheological properties; sensory properties; hexanal; pentanal

INTRODUCTION

Fermented soy products have been perceived as healthy food and considered an important part of the diet. Furthermore, the incorporation of probiotic bacteria as dietary adjuncts has given rise to increased consumption of probiotic products in Europe, the United States, and Asia (*1–3*). Soy-based foods may provide additional benefits for the consumer due to their hypolipidemic, anticholesterolemic, and antiatherogenic properties and reduced allergenicity (*4, 5*). Consequently, soymilk-based yogurt offers a considerable appeal to a growing segment of consumers with certain dietary and health concerns. Probiotic bacteria are defined as “live microorganisms which when administered in adequate amounts confer health benefits on the host” (*6*). They are normally marketed as nutraceuticals in forms of capsules and powders or added to yogurt, which is a popular vehicle for incorporation of probiotic microorganisms (*7*). The diverse range of “functional” food products currently available on the market reflects the convenience of using food as a delivery system for probiotic microorganisms (*8*). Much of the research concerning the health-promoting effect of soy products has focused on isoflavones. In addition to isoflavones, soy is an important source of many other nutrients including dietary fiber, oligosaccharides, proteins, trace minerals, and vitamins, which could influence the host’s well-being (*9*). An important physiological role can be attributed to soy oligosaccharides, which could meet the standards of a prebiotic. Prebiotics have been used to promote the growth and activity of beneficial microorganisms *in vitro* (*10*) and in the large intestine (*11*). A prebiotic is a nondigestible food ingredient that affects the host beneficially by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve the health of the host (*12*). Some lactic acid bacteria (LAB) have been reported to grow slowly or poorly in soymilk (*13*) and produce low levels of organic acids (*1*). Therefore, to improve the growth of probiotic bacteria and production of organic acid, soymilk needs to be supplemented with various prebiotics such as raffinose or inulin or a combination of glucose and raffinose (*14–16*).

Soymilk, the aqueous extract of soybean, originates from Asia and presents a nutritious beverage. Western populations generally dislike the flavor profile of traditional soymilk because of its aftertaste, often described as “beany” due to the presence of hexanal and pentanal (*17*). The formation of these aldehydes results mainly from the hydroperoxidation of polyunsaturated fatty acids catalyzed by lipoxygenase (*18*). Oriental methods of soymilk manufacture establish conditions for the oxidation to occur during the initial soaking and grinding of soybeans. Commercial methods implement steps that either prevent the formation of undesirable volatile compounds (inactivation of lipoxygenase by heating) or remove the residual off-flavors using deodorizing techniques (*1*). Lactic acid fermentation may be used as a means to reduce beany flavors and antinutritional factors such as phytic acid in soybean products. Furthermore,

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Table 1. Experimental Plan

soy yogurt	supplementation	culture
control	none	*L. delbrueckii* ssp. *bulgaricus* Lb1466 and *S. thermophilus* St1342
	2% inulin	*L. delbrueckii* ssp. *bulgaricus* Lb1466 and *S. thermophilus* St1342
	1% raffinose + 1% glucose	*L. delbrueckii* ssp. *bulgaricus* Lb1466 and *S. thermophilus* St1342
probiotic	none	*L. delbrueckii* ssp. *bulgaricus* Lb1466 and *S. thermophilus* St1342, *L. acidophilus*L10, *L. casei* L26, and *B. animalis* B94
	2% inulin	*L. delbrueckii* ssp. *bulgaricus* Lb1466 and *S. thermophilus* St1342, *L. acidophilus*L10, *L. casei* L26, and *B. animalis* B94
	1% raffinose + 1% glucose	*L. delbrueckii* ssp. *bulgaricus* Lb1466 and *S. thermophilus* St1342, *L. acidophilus*L10, *L. casei* L26, and *B. animalis* B94

in an attempt to reduce the aftertaste in soymilk and improve sensory attributes, sweeteners such as sucrose are added (*19, 20*).

The preparation of a soy yogurt-like product with *Lactobacillus* and *Bifidobacterium* appears to be an interesting approach to the development of new fermented soy products containing probiotic cultures (*21*). In addition, the texture and taste of soy yogurt are essential attributes for product acceptability by the consumers. Consequently, it is important that the probiotic culture contributes to good sensory properties of the final product (*22*). There are very few studies on the texture of soy yogurt containing probiotics. Supplementation with either hi-maize or inulin has been shown to affect firmness and viscosity of yogurt (*10*) as well as the growth of starter culture (*23*). However, not much information is available in the literature about the effect of supplementation of inulin, raffinose, or glucose on growth of microorganisms in soymilk and rheology and sensory attributes of soy yogurt. Rheological measurements have been widely used to characterize the structure of fermented milk/soymilk gels. The most frequently determined parameters of such testing are the elastic or storage modulus (G'), which is a measure of energy stored per oscillation cycle, the viscous or loss modulus (G''), which is a measure of the energy dissipated as heat per cycle, and the loss tangent, $\tan \delta = G''/G'$ (*3*).

Although some published studies have dealt with yogurt production from soymilk, not much data are available providing a complete assessment of the product including supplementation with carbohydrates and their effects on viability of probiotic organisms, removal of aldehyde and sensory attributes, and rheological properties of the product. Therefore, the aims of the present study were to examine organic acid production and aldehyde metabolism in soymilk by probiotic organisms to assess rheological and sensory properties of soy yogurt during cold storage for 21 days.

MATERIALS AND METHODS

Substrates and Chemicals. Sanitarium organic soymilk (simply soy; Sanitarium, NSW, Australia), fresh soybean drink (Fortune Beancurd Manufacturer P/L, Springvale, Victoria, Australia), and inulin (Raftilin-eST; ORAFTI, Oreye, SA, Australia) were provided by the respective companies. L-Cysteine hydrochloride, sorbitol, bacteriological agar, nalidixic acid, neomycin sulfate, lithium chloride, paromomycin sulfate (NNLP), L(+)-lactic acid, glacial acetic acid, propionic acid, butyric acid, hexanal, pentanal, and D(+)-raffinose were purchased from Sigma Chemical Co. (St. Louis, MO). Reinforced clostridia agar (RCA), glucose, yeast extract, and bacteriological peptone were purchased from Oxoid (West Heidleberg, Victoria, Australia) and sulfuric acid (H_2SO_4) and ethanol were from Merck (Merck KGaA, Darmstadt, Germany), while de Man, Rogosa, and Sharpe (MRS) agar was purchased from Amyl Media (Dandenong, Victoria, Australia).

Bacterial Strains and Culture Conditions. *Lactobacillus acidophilus* LAFTI L10 (L10), *Lactobacillus casei* LAFTI L26 (L26), and *Bifidobacterium animalis* ssp. *lactis* LAFTI B94 (B94) were provided by DSM Food Specialties (Moorebank, NSW, Australia). The selected probiotic microorganisms used in this study have been investigated extensively and are shown to possess several important probiotic properties (*24*). *Streptococcus thermophilus* St1342 and *Lactobacillus delbrueckii* ssp. *bulgaricus* Lb1466 were obtained from the Victoria University Culture Collection (Werribee, Australia). Each organism was stored at −80 °C prior to use. Sterile 10 mL portions of de Man, Rogosa, and Sharpe (MRS) broth (Sigma) were inoculated with 1% of each organism and incubated at 42 °C for *L. delbrueckii* ssp. *bulgaricus* Lb1466 and 37 °C for *S. thermophilus* St1342, *L. acidophilus* LAFTI L10, *B. animalis* ssp. *lactis* LAFTI B94, and *L. casei* LAFTI L26. For the propagation of *Bifidobacterium*, sterile MRS broth was supplemented with 0.05% L-cysteine hydrochloride to provide anaerobic conditions and stimulate their growth (*25*). The activated organisms after three successive transfers were used for the production of soy yogurt. The cultures were prepared by inoculating each organism in 10 mL aliquots of sterilized Sanitarium organic soymilk supplemented with 2% (w/v) glucose and 1% (w/v) yeast extract for the manufacturing of the fermented soymilk.

Preparation of Soy Yogurt Batches. Six batches of soy yogurt were prepared using the method described previously (*21*). Each batch was made with 2 L of commercial soymilk with 2% (w/v) inulin or 1% (w/v) each of raffinose and glucose or without any supplementation according to the experimental plan shown in **Table 1**. Three batches of soymilk were made with yogurt culture only whereas the other three batches were made with yogurt and probiotic cultures. All experiments were conducted under aseptic conditions in a laminar flow. Inoculated soymilk batches were then poured into 70 mL sterile clear/transparent plastic cups with lids (30 mL per cup) and incubated at 42 °C until the pH of 4.50 was reached. The products were removed from the incubator and stored at 4 °C for 28 days. Viability of bacteria, expressed as log colony forming units per milliliter (cfu/mL), and metabolic activity measured as production of organic acid and aldehyde content in product were determined 12 h postfermentation and subsequently at weekly intervals. Sensory evaluation of the products was assessed at day 1 and day 21 whereas rheological properties of soy yogurts were determined at day 10 of storage at 4 °C.

Determination of Viability of Probiotics during Cold Storage. The colony counts of *S. thermophilus* St1342, *L. delbrueckii* ssp. *bulgaricus* Lb1466, *L. acidophilus* L10, *L. casei* L26, and *B. animalis* ssp. *lactis* B94 were determined just after inoculation, at the end of fermentation (final pH 4.5), and during storage at 4 °C as described previously using the pour plate technique (*21*). M17 agar was used for the selective enumeration of *S. thermophilus* St1342 incubated at 37 °C aerobically for 72 h and reinforced clostridia agar (RCA), pH 5.3 (oxoid), for *L. delbrueckii* ssp. *bulgaricus* Lb1466 (*26*). *L. acidophilus* L10 was enumerated in MRS–sorbitol agar (*26*) whereas MRS–nalidixic acid, neomycin sulfate, lithium chloride, and paramomycin sulfate (NNLP) agar was used for enumeration of *B. animalis* ssp. *lactis* B94 (*27*). The viable counts of *L. casei* L26 were enumerated using *L. casei* agar (*28*) incubated at 37 °C anaerobically for 72 h.

Measurement of pH. The pH of samples was determined using a pH meter (model 8417; HANNA Instruments, Singapore). The pH of all batches of soy yogurt was recorded at the end of fermentation, 12 h postfermentation, and subsequently at weekly intervals. The measurements were performed in triplicate for each soy yogurt sample.

Quantification of Organic Acids. The determination of lactic acid, acetic acid, propionic acid, and butyric acid was performed according to the method of Donkor et al. (*10*) with some modifications. A 1.5 g aliquot of soy yogurt was mixed with 40 μL of 15.5 M nitric acid and then diluted with 0.5 mL of the mobile phase of 0.001 M H_2SO_4. The resulting mixture was centrifuged for 30 min at 14000*g* using the Eppendorf 5415C centrifuge (Crown Scientific, Melbourne, Australia) for removal of protein. The supernatant was filtered through a 0.45 μm membrane filter (Schleicher & Schuell GmbH, Dassel, Germany) into a high-performance liquid chromatography (HPLC) vial for the analysis of organic acids. The separation of organic acids was achieved using a Varian HPLC (Varian Analytical Instruments, Walnut Creek, CA) fitted with an Aminex HPX-87H, 300 × 7.8 mm ion-exchange column (Bio-Rad Life Science Group, Hercules, CA) and a guard column maintained at 65 °C. The flow rate was kept constant using 0.001 M H_2SO_4 as the mobile phase, at 0.6 mL/min. A UV/visible detector was used at 220 nm. A 20 μL injection volume was used for both samples and standards with the retention time for L(+)-lactic acid, glacial acetic acid, propionic acid, and butyric acid (Sigma) at 12.60, 14.25, 16.71, and 18.78 min, respectively. Lactic acid, acetic acid, propionic acid, and butyric acid were quantified as described previously (*21*) using standard curves obtained, and organic acid levels were calculated back to milligram per milliliter of soymilk.

Headspace (HS) Analysis of Aldehydes. The levels of 1-hexanal and pentanal were determined in soy yogurt and in two types of commercial soymilk using the method of Tsangalis and Shah (*16*) and Scalabrini et al. (*29*) with some modifications. Frozen (−20 °C) soy yogurt and soymilk samples were thawed, and 2 g of sodium chloride was added to 5 mL of each sample in an air-tight headspace (HS) bottle. The samples were heated at 40 °C for 45 min. Analysis was performed using a gas chromatograph equipped with a flame ionization detector (FID) (Varian) and a high-resolution gas chromatography (GC) column, DB-1 (29 m × 0.32 mm fused-silica column coated with a 0.25 μm film) (J & W Scientific, Folsom, CA). The carrier gas was hydrogen (H_2) at 0.36 bar, and a flow rate of 2.5 mL/min was used. For the FID detector, H_2 and instrument air were maintained at flow rates of 40 and 450 mL/min, respectively. The column was maintained at 40 °C during transfer of the HS gases. After 2 min, the column was programmed to heat at the rate of 7 °C/min to 140 °C, held for 1 min, and then heated again at the rate of 10 °C/min to 230 °C. The HS gas injection volume (2 mL) was used for both samples and standards with retention times of 2.36 and 3.79 min for pentanal and hexanal, respectively.

Rheological Measurements. The rheological properties of soy yogurts were measured using a controlled-stress rheometer (Physica MCR 301; Anton Paar, GmbH, Germany), equipped with a temperature and moisture regulating hood and a cone and plate geometry (CP50-1, 50 mm diameter, 1° angle, and 0.02 mm gap). The temperature was regulated by a viscotherm VT 2 circulating bath and controlled with a Peltier system (Anton Paar) and kept constant at 5 °C with an accuracy of ±0.1 °C. The data of all rheological measurements were analyzed with the supporting software Rheoplus/32 v2.81 (Anton Paar).

Viscoelastic Properties and Flow Behavior. After gentle stirring for 10 s with a plastic spoon, a small sample of soy yogurt was deposited on the inset plate. After setting the module into a measuring position, the sample was presheared at a preselected shear rate of 500/s to avoid any bias due to structural memory and subsequently rested for 5 min to allow for a structural rebuilding. The newly formed structure would be governed by the system composition. The sample was then subjected to a small amplitude oscillatory measurement (SAOM). The magnitude of strain was verified prior to experiments by conducting an amplitude test to ensure that the test was performed in the linear viscoelastic domain. First, a frequency sweep test was performed using a frequency ramp from 0.1 to 100 Hz at a constant strain of 0.1% to ascertain viscoelastic properties. This test was followed by a shear rate sweep by increasing the shear rate from 0 to 100/s (upward curve), followed by decreasing the shear rate from 100 to 0/s (downward curve) to assess the flow behavior of the soy yogurt. The time required for the upward or downward shear rate ramp was set at 3 min (for a total time of 6 min).

Flow Behavior Model. The model used to fit the flow behavior data was that of Ostwald deWaele, also known as the Power law, and is represented by the equation:

$$\eta_a = \tau/\gamma = k\gamma^{n-1}$$

where η_a is the apparent viscosity (Pa s), τ is the shear stress (Pa), γ is the shear rate (s^{-1}), k is the consistency index (Pa s^n), and n is the flow behavior index. The larger the value of k, the thicker the product and therefore more viscous the fluid. In this model the parameter n constitutes a physical property that characterizes a non-Newtonian behavior, and when $n < 1$, the fluid is pseudoplastic (*30*).

Sensory Evaluation. The sensory properties of set-style probiotic and control soy yogurt batches were evaluated by an untrained panel of 30 assessors recruited from students and staff members of the School of Molecular Sciences, Victoria University, Werribee Campus. The ethics approval was obtained from the Human and Animal Ethics Committee of Victoria University. The products were evaluated at day 1 and day 21 of storage at 4 °C. The samples were served at 7–10 °C in plastic cups and were coded with three-digit numbers. Water was available for panel members to rinse their pallet between samples. Crackers were also supplied to aid in removing any carryovers between tasting (*20*). A test form comprising five sensory attributes, namely, appearance, mouth feel, beany flavor, acid intensity, and overall acceptance, was given to each assessor. A structured nine-point hedonic scale ranging from “like extremely”, “extremely intense”, or “high” rating nine points to “dislike extremely”, “absent”, or “low” rating one point was used to numerically describe the sensory properties. Two sensory evaluation sessions were performed by the same group of panelists in order to assess the acceptability of the products compared to the controls as affected by supplementation with glucose or prebiotic and the presence of probiotic organism. The scores were analyzed statistically using a two-way ANOVA test and Tukey’s test for multicomparison of the means (*31*).

Statistical Analysis. The experiments were organized as a random, full factorial design exploring the influence of probiotic cultures, supplementation with prebiotics and glucose, and time as the main effects. All experiments were replicated three times and subsampled at least twice ($n = 6$). Results were analyzed as a repeated measure design using a general linear model procedure of the SAS system (*32*). Where appropriate, one- or two-way ANOVA and correlational analyses were employed using Microsoft Excel StatPro (*33*), and the multicomparison of means was assessed by Tukey’s test. The level of significance was preset at $p \leq 0.05$.

RESULTS AND DISCUSSION

Cell Growth. The viable population of each bacterium at the end of fermentation compared to its initial number is presented in **Table 2**. The increase in the number of *S. thermophilus* St1342, *L. delbrueckii* ssp. *bulgaricus* Lb1466, *L. acidophilus* L10, *L. casei* L26, and *B. animalis* B94 after fermentation and storage was defined as $\Delta N = \log N - \log N_0$, where ΔN is the increase in the number of bacteria, N_0 the number of bacteria just after inoculation of soymilk, and N the number of bacteria at the end of fermentation. A high number of the probiotic culture at the end of fermentation is one of the prerequisites to maintain high levels of the probiotic organism during storage (*7*). Hence, it was important to establish conditions that would promote growth of these microorganisms in soy yogurt. Therefore, soymilk was supplemented with 2% inulin or 1% each of raffinose and glucose to investigate the effect of these carbohydrates on viability of probiotic organisms, and results were compared with those in the soymilk without any supplementation. In an earlier study,*S. thermophilus* St1342, *L. delbrueckii* ssp. *bulgaricus* Lb1466, *L. acidophilus* L10, *L. casei* L26, and *B. animalis* B94 were shown to possess α-galactosidase activity in the presence of oligosaccharides (*34*). As a result, these microorganisms showed improved growth in soymilk during fermentation and storage at 4 °C. Previous studies have also shown that the supplementation of 2% inulin or 1% each of raffinose and glucose substantially stimulated growth of probiotic bacteria in milk during fermentation at 42

Table 2. Population of Bacteria in Soy Yogurt Batches before and after Fermentation and during Storage at 4 °C for 28 Days

treatment	microorganism	CFU/g of the product at storage period, days					
		0	1	7	14	21	28
C, soymilk	St1342	7.57^{Aa}	8.63^{Bb}	8.66^{Cb}	8.54^{Ab}	8.64^{Cb}	8.76^{Ab}
	Lb1466	7.51^{Aa}	8.68^{Bb}	8.63^{Cb}	8.58^{Ab}	8.67^{Cb}	8.83^{Ab}
C, soymilk + 2% inulin	St1342	7.51^{Aa}	8.90^{Ab}	8.67^{Cb}	8.64^{Ab}	8.71^{Ab}	8.91^{Ab}
	Lb1466	7.51^{Aa}	8.76^{Ab}	8.74^{Cb}	8.60^{Ab}	8.73^{Ab}	8.82^{Ab}
C, soymilk + 1% raffinose + 1% glucose	St1342	7.54^{Aa}	9.15^{Cb}	9.09^{Ab}	9.20^{Bb}	9.18^{ABb}	9.29^{Bbc}
	Lb1466	7.52^{Aa}	9.14^{Cb}	9.06^{Ab}	9.16^{Bb}	9.15^{ABb}	9.28^{Bbc}
P, soymilk	St1342	7.45^{Aa}	8.86^{Ab}	8.93^{Ab}	8.82^{Ab}	8.72^{Ab}	9.09^{Ab}
	Lb1466	7.43^{Aa}	8.81^{Ab}	8.92^{Ab}	8.66^{Ab}	8.92^{Ab}	8.91^{Ab}
	L10	7.54^{Aa}	8.93^{Ab}	8.94^{Ab}	8.82^{Ab}	9.00^{Ab}	9.04^{Ab}
	L26	7.53^{Aa}	8.89^{Ab}	8.93^{Ab}	8.83^{Ab}	8.96^{Ab}	9.06^{Ab}
	B94	7.18^{Aa}	8.78^{Ab}	8.98^{Ab}	8.86^{Ab}	9.09^{Abc}	8.99^{Ab}
P, soymilk + 2% inulin	St1342	7.52^{Aa}	9.08^{Ab}	9.21^{Bb}	8.87^{Ab}	8.83^{Abc}	9.10^{Ab}
	Lb1466	7.42^{Aa}	8.98^{Ab}	9.25^{Bbc}	8.76^{Abd}	9.06^{Ab}	8.92^{Abd}
	L10	7.55^{Aa}	9.06^{Ab}	9.18^{Bb}	8.86^{Abd}	9.12^{ABb}	9.06^{Ab}
	L26	7.47^{Aa}	9.04^{Ab}	9.24^{Bbc}	8.90^{Abc}	9.04^{Ab}	9.04^{Ab}
	B94	7.25^{Aa}	8.93^{Ab}	9.24^{Bbc}	8.83^{Ab}	9.12^{ABbce}	9.03^{Abc}
P, soymilk + 1% raffinose + 1% glucose	St1342	7.48^{Aa}	9.06^{Ab}	9.04^{Ab}	8.83^{Abd}	8.97^{Abcd}	9.13^{Abc}
	Lb1466	7.39^{Aa}	9.02^{Ab}	9.07^{Ab}	8.66^{Abc}	9.14^{ABb}	8.99^{Ab}
	L10	7.45^{Aa}	9.07^{Ab}	8.99^{Ab}	8.80^{Ab}	9.04^{Ab}	9.11^{Ab}
	L26	7.51^{Aa}	9.07^{Ab}	9.03^{Ab}	8.83^{Abc}	9.12^{ABb}	9.13^{Ab}
	B94	7.25^{Aa}	8.93^{Ab}	9.03^{Ab}	8.67^{Abc}	9.09^{Ab}	8.85^{Abc}
SEM				0.08			

^{abcde}Means in the same row with different small letter superscripts are significantly different; ^{ABC}Means in the same column with different capital letter superscripts are significantly different; C = control; P = probiotic; St1342 = *S. thermophilus*, Lb1466 = *L. delbrueckii* subsp. *bulgaricus*, L10 = *L. acidophilus*, B94 = *B. animalis* ssp. *lactis*, and L26 = *L. casei*.

°C (*10*) and in MRS incubated at 37 °C for 48 h (*34*). Despite the slow growth of the bacteria in soy yogurts, cell viability was maintained above 8 log cfu/g of product for all microorganisms throughout the storage. Similar results were reported by Donkor et al. (*21*) in soy yogurt supplemented with 1% α-lactose monohydrate.

pH Changes. The initial pH of soymilk was 7.1 and was slightly higher than previously reported (*35*), and the pH of soy yogurts at the end of fermentation was 4.50. The changes in pH during fermentation were found to vary with the starter cultures and substrate concentration (carbon source). Fermentation was monitored up to 15 h for unsupplemented soymilk, and since prolonged incubation is usually not desirable for commercial production in the yogurt industry, supplementation to improve fermentation time may be essential.

Changes in pH of soy yogurts before and after fermentation and during storage are presented in **Figure 1**. The control soy yogurt supplemented with 1% each of raffinose and glucose exhibited the highest pH decline during fermentation and storage. Similar observations were reported in MRS supplemented with a mixture of glucose and raffinose which stimulated higher ($p < 0.05$) production of lactic acid and thus lowered the pH of the medium (*34*). By the end of the storage period, postacidification caused significant ($p \leq 0.05$) pH decline during storage at 4 °C.

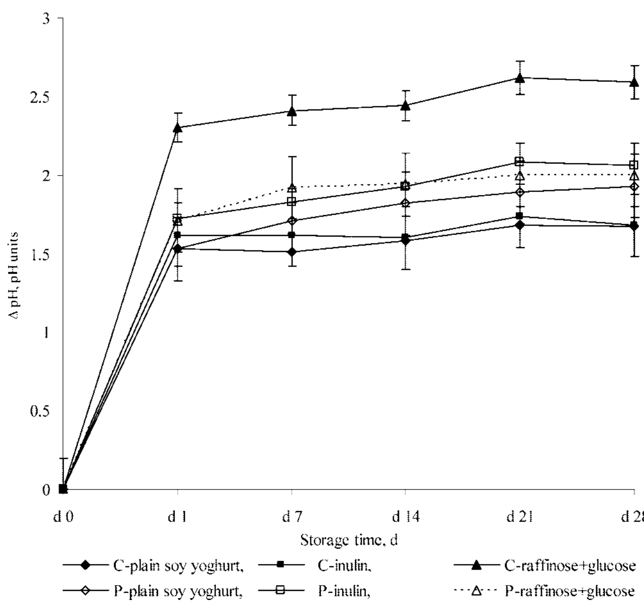

Figure 1. pH changes (ΔpH) during manufacture and storage of plain soy yogurt and soy yogurt supplemented with 2% inulin or 1% each of raffinose and glucose. C = control soy yogurt with only *S. thermophilus* St1342 and *L. belbrueckii* subsp. *bulgaricus* Lb1466; P = probiotic soy yogurt with *S. thermophilus* St1342, *L. belbrueckii* ssp. *bulgaricus* Lb1466, *L. acidophilus* L10, *B. animalis* ssp. *lactis* B94, and *L. casei* L26 (error bars present a pooled standard error of the mean).

Organic Acids. The mean concentrations (mg/mL) of organic acids in the control and probiotic soy yogurt during storage at 4 °C are shown in **Table 3**. Extent of acidification of soymilk depends on the strains of yogurt cultures and other associated cultures employed (*36*). It is evident that high concentration of organic acids compared to unfermented soymilk was a result of the added starter culture. Patel et al. (*37*) reported that better acid and flavor production was obtained in soymilk with a combination of *S. thermophilus* HST and *L. delbrueckii* ssp. *bulgaricus* LBW than that by *S. thermophilus* B 3641 and *L. delbrueckii* ssp. *bulgaricus* B 548. The concentration of lactic acid in all of the products was substantially higher than the concentration of acetic, propionic, or butyric acid after fermentation and during 28 day storage at 4 °C. However, despite the low production of acetic acid in soy yogurt, its concentration was considerably higher than the concentration of propionic or butyric acid in all of the products (**Table 3**). The results showed that the predominant organic acids produced during fermentation of soymilk were lactic and acetic acids. Similar lactic and acetic acid production in soy yogurt with probiotic microorganisms was reported in a previous study (*21*).

There were remarkable differences in lactic and acetic acid contents of the control and probiotic yogurts (**Table 3**).

Table 3. Organic Acid Contents of Soy Yogurt Made with or without Probiotics during Storage at 4 °C

treatment	storage time, days	organic acids, mg/mL			
		lactic	acetic	propionic	butyric
C, soymilk	0	0.28[a]	0.06[a]	0.02[a]	0.03[a]
	1	3.65[b]	1.19[b]	0.05[ab]	0.45[b]
	7	3.97[b]	1.37[b]	0.06[b]	0.49[b]
	14	3.94[b]	1.12[bd]	0.07[b]	0.56[b]
	21	4.02[c]	1.49[c]	0.07[b]	0.47[b]
	28	5.84[d]	1.47[c]	0.10[c]	0.82[bc]
C, soymilk + 2% inulin	0	0.27[a]	0.06[a]	0.04[a]	0.06[a]
	1	3.95[b]	0.99[b]	0.05[a]	0.58[b]
	7	4.24[b]	1.08[b]	0.06[a]	0.66[b]
	14	3.01[b]	1.31[c]	0.06[a]	0.66[b]
	21	4.09[b]	1.30[c]	0.06[a]	0.66[b]
	28	4.19[b]	1.29[c]	0.06[a]	0.71[b]
C, soymilk + 1% raffinose + 1% glucose	0	0.26[a]	0.10[a]	0.04[a]	0.06[a]
	1	6.20[b]	0.28[a]	0.06[ab]	0.06[a]
	7	7.49[b]	0.28[a]	0.07[ab]	0.08[a]
	14	8.27[c]	0.33[b]	0.08[ab]	0.09[a]
	21	9.39[d]	0.40[b]	0.10[b]	0.08[a]
	28	10.03[d]	0.35[b]	0.10[b]	0.08[a]
P, soymilk	0	1.48[a]	0.11[a]	0.09[a]	0.09[a]
	1	6.60[c]	0.93[b]	0.09[a]	0.52[b]
	7	8.70[c]	0.97[b]	0.10[a]	0.58[b]
	14	9.09[c]	1.01[b]	0.11[a]	0.59[b]
	21	9.47[c]	1.11[bc]	0.11[a]	0.64[c]
	28	11.25[d]	1.23[c]	0.12[a]	0.58[b]
P, soymilk + 2% inulin	0	1.37[a]	0.09[a]	0.09[a]	0.12[a]
	1	7.27[b]	0.58[b]	0.10[a]	0.33[b]
	7	8.70[c]	0.55[b]	0.10[a]	0.40[b]
	14	10.36[d]	0.76[c]	0.12[a]	0.48[b]
	21	10.97[d]	0.77[c]	0.13[a]	0.57[b]
	28	11.62[d]	0.69[b]	0.13[a]	0.59[bc]
P, soymilk + 1% raffinose + 1% glucose	0	1.50[a]	0.10[a]	0.08[a]	0.12[a]
	1	7.29[b]	0.21[a]	0.08[a]	0.31[b]
	7	8.92[c]	0.23[a]	0.11[a]	0.41[b]
	14	10.20[c]	0.25[a]	0.11[a]	0.48[b]
	21	10.41[d]	0.34[b]	0.11[a]	0.56[b]
	28	11.28[d]	0.23[a]	0.12[a]	0.59[bc]
SEM		0.49	0.07	0.01	0.09

[abcd]Means in the same column per treatment with different small letter superscripts are significantly different; P = probiotic; C = control.

Supplementation with 2% inulin and 1% each of raffinose and glucose influenced the production of lactic acid in both the control and probiotic soy yogurts as the concentration values were substantially higher than those with no supplementation (**Table 3**). Favaro Trindade et al. (*20*) also showed the enhancement in the acidification rate by starter cultures grown in soymilk fortified with 2% sucrose. Consequently acid-induced soymilk gel formation occurred faster in the soymilk supplemented with raffinose/glucose or inulin, reaching a pH of 4.5 in 5.30 and 7.30 h, respectively, compared to the 10 h fermentation time for the soymilk without any supplementation. A similar pattern was observed for the control samples with fermentation times of 7.30 and 12 h for raffinose/glucose and inulin, respectively, as compared to 15 h for control soy yogurt without any supplementation. Favaro Trindade et al. (*20*) suggested an incubation time of 6 h for the manufacture of soy yogurt for the best sensory quality. The production of butyric acid or propionic acid did not depend on supplementation and that all cultures appeared to be capable of producing butyric acid in soymilk. Similar results were reported by Donkor et al. (*10*) and Fernandez-Garcia et al. (*38*) in yogurts supplemented with prebiotics or fiber.

Pentanal and Hexanal Content in Soy Yogurt. The concentration of pentanal and hexanal before and after fermentation at day 1 and day 21 of storage at 4 °C is shown in **Figure 2**. The results also include the analysis of the levels of pentanal and hexanal in commercial soymilk (Sanitarium organic soymilk and fresh soybean drink). The commercial soymilk samples did not differ significantly ($p > 0.05$) in pentanal and hexanal concentrations, indicating that the content of these aldehydes were not reduced substantially during manufacturing practices (**Figure 2**). Results of our study also showed that soymilk contained substantially higher levels of hexanal than pentanal. Various studies have reported similar findings (*16*, *29*, *39*). In general, pentanal and hexanal concentrations decreased significantly ($p < 0.05$) for all batches of soy yogurt after fermentation especially those with inulin or glucose/raffinose supplementation; however, the reduction during storage was substantially higher in the batches with probiotic than in the control soy yogurts (**Figure 2**). Probiotic organisms possess complex enzyme systems, which may be responsible for metabolizing aldehydes (*40*). Apparently hexanal was metabolized more than pentanal, likely due to its higher concentration in soymilk (**Figure 2**). Similarly, Tsangalis and Shah (*16*), Scalabrini et al. (*29*), and Desai et al. (*39*) reported substantial metabolism of hexanal and pentanal in soymilk fermented with *Bifidobacterium* sp. for 48 h at 37 °C.

Rheological Properties of Soy Yogurt. Small amplitude shear stress oscillatory testing was applied to set-type soy yogurt during cold storage. The mechanical spectra of soy yogurt with or without inulin or raffinose and glucose are represented in **Figures 3** and **4**. Storage (G') and loss modulus (G'') characterize the degree of solid-like (elastic) and liquid-like (viscous) character of a gel, respectively (*3*). Products showed a clear gel-like behavior, in which G' was higher than G'' for all samples (**Figure 4**). The storage modulus showed a weak frequency dependency. The magnitude of storage modulus of the soy yogurt samples showed two closely paired G' values for the six types of products (**Figures 3** and **4**). Control and probiotic soy yogurts supplemented with raffinose and glucose exhibited the highest G' followed by the probiotic soy yogurt and that supplemented with inulin and control soy yogurt and control soy yogurt with inulin (**Figure 3**). The differences in storage modulus reflected the gelation characteristics within the different soy yogurts. The supplementation of raffinose and glucose may have influenced the metabolic activities of the microorganisms and may have affected the G' values. The low G' indicated by the low (solid) elastic nature of control soy yogurts with or without inulin supplementation was further confirmed by sensory evaluation (**Table 5**).

Flow Behavior. The values of the flow behavior index (n) and the consistency index (k) determined graphically by plotting shear rate (γ) versus apparent viscosity (η_a) for all of the soy yogurts are presented in **Table 4**. The apparent viscosity of the soy gel produced by the bacteria was determined, and the dependence of the rheological parameters was observed in the soy yogurt in the presence or absence of supplementation. The rheological parameters n and k varied among the various treatments of soy yogurts in the presence of the respective carbon sources at 5 °C. The experimental data fitted well to the Power law with R^2 values ranging from 0.98 to 0.99 for the ascending curves and from 0.94 to 0.99 for the descending curves. High k values and low n values ($n < 1$) were recorded for all soy yogurts, which indicated that the products were more viscous and had pseudoplastic properties (**Table 4**). Similar rheological behavior was reported by Bueno and Garcia-Cruz (*30*) in fermented broth with polysaccharide-producing microorganisms free of soil in the presence of glucose and sucrose.

In relation to the carbon sources used, it appeared that the presence of supplementation did not have a significant ($p >$

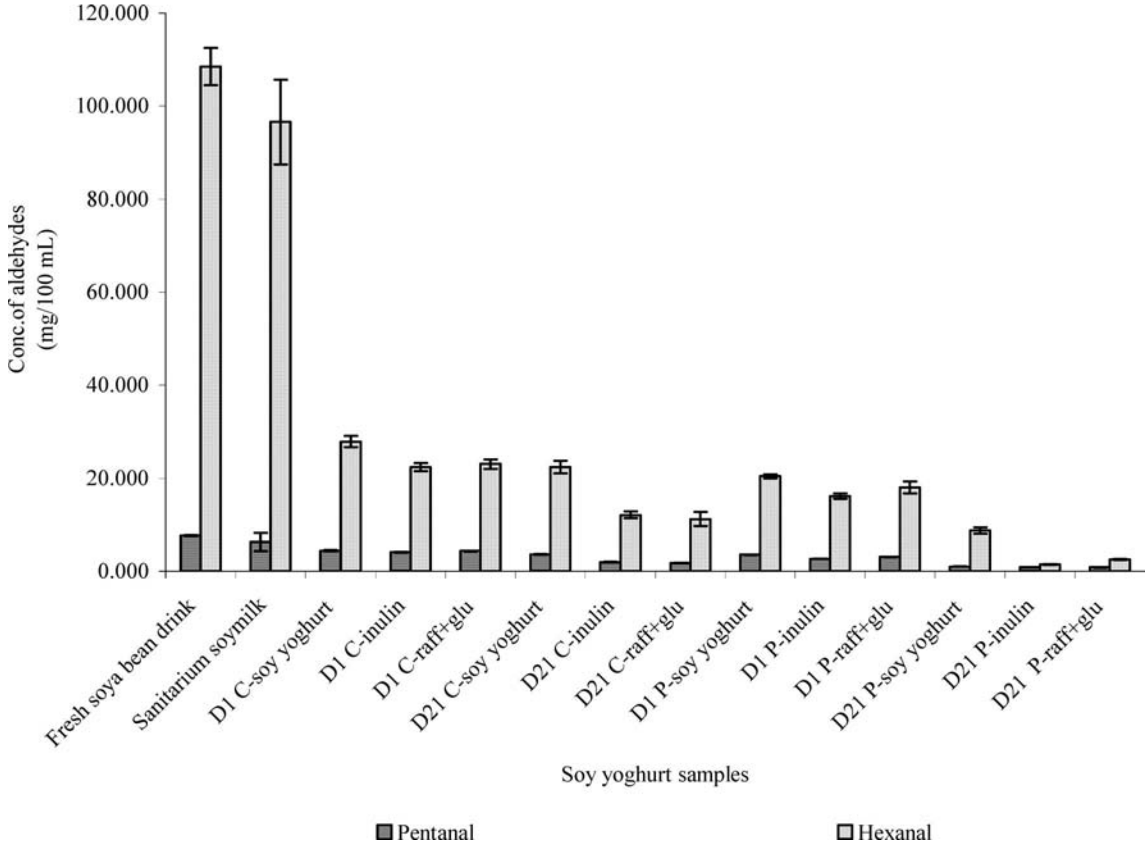

Figure 2. Concentration of hexanal and pentanal in fresh soymilk and soy yogurt supplemented with or without inulin or raffinose and glucose (raff + glu) fermented with yogurt culture or yogurt culture and probiotic organisms. D1 = day 1; D21 = day 21; C = control soy yogurt containing yogurt culture (*S. thermophilus* St1342 and *L. belbrueckii* ssp. *bulgaricus* Lb1466); P = probiotic soy yogurt containing yogurt culture, *L. acidophilus* L10, *B. animalis* ssp. *lactis* B94, and *L. casei* L26. Error bars represent standard deviation.

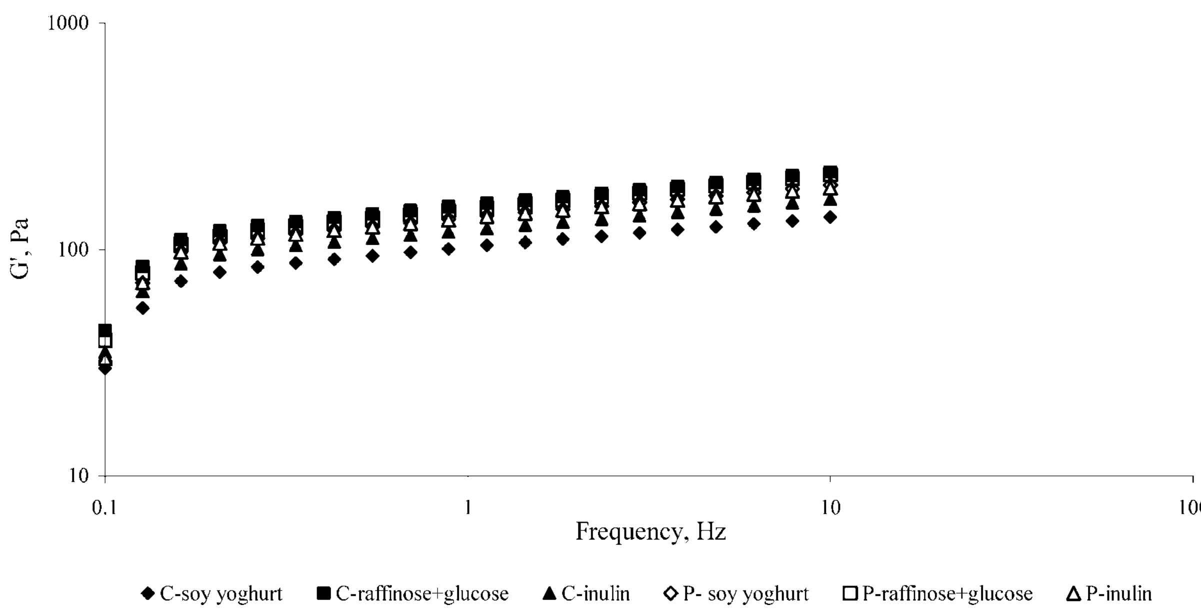

Figure 3. Effect of supplementation of soy yogurt with inulin or raffinose and glucose on the storage modulus (*G′*) compared to soy yogurt without any supplementation after 10 days of storage at 4 °C. C = control soy yogurt containing yogurt culture (*S. thermophilus* St1342 and *L. belbrueckii* ssp. *bulgaricus* Lb1466); P = probiotic soy yogurt containing yogurt culture and probiotic organisms (*L. acidophilus* L10, *B. animalis* ssp. *lactis* B94, and *L. casei* L26).

0.05) effect on k for both upward and downward curves compared to probiotic soy yogurt without any supplementation. There were also no significant ($p > 0.05$) changes in the k values for the control soy yogurts irrespective of supplementation, which was opposite from higher ($p < 0.05$) k values observed for probiotic soy yogurt. A higher consistency index value may be attributed to the metabolic activity of probiotic organisms which contributed to greater pseudoplastic properties of newly formed gels. Our findings contradicted those of Bueno and Garcia-Cruz (*30*) and Charles (*41*), who reported that the cellular contribution in fermented broths was insignificant.

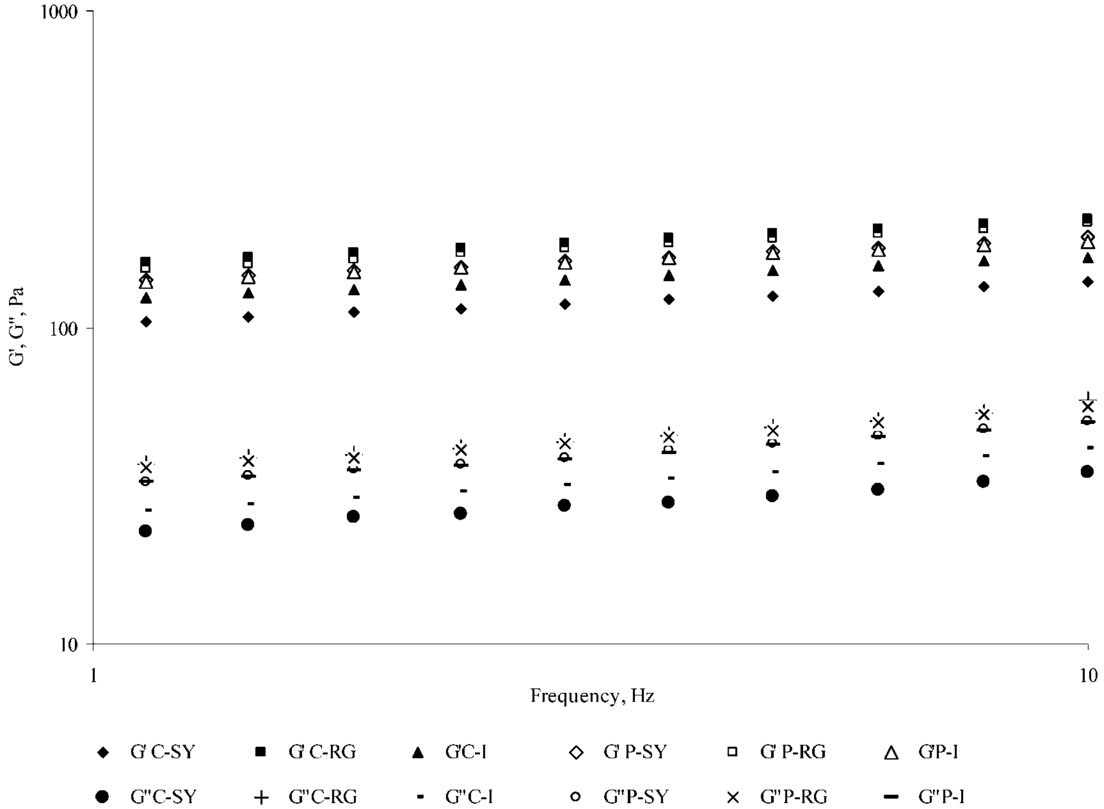

Figure 4. Frequency sweeps of soy yogurt (SY) batches with or without inulin (I), or raffinose and glucose (RG) after 10 days of storage at 4 °C. C = control soy yogurt containing yogurt culture (*S. thermophilus* St1342 and *L. belbrueckii* ssp. *bulgaricus* Lb1466); P = probiotic soy yogurt containing yogurt culture and probiotic organisms (*L. acidophilus* L10, *B. animalis* ssp. *lactis* B94, and *L. casei* L26); G' = storage and G'' = loss modulus.

Table 4. Flow Behavior of Soy Yogurt at 5 °C after 10 Days of Storage at 4 °C

treatment	up			down		
	k (Pa s^n)	n	R^2	k (Pa s^n)	n	R^2
C, soymilk	9.67^b	0.21^a	0.98	6.33^a	0.32^a	0.99
C, soymilk + 2% inulin	10.05^b	0.19^b	0.99	6.54^a	0.32^{ab}	0.94
C, soymilk + 1% raffinose + 1% glucose	9.98^b	0.20^a	0.98	6.84^a	0.31^{ab}	0.99
P, soymilk	12.87^a	0.18^b	0.98	8.54^a	0.26^b	0.99
P, soymilk + 2% inulin	10.51^b	0.20^a	0.98	7.14^a	0.31^{ab}	0.99
P, soymilk + 1% raffinose + 1% glucose	10.24^b	0.20^a	0.98	6.99^a	0.32^{ab}	0.99
SEM	0.53	0.004	0.00	0.78	0.01	0.00

[ab]Means in the same column with different small letter superscripts are significantly different; C = control; P = probiotic.

Sensory Evaluation. The sensory ratings of individual products for appearance, mouth feel, beany flavor, acidity, and overall likeness are presented in **Table 5**. The treatment effect was associated with the supplementation used in every case with the exception of the soymilk without any supplementation. The acid character described by flavor was associated with different bacterial growth as well as supplementation in some cases. For example, probiotic fermented soymilk was more acidic than the corresponding product made with yogurt culture only. La Torre et al. (*42*) made similar observations in yogurts made with commercial probiotic and starter cultures. For the appearance of all of the products with the exception of control soymilk without supplementation, which apparently had a weak gel after fermentation and during storage, 36.7% of panelists declared "liked very much" (**Table 5**). In addition, panelists observed that all of the soy yogurts did not show syneresis after fermentation and during storage at 4 °C. Liu (*1*) also reported of appearance and physical property acceptance of soy yogurt by consumer panels. This was in contrast to Favaro Trindade et al. (*20*), who reported excessive syneresis in soy yogurt produced with 5° Brix soymilk after 20 h and suggested the likely cause to be the use of 13 MPa of pressure for homogenization. Panelists recorded significant differences ($p < 0.05$) in mouth feel among the products and selected control soy yogurts supplemented with inulin, raffinose and glucose, or probiotic soy yogurt supplemented with raffinose and glucose over probiotic soy yogurt with no supplementation as having better mouth feel (**Table 5**). Soy yogurts exhibited more beaniness in the first day than that at 21 days of storage. On the other hand, plain soy yogurts (control and probiotic) registered substantially higher aftertaste characteristics. The reduction in the beaniness of the products could be the result of bacterial metabolism of alkylic aldehydes due to better growth in the presence of supplementation. However, the absence of sweeteners in the products made the presence of residual beaniness detectable by the panelists. Previous studies have used sucrose as a sweetener to mask the beany flavor detected in soy-derived products (*19*, *20*). GC analysis similarly showed a significant reduction in the aldehyde content at day 21 compared to day 1 (**Figure 2**). Furthermore, the panelists recorded high acidity ($p < 0.05$) in the probiotic soy yogurts especially in those supplemented with inulin or raffinose and glucose than

Table 5. Results of Sensory Evaluation of Soy Yogurt with Different Supplementation during Storage at 4 °C

attributes	treatments					
	C, soymilk	C, soymilk + 2% inulin	C, soymilk + 1% raffinose + 1% glucose	P, soymilk	P, soymilk + 2% inulin	P, soymilk + 1% raffinose + 1% glucose
day 1						
appearance	5.62 ± 0.15^{b}	6.91 ± 0.15^{a}	6.86 ± 0.15^{a}	6.86 ± 0.15^{a}	6.06 ± 0.15^{ab}	6.59 ± 0.15^{a}
mouth feel	5.62 ± 0.13^{bc}	5.71 ± 0.13^{bc}	6.42 ± 0.13^{a}	4.95 ± 0.13^{bcd}	4.91 ± 0.13^{bcd}	6.19 ± 0.13^{ab}
beany flavor	6.55 ± 0.13^{a}	6.46 ± 0.13^{a}	6.24 ± 0.13^{a}	6.28 ± 0.13^{a}	5.75 ± 0.13^{b}	5.17 ± 0.13^{b}
acidity	3.08 ± 0.16^{bc}	3.04 ± 0.16^{bc}	6.19 ± 0.16^{a}	5.22 ± 0.16^{b}	6.15 ± 0.16^{a}	5.88 ± 0.16^{a}
overall likeness	5.08 ± 0.13^{b}	5.62 ± 0.13^{a}	5.35 ± 0.13^{ab}	4.37 ± 0.13^{c}	4.15 ± 0.13^{c}	5.13 ± 0.13^{ab}
day 21						
appearance	4.25 ± 0.17^{c}	6.43 ± 0.17^{ab}	6.01 ± 0.17^{b}	6.91 ± 0.17^{a}	5.95 ± 0.17^{b}	6.11 ± 0.17^{b}
mouth feel	5.69 ± 0.11^{a}	5.74 ± 0.11^{a}	5.69 ± 0.11^{a}	4.30 ± 0.11^{bc}	5.15 ± 0.11^{b}	4.89 ± 0.11^{b}
beany flavor	6.16 ± 0.10^{a}	5.85 ± 0.10^{b}	5.80 ± 0.10^{b}	5.74 ± 0.10^{b}	5.37 ± 0.10^{c}	5.11 ± 0.10^{c}
acidity	3.18 ± 0.14^{bc}	3.72 ± 0.14^{bc}	6.54 ± 0.14^{b}	7.76 ± 0.14^{a}	7.55 ± 0.14^{a}	7.39 ± 0.14^{a}
overall likeness	5.90 ± 0.10^{a}	5.74 ± 0.10^{a}	4.94 ± 0.10^{b}	3.56 ± 0.10^{bc}	4.30 ± 0.10^{bcd}	3.93 ± 0.10^{bc}

C = control with *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* only; P = probiotic with *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus*, *L. acidophilus*, *B. animalis* ssp. *lactis*, and *L. casei*; [abcd]Means in the same row with different small letter superscripts are significantly different.

Table 6. Percentage of Panelists' Response to the Sensory Attributes in Soy Yogurt during Storage at 4 °C

overall likeness	% of panelist response	
	control soy yogurt	probiotic soy yogurt
day 1		
like extremely	3.33	0.00
like very much	15.56	6.67
like slightly	31.11	26.67
neither like or dislike	18.89	22.22
dislike slightly	18.89	20.00
dislike very much	10.00	20.00
dislike extremely	2.22	4.44
total	100	
day 21		
like extremely	2.67	0.00
like very much	17.33	4.00
like slightly	30.67	16.00
neither like or dislike	24.00	17.33
dislike slightly	17.33	30.67
dislike very much	8.00	22.67
dislike extremely	0.00	9.33
total	100	

the control soy yogurts (**Table 5**). The possible explanation could be that the supplementation promoted bacterial growth and increased acid production (**Table 3**).

The percentage of panelists' response to the sensory evaluation with respect to the various attributes is presented in **Table 6**. At the first day of storage at 4 °C, the mean score values for the probiotic and control soy yogurt samples were 4.55 and 5.35, which corresponded to "dislike slightly" and "neither like or dislike" ratings. For the control, 31.1% of panelists declared "like slightly" and 18.9% "neither like or dislike". The corresponding ratings for the probiotic products were 26% "like slightly" and 18.9% "neither like or dislike". Storage for 21 days did not bring any significant changes in the evaluation scores of the control soy yogurts, but substantial changes were detected by panelists in the probiotic soy yogurts (**Table 6**). In this case, the distribution of acceptability responses for the control products were 30.9% "like slightly" and 21.4% "neither like or dislike", whereas for the probiotic soy yogurts the distribution was "like slightly" 21.3%, "neither like or dislike" 19.8%, "dislike slightly" 25.3%, and "dislike very much" 21.3%. Overall, based on the acceptability mean scores, the control soy yogurt appeared to be acceptable by the consumer panel as opposed to the slight dislike of the probiotic soy yogurt. The slight dislike of the probiotic soy yogurt could likely be due to high production of organic acids by probiotic organisms. Adoption of recommended techniques to eliminate the antinutritional factors of soybean, use of sweetening agents, manipulation of starter combinations, and addition of flavors are recommended to overcome the problems of bitterness and the objectionable bean flavor in the product (*36*).

Conclusion. The starter cultures used in the production of soy yogurts played an important role in acid production and flavor of the product. The addition of inulin, raffinose, or glucose to soy yogurt effectively overcame the low acid production frequently faced in soy-based yogurt and promoted bacterial cell growth. Furthermore, the probiotic bacteria metabolized alkylic aldehydes that are responsible for beany flavor and produced low concentrations of acetic acid which provides a vinegary flavor. The appropriate carbohydrate concentrations in soymilk can improve the texture quality as well as sensory characteristics without sacrificing flavor quality. Overall, based on the acceptability mean scores, the control soy yogurt appeared to be more acceptable by the consumer panel (30.9% "like slightly") than probiotic soy yogurt (25.3% "dislike slightly"). However, in terms of mouth feel, the supplemented products either with inulin or raffinose/glucose in control and probiotic soy yogurts were acceptable. Rheological tests have shown that all soy yogurt samples irrespective of supplementation exhibited solid-like gel characteristics, but supplementation with raffinose/glucose produced firmer soy yogurts. The probiotic soy yogurts showed more viscous and pseudoplastic properties than the control soy yogurts. The overall visual appearance and rheological properties are important physical attributes, which contributed to the overall sensory perception and functionality of these products.

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