Qualitative Aspects of Probiotic Flavored Soymilk-Based Yogurt

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ABSTRACT: *In this study, the effects of soymilk to cow's milk ratio (0:100, 25:75, 50:50, 75:25, 100:0), kind of commercial starter culture (ABY-1 or ABY-2 containing Bifidobacterium lactis BB-12, Lactobacillus acidophilus LA-5, and yogurt bacteria, and natural fruit concentrations (kiwi, pear, strawberry or apricot) on biochemical characteristics, viability and sensory properties of probiotic flavored soy-yogurt were investigated. These properties were analyzed during and at the end of fermentation as well as during the cold storage (21 days). The highest viability of probiotics ($p<0.05$) was observed when ABY-1 starter culture with soymilk to cow's milk ratio of 50:50 was applied, whilst the best sensory attributes were related to the treatment with the highest cow's milk content and ABY-1 starter culture. Considering all aspects, the treatment of ABY-1/50:50 (cow's milk: soymilk) which renders the highest viability and acceptable sensory properties was selected as an optimum. Viability was over 10^8 cfu/mL during the storage period (5˚C. 21 days) for all treatments of ABY-1/50:50 containing fruit concentrates. Those which had apricot and strawberry flavorings showed the best sensory acceptance.*

KEYWORDS: *Bifidobacterium lactis*; *Lactobacillus acidophilus; Probiotic; Yogurt ; Viability.*

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INTRODUCTION

Soybean products are a valuable source of nutrients such as proteins, dietary fiber, unsaturated fatty acids, vitamins, oligosaccharides, minerals, and bioactive substances, such as isoflavones [1-3]. Soybeans are functional foods due to their high health benefits including hypolipidemic, anticholesterolemic, and antiatherogenic properties, and reduced allergenicity [4]. However, the consumption of soy-based products due to their unpleasant beany flavor (mainly due to the presence of hexanol and pentanol) has been limited in the world. This typical off-flavor plays an important role to reduce the popularity of these products [4-6]. On the other hand, the presence of oligosaccharides such as raffinose and stachyose makes gastrointestinal diseases such as stomach discomfort and flatulence [5]. Soy fermentation is an ancient green technology used to develop soy-derived foods [7]. It is also an excellent solution that improves the taste and texture of this food, reduces digestive problems, increases health properties, and reduces the anti-nutritional factors [5-8]. Soymilk, due to its desirable nutrients such as proteins, raffinose, acidosis, free amino acids, and vitamins is a very good culture for the growth of probiotic bacteria [7, 9, 10].

Probiotics are defined as live microorganisms that when administered in vulnerable populations, exert health benefits upon the host by contributing to the microbial balance of the intestine [8, 11]. Lactobacilli and bifidobacteria are the most important probiotic microorganisms [12, 13]. Antitumor effects, anti-inflammatory properties, blood pressure decrease, ability to maintain the integrity of the gut, ability to counter effects of alcohol-induced liver injury, and effects to decrease or prevent intestinal disorders such as diarrhea and lactose intolerance had reported for probiotics properties [14]. In probiotic products, the viability of microorganisms is the most important product quality parameter in food products [12]. A lot of studies have been done on the growth of probiotics in soymilk, and in many of them, it has been shown that *Bifidobacterium* especially was well grown in soybean products [10]. In addition, many studies have shown that fermentative soybean products compared to non- fermentative have a higher content of peptides [15] and isoflavones, but less content of oligosaccharides [16, 17]. The health-enhancing effects of probiotic-fermented soy foods like hypocholesterolemia, antihypertension, improvement of immunity, alleviation of lactose intolerance, reduction of ovarian cancer and cardiovascular disease risks have been well studied [18]. Yogurt is the most acceptable and practical probiotic product [19, 20]. However, it isn't a suitable vehicle for transmission of probiotic micro-organisms into the body from a probiotic viability point of view due to its high acid and low pH nature [21], especially for bifidobacteria [19]. Therefore, by mixing cow's milk with soy milk, not only the undesirable flavor is significantly reduced, but also the fermentability of media is considerably decreased due to the attendance of various sugars in soymilk such as sucrose, raffinose, and stachyose. On the other hand, by adding soymilk to cow's milk, the viability of probiotics was increased by reducing the reduction potential [21] and increasing buffering capacity [10]. Thus, this work was performed to determine the effects of a combination of soymilk and cow's milk, the type of commercial starter culture (ABY-1 or ABY-2 containing *Bifdobacterium lactis* BB-12, *Lactobacillus acidophilus* LA-5, and yogurt bacteria), and natural fruit flavoring foods (kiwi, pear, strawberry, and apricot) on biochemical, microbiological and sensory properties of probiotic soy-based yogurt at the end of fermentation as well as during the cold storage (5˚C, 21 days).

EXPERIMENTAL SECTION

Starter cultures

Two types of commercial lyophilized DVS pouches consisting *Lactobacillus acidophilus* LA-5, *Bifdobacterium lactis* BB-12, *Streptococcus thermopilus* and *Lactobacillus bulgaricus* (ABY) were used as a starter culture. These types were ABY-1 (Medium fermenting culture, giving a medium flavor and a high viscosity; ideal for the creamy set or stirred yogurts) and ABY-2 (fast fermenting culture, giving a medium flavor and a medium viscosity; ideal for the creamy set or stirred yogurts). They were procured from Chr-Hansen Co. (Horssholm, Denmark). The starter cultures were stored at -18°C up to utilization based on the manufacturer's guidelines. Fruit concentrate and non-sugar soymilk were supplied by FRu lact Company (Spanish) and Maxsoy Company (Karaj, Iran) respectively. The stabilizer was provided by PREMIX (Spanish) and the skim milk powder was supplied from NZNP Company (Waikato, New Zealand).

Sample preparation

The various ratios of soymilk to reconstituted skim milk (100:0, 75:25, 50:50, 25:75, or 0:100) were made by mixing reconstituted skim milk and soymilk. The samples were heated up to 85°C for 30 min. Then, they were cooled up to inoculation temperature (40°C) and starters (probiotic and yogurt bacteria) were inoculated according to the manufacturer's instructions. The initial population of probiotics was 1×10^7 CFU/mL. Samples were incubated at 40°C until the pH 4.5±0.02 was achieved. The samples were cooled in two steps (first, rapidly up to 15°C, and second, up to 5°C). Changes in redox potential, titrable acidity, and pH were evaluated during fermentation every 30 min. In addition, the quantity of lactic and acetic acids, sensory characteristics, and viability of probiotic microorganisms were assessed in treatments at the end of fermentation. Then, after choosing the optimal treatment in terms of the viability of probiotics, different fruit concentrations (kiwi, pear, strawberry, and apricot), (12% w/w) were added to it at 5°C followed by determination of biochemical, microbiological, and sensory characteristics during the cold storage (5˚C, 21 days) per 7-day intervals.

Microbiological analysis

Selective enumeration of probiotic micro-organisms (*Bifdobacterium lactis* BB-12 and *Lactobacillus acidophilus* LA-5) was carried out by using MRS-bile agar medium (Merck, Darmstadt, Germany [22]. The plates were incubated under anaerobic and aerobic conditions at 37°C for 72 h. The viability proportion index (VPI) of probiotic bacteria was computed according to *Shafiee et al* [23].

Biochemical Analyses

The redox potential (Eh) and pH values of treatments were determined with pH meter (HANNA, Milan, Italy) at room temperature. The titrable acidity was determined by methods of *Mortazavian et al.* 2011 [24]. Quantification of acetic and lactic acids was measured by HPLC device (CE 4200, Cambridge, UK), using Jasco UV- 980 detectors at 254 nm and a C18 column (Duren, Germany) according to *Mortazavian et al*. (2010). In order to the exploitation of acids, 5.0 g of soy-yogurt was added to 20 mL with 0.1 N H_2SO_4, homogenized and centrifuged at 3000 *g* for 15 min. After filtering the supernatant through a 0.45 µm filter, it was used for analysis. Elution was accomplished isocratically with sulfuric acid 0.009 N as the mobile phase at the flow rate 0.5 mL/min. Quantification of acetic lactic acids was obtained by external standard absorbance recorded in the chromatograms.

The parameters of mean pH drop rate, mean acidity increase rate, and mean redox potential increase rate were calculated according to the method of *Mortazavian et al.* as follows:

pH drop rate = (final pH value - initial pH value) /Storage time.

Acidity increase rate = (final acidity value - initial acidity value)/storage time.

Redox potential increase rate = (final value – initial value)/storage time.

Sensory evaluation

The evaluation of sensory parameters was carried out by 10 trained panelists who were members of Maxsoy Co., on the 1st and the 21st days of storage. They were compared using the scoring methodology. Each panelist was provided with a questionnaire and asked to evaluate each treatment according to flavor, appearance (color and syneresis), and texture by using a 5-point hedonic scale as follows: 0 = inconsumable, 1 = unacceptable, 2 = acceptable, 3 = satisfactory and 4 = excellent. Coefficients 4, 3, and 2 were devoted to flavor, texture, and appearance, respectively.

Statistical analysis

This research was done according to a completely randomized design. the ranked orders of means were performed at the significance level of 0.05 ($p<0.05$) using a two-way analysis of variance (ANOVA) by SPSS software (version 23, Chicago, USA) All analyses were implemented in triplicate

RESULTS AND DISCUSSION

Biochemical properties

Changes in pH drop, acidity increase, and redox potential of treatments during fermentation are shown in Fig. 1. Also, Table 1 shows the mean acidity increase rate, mean redox potential increase rate, mean pH drop rate, incubation time, lactic and acetic acid contents (%), and final titrable acidity, in different samples during fermentation or at the end of fermentation. According to Fig. 1, three obvious phases, namely lag, exponential and stationary was observed, and 100:0/ABY-1 treatment

Table 1: Acidity increase rate, redox potential increase rate and mean pH drop rate throughout the fermentation or at the end of fermentation.

Treatments	Paramerets							
Cow milk/soy milk ratio	M-pH-DR (pH/min)	M-A-IR (D/min)	M-RP-IR (mV/min)	incubation time (min)	Final acidity (D)	Fermentation peak time (min)	Acid lactic (%)	Acid acetic (%)
100 : 0/1	0.005^{e}	0.23^{c}	0.32^{e}	360^{b}	106^{a}	120-150	0.9^{a}	0.14^{e}
75: 25.1	0.008^{b}	0.32^{a}	0.49^{ab}	240^{f}	97^{b}	120-150	0.81^{b}	0.07^{g}
50: 50.1	0.009^{a}	0.27^{b}	0.52^{a}	220^{g}	86.3^{c}	60-90	0.66^{c}	0.22^{c}
25: 75.1	0.008^{b}	0.21^{cd}	0.51^{a}	250^{e}	72.3^{e}	90-120	0.56^{d}	0.26^{b}
0: 100.1	0.006^{d}	0.19^{d}	0.42^{c}	300^{c}	84.1^{cd}	90-120	0.52^{e}	0.30^{a}
100. 0.2	0.004^{f}	0.20^{cd}	0.28^{f}	390^{a}	100^{ab}	240-270	0.87^{ab}	0.12^{e}
75: 25.2	0.006^{d}	0.23^{c}	0.41^{cd}	300^{c}	89^{c}	180-210	0.71^{c}	0.17^{cd}
50: 50.2	0.007^{c}	0.23^{c}	0.42^{c}	285^{d}	86^{c}	150-180	0.69^{c}	0.15^{e}
25: 75.2	0.007^{c}	0.20^{cd}	0.44^{c}	300^{c}	72.5^{e}	120-150	0.51^{e}	0.19^{cd}
0: 100.2	0.006^{d}	0.18^{d}	0.46^{ab}	300^{c}	72.9^{e}	150-180	0.55^{d}	0.16^{d}

**/1: ABY-1, /2: ABY-2, M-pH-DR = mean pH drop rate; M-A-IR = mean acidity increase rate; M-RP-IR = mean redox potential increase rate. Means in the same column with different letters are significantly different (p\0.05). ** ABY = Lactobacillus acidophilus LA-5, Bifdobacterium lactis BB-12, and yogurt bacteria.*

had the longest stationary phase (150 min). A long stationary phase indicated undesirable conditions for a starter. The rate of pH decrease and acidity increase at the beginning of fermentation were ordinarily slight, which could be explained by the high buffering capacity of cow's milk and the lack of available forms of vital growth factors. 50:50/ABY-1 had a top point of pH reduction and increase in acidity and redox potential was in 60-90 minutes from the start of incubation, and it was reduced in comparison with 100:0/ABY-1 (Fig. 1), due to decreasing in cow's milk and casein contents as well as the buffering capacity. Pursuant to Table 1, the lowest and longest time of incubation belonged to 50:50/ABY-1 (220 min) and 100:0/ABY-2 (390 min), respectively ($p<0.05$). Besides, 100:0/ABY-2 showed a longer lag phase because of the disability of yogurt bacteria in ABY-2 culture (than that of ABY-1) in consuming nutritious components, a greater need for the growth factors, and high buffering capacity of cow's milk. The longest logarithmic phase related to 75:25/ABY-2 treatment indicated poor activity of yogurt bacteria in ABY-2 culture in comparison with ABY-1. According to Table 1, the greatest mean pH decrease rates were obtained for 50:50/ABY-1 and the greatest mean acidity and redox potential increase rates were observed in 50:50-ABY-1 and 75:25/ABY-1 ($p < 0.05$). The 00:0/ ABY-2 treatment showed the least ($p<0.05$) mean pH drop rate and redox potential increase rates. Thus, the addition of soymilk at 25% and 50% levels led to an increase in mean acidity increase rate and mean pH drop rate, but high contents of soymilk (100%) led to a reduction in mentioned indicator and it justified the shortest fermentation time of 75:25/ABY-1 and 50:50/ABY-1 treatments. In fact, the addition of soymilk to cow's milk can improve the milk environment for the growth of starter cultures [25]. However, a high amount of soymilk (100%), caused a shortage of essential nutrients for the starter bacteria found in cow's milk [26].

According to Table 1, the most and the least final titrable acidity was related to 100:0/ABY-1 and 25:75/ABY-1 and there was no significant difference in final acidity in 25:75/ABY-1, 25:75/ABY-2, and 0:100/ABY-2. It could be understood that by increasing the soymilk content, final acidity was reduced and it was obvious that the content of soymilk on biochemical characteristics of fermentation was more effective than the type of starter culture.

The highest amount of lactic acid ($p < 0.05$) is related to 100:0/ABY-1 due to the high content of lactose and significant growth of *L. delbrueckii* ssp. *bulgaricus* (Table 1), (Liu JR, Lin CW, 2000m). The 0:100/ABY-1 and 25:75/ABY-2 showed the least lactic acid that had a significant difference from the other samples ($p<0.05$).

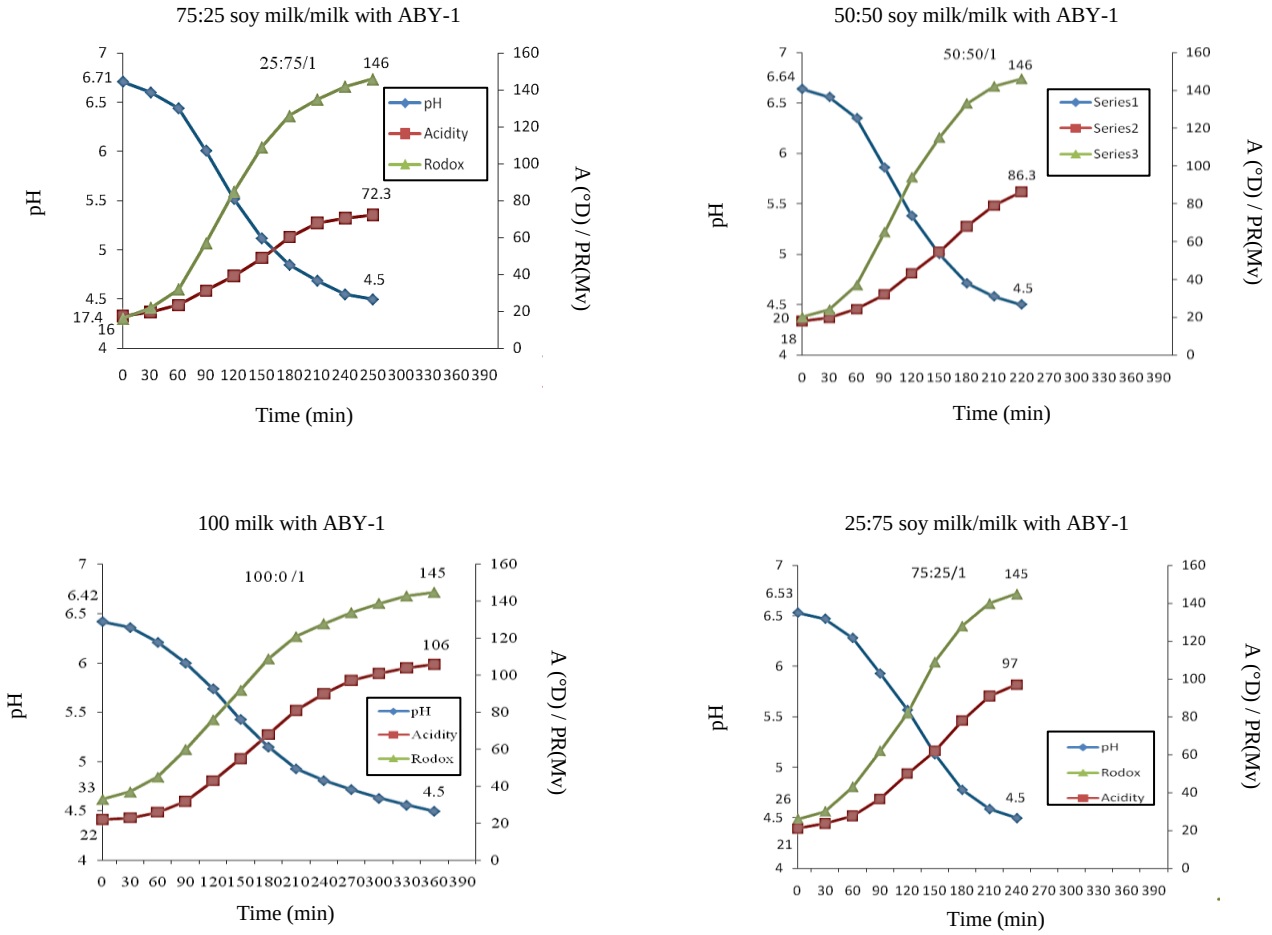

Fig. 1: Acidity increase, redox potential, and pH drop, during fermentation.

Murti et al. reported that *L. delbrueckii* ssp. *bulgaricus* is unable to ferment sucrose and galactoside sugar (raffinose and stachyose) in soymilk [27]. Hence, the addition of soymilk led to diminished content of lactic acid. As shown in Table 1, the greatest and lowest content of acetic acid ($p<0.05$) was related to 0:100/ABY-1 and 75:25/ABY-1, respectively. Because one of the main products of fermentation by *B. lactis* BB-12 is acetic acid, the relatively high content of acetic acid represented that the growth condition for *Bifdobacterium* should have been suitable by increasing the soymilk proportion. In 75:25/ABY-1, the condition was not suitable for *Bifdobacterium*, and they could not compete with *L. delbrueckii* ssp. *bulgaricus*, so their metabolite is reduced [28].

Viability of probiotic bacteria at the end of fermentation

The viability of probiotic bacteria in different samples at the end of fermentation is shown in Table 2. The results showed that the highest and the lowest viability of L. acidophilus LA-5 were observed in 25:75/ABY-2 and 100:0/ABY-2, respectively ($p<0.05$). This indicated that ABY-2 starter needed more available growth factors due to low proteolytic activity (according to Chr-Hansen's document), and increasing the soymilk: cow's milk proportion provided available growth factors. Therefore, the growth and viability of probiotics increased. The highest viable count of B. lactis BB-12 was observed in 25:75/ABY-2 and 50:50/ABY-1 ($p < 0.05$), probably because B. lactis BB-12 requires a combination of growth factors for survival and growth that are provided better in mixed source than single sources (cow's milk or soymilk). The lowest viability for B. lactis BB-12 was related to 100:0/ABY-2 treatments and it represented that the Bifidobacterium in ABY-2 had slow growth and needed more growth factors. In fact, due to the lower growth of yogurt bacteria in ABY-2 starters in comparison with ABY-1, proteolytic properties activity

Table 2: Viability of probiotic microorganisms and their relevant viability proportion index in different treatments at the end of fermentation.

Treatments		Primary population (log cfu/mL)			Final population (log cfu/mL)					GPI*
Cow milk/soy milk ratio	pH	A	B	A+B	A	B	A+B	A*	B*	A+B
0:100/1**	4.5	7.47	7.84	8.00	8.17^{bB}	8.44^{aA}	8.62^{a}	1.09	1.07	1.07
25:75/1	4.5	7.47	7.84	8.00	7.29^{deB}	8.14^{cdA}	8.19^{b}	0.97	1.04	1.02
50:50/1	4.5	7.47	7.84	8.00	7.56^{cB}	8.31^{bA}	8.37^{c}	1.01	1.06	1.04
75:25/1	4.5	7.47	7.84	8.00	7.39^{dB}	8.20^{cA}	8.26^{cd}	0.98	1.02	1.03
100:0/1	4.5	7.47	7.84	8.00	7.56^{cB}	8.14^{cdA}	8.24^{d}	1.01	1.04	1.03
0:100/2	4.5	7.47	7.84	8.00	7.00^{fB}	7.54^{fa}	7.65^{e}	0.93	0.96	0.95
25:75/2	4.5	7.47	7.84	8.00	8.21^{abB}	8.36^{abA}	8.59^{a}	1.09	1.06	1.07
50:50/2	4.5	7.47	7.84	8.00	8.17^{cA}	8.17^{cA}	8.26^{cd}	1.00	1.04	1.03
75:25/2	4.5	7.47	7.84	8.00	8.39^{aA}	8.39^{aA}	8.62^{a}	1.11	1.07	1.07
100:0/2	4.5	7.47	7.84	8.00	8.00^{eA}	8.00^{eA}	8.50^{a}	1.11	1.02	1.06

Means shown with different small and capital letters represent significant differences ($p < 0.05$) ***in the same columns (among the treatments) and rows (between the two probiotic bacteria in each treatment), respectively.***
****/1: ABY-1, /2: ABY-2 *(A :LA-5 Lactobacillus acidophilus) ،(B :Bb-12 Bifidobacterium) ،(GPI :Growth factor (Averages highlighted in capital and small English words are significantly different (p <0.05).***
A = L. acidophilus; B = B. lactic; A + B = total probiotic

of yogurt bacteria reduced and there were not enough available growth factors, which led to its weak growth. Due to mentioned reason, the stationary phase became longer, causing more death of probiotic bacteria.

Wang et al. (2003) reported that soymilk could provide conditions simultaneously for Bifdobacterium as well as S. thermophilus and L. delbrueckii ssp. bulgaricus. Also, Chaou et al. (1999) understood that adding supplements such as isomaltoligosaccharides, glucose, galactose, and soy oligosaccharides led to the increased number of Bifidobacterium. According to the totality of probiotics, the greatest viability was related to 25:75/ABY-2 ($p<0.05$), and it indicated that prebiotic substances found in soy milk could support probiotic growth. The lowest total viability of probiotics was observed in 100:0/ABY-2. This could be attributed on one hand to a lack of available forms of vital growth factors for ABY-2 bacteria and on the other hand, to minor disability of these bacteria to utilize nutritious substances of cow's milk. Therefore, by increasing the growth factors via adding soy milk, viability was increased. High viability and population of probiotics indicated further compatibility of ABY-1 bacteria with cow's milk medium. Totally, the population of bacteria in all treatments was in the normal range. The viability of L. acidophilus LA-5 was higher in treatments containing 100% cow's milk, indicating that L. acidophilus could tolerate more acidic conditions in comparison with B. lactis BB-12. The Viability Proportion Index (VPI) and Growth Proportion Index (GPI) of Bifidobacterium increased by raising the soymilk content in treatments, which showed the capability of this bacteria to use α-galactoside sugar (raffinose and stachyose) as well as probably the side effect of produced acetic acid by this bacterium on the viability of Lactobacillus. These results are in accordance with Shah's research in 1997 [29]. Also, *Dave* and *Shah* (1997) figured out that the viability of probiotics depends upon the inoculation volume, growth factors, inhibitors, time of fermentation and temperature of storage, osmotic pressure, availability of nutrient components, and incubation temperature [30]. According to the effect of mixing milk ratio on the viability of probiotics, there was a significant difference ($p<0.05$) in the population of B. lactis BB-12, L. acidophilus LA-5, and the sum of the two probiotics between 100:0/ABY-1 and 75:25/ABY-1. In fact, due to the presence of a nutritional medium of soymilk and fermentative carbohydrate lactose (cow's milk) in 75:25/ABY-1, yogurt bacteria grew rapidly and due to the shorter time of fermentation in this treatment (in comparison to 100:0/ABY-1), there was not enough time for probiotic growth. At the same time that probiotic bacteria entered to logarithmic phase, yogurt

Table 3: Sensory evaluation of treatments at the end of fermentation.

Treatments	Parameters			
Cow milk/soy milk ratio	Taste	Texture	Appearance	Final score
100:0/1 (T1)	28.5^{a}	13.2^{a}	8.8^{a}	56.8^{a}
75:25/1(T2)	14.4^{c}	10.8^{b}	6.8^{b}	32^{d}
50:50/1 (T3)	18^{b}	10.8^{b}	8^{ab}	36.8^{c}
25:75/1(T4)	8.4^{f}	6.6^{de}	4.4^{d}	19.4^{h}
0:100/1(T5)	10.8^{e}	8.4^{d}	4^{bc}	25.2^{fg}
100:0/2 (T6)	28.8^{a}	12^{ab}	7.2^{ab}	48.4^{b}
75:25/2 (T7)	15.6^{bc}	9.6^{c}	6.4^{b}	31.6^{d}
50:50/2(T8)	15.6^{e}	10.2^{bc}	6.4^{b}	27.4^{f}
25:75/2 (T9)	10.8^{ef}	7.8^{d}	5.2^{c}	21.4^{f}
0:100/2 (T10)	8.4^{cd}	10.8^{b}	7.1^{b}	30^{de}

Means in the same column with different letters are significantly different (P < 0.05).
**/1: ABY-1, /2: ABY-2*

bacteria-produced acid rapidly, and reduced pH composition. Regarding Growth Proportion Index (GPI), the viability of L. acidophilus LA-5 was higher which is in accordance with *Mortazavian et al.* (2006) [19].

Sensory evaluation

The averages of sensory attributes are shown in Table 3. According to this table, the most overall acceptance related to 100:0/ABY-1, and then, 100:0/ABY-2. 25:75/ABY-1 had the least acceptance, and there was a significant difference between 75:25/ABY-1 and 75:25/ABY-2 in texture so the ABY-1 starter caused better texture. Although there was no significant difference between the 50:50/ABY-1 and 50:50/ABY-2 in appearance, flavor, and texture. No significant difference was observed in texture between 25:75-ABY-1 and 25:75-ABY-2, however, 25:75-ABY-2 had lesser syneresis. In fact, in a high portion of soymilk, ABY-2 starter could make better texture and appearance with lower syneresis, due to an increase in the dry matter. 0:100/ABY-1 and 0:100/ABY-2 treatments showed no significant difference in appearance, but in treatment with ABY-2, the texture became better ($p < 0.05$). Totally, it could be concluded that in 100% soymilk culture, ABY-2 showed better sensory attributes, but in lower portions (under 75% soymilk), ABY-1 had more ability to make better sensory attributes better. Based on the results of sensory evaluation, 100:0/ABY-1, and after that, 100:0/ABY-2 had the highest total score. Therefore, the treatment of 50:50-ABY-1 was selected as the optimal treatment to add different types of fruit juice concentrates (pear, apricot, kiwi, and strawberry) in the next step.

Biochemical properties during refrigerated storage

Fig. 2 illustrates changes in pH drop, titrable acidity increase, and redox potential increase of samples during 21-day refrigerated storage. According to Fig. 2, the samples with various kinds of fruit concentrates showed different redox potential increases, acidity, and pH drop during the mentioned period. As was evident, the pH value was reduced and titrable acidity and redox potential increased during storage and it is in accordance with the results of *Kailasapathy* (2006) [31] reported that pH value of probiotic yogurt containing *L. acidophilus* and *B. lactis* reduced significantly during refrigerator storage. The maximum and minimum changes in pH drop at the end of storage were observed in the sample with kiwi and apricot as flavoring agents, respectively. Due to a lack of nutritious substance and stopping in growth and fermentation, the control sample showed the least drop pH value. Also, the most and least redox potential were related to samples with kiwi and apricot flavoring, respectively; and it could be explained by the presence of reductive sugar in fruit pulp especially fructooligosaccharides [32] that induced probiotic and yogurt bacteria to grow. Finally, it was observed that starter

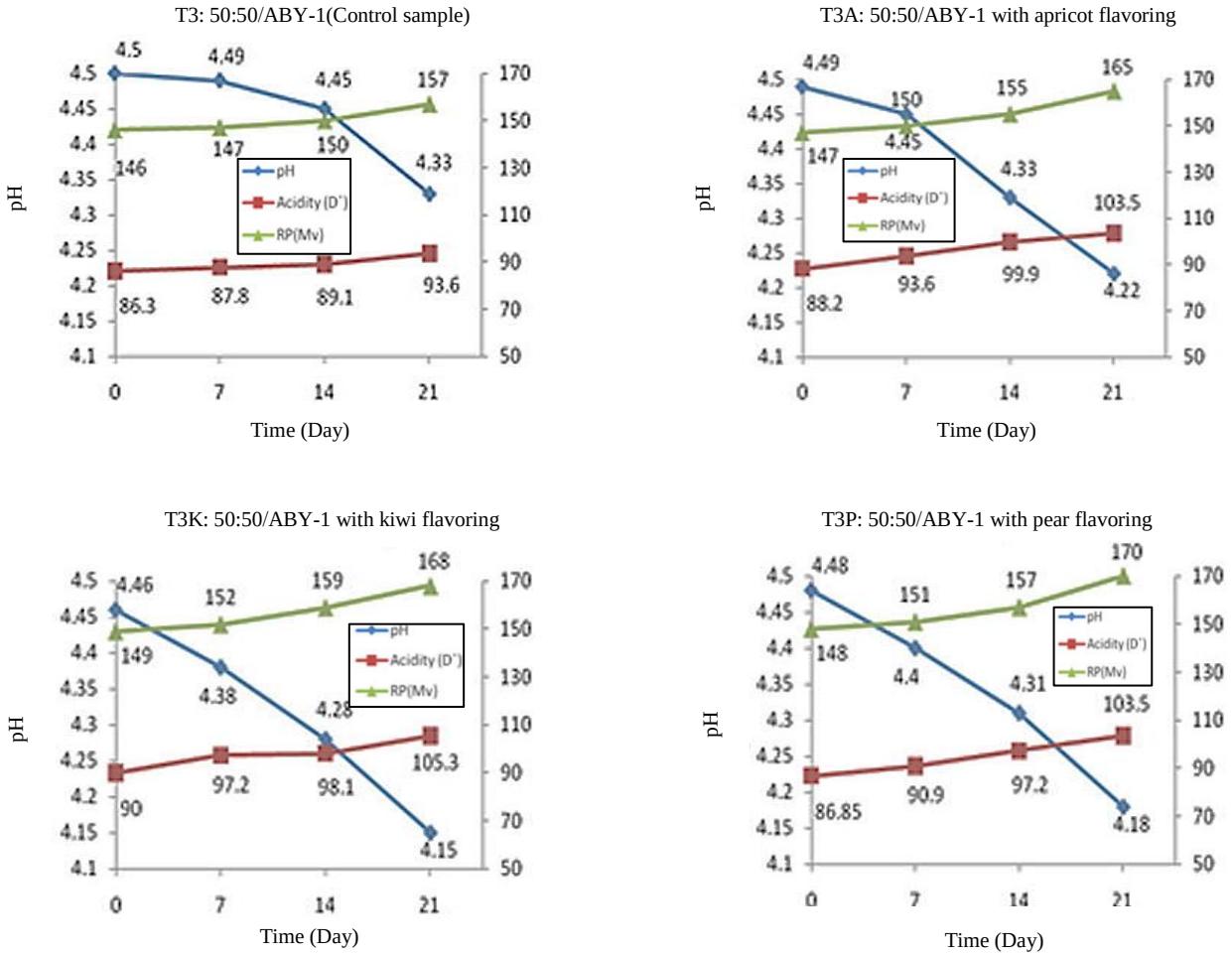

Fig. 2: Changes in pH drop, acidity increase, and redox potential increase in treatment 50:50/ABY-1 (T3) flavored with apricot (T3A), kiwi (T3K), and pear (T3P) during refrigerated storage at 5 °C.

bacteria increased pH drop value, redox potential, and titrable acidity in flavored treatment with kiwi and apricot and showed more ability for growth and survival in this culture composition.

Viability of probiotics during refrigerated storage

Table 4 indicates the viability of *L. acidophilus* LA-5 and *B. lactis* Bb-12 in flavored treatments during 21 days of cold storage. There was a significant difference in all flavored samples in terms of viability with control treatment ($p<0.05$); and in samples that contained flavoring, viability was significantly ($p<0.05$) higher than control. The greatest and the lowest viabilities of *L. acidophilus* LA-5 were related to 50:50/ABY-1 with apricot flavoring and 50:50/ABY-1 with strawberry flavoring, respectively. Previous studies have shown that prebiotic compounds such as inulin and fructooligosaccharides in fruit concentrates, lactulose and galactooligosaccharides in milk, and soymilk carbohydrates (raffinose and stachyose) could have synergistic effects on the viability of *L. acidophilus* LA-5 [9, 33]. *Capela et al*. (2006) reported that Hi-maize, inulin, and fructooligosaccharides supplements had positive effects on the viability of *L. rhamnosus*, *L. casei*, and *L. acidophilus* in yogurts [34]. Also, Rybka (2001) reported that *L. acidophilus* survived sufficiently during 28 days of storage [35], and showed more viability than yogurt bacteria and *Bifdobacterium* during storage. As it is shown in Table 4, *L. acidophilus* LA-5 had higher resistance to acidic conditions during fermentation. The greatest viability of *B. lactis* BB-12 was in 50:50/ABY-1 with peach flavoring and 50:50/ABY-1 with strawberry flavoring. 50:50/ABY-1 with kiwi flavoring showed the lowest viability of *B. lactis* BB-12 due to high redox potential and titrable acidity, and low

Table 4: Viability of Lactobacillus acidophilus LA-5.

Treatments	LA-5 During storage time			Final viability
Storage time(day)	1	7	14	21
T3(control)	7.56^{aA}	6.47^{dB}	6.41^{dC}	6.21^{dD}
T3P	7.56^{aC}	8.80^{bA}	8.83^{bA}	8.75^{bB}
T3S	7.56^{aD}	7.74^{cB}	7.80^{cA}	7.70^{cC}
T3A	7.56^{aD}	9.00^{aC}	9.30^{aA}	9.20^{aB}
T3K	7.56^{aC}	8.65^{bB}	7.84^{bA}	8.68^{bB}

**T3: Treatment 50:50-ABY-1, T3A: T3 flavored with apricot, T3K: T3 flavored with kiwi ,*
T3P: T3 flavored with pear and T3S: T3 flavored with strawberry

Table 5. Viability of Bifdobacterium lactis Bb-12

Treatments	Bb-12 During storage time			Final viability
Storage time(day)	1	7	14	21
T3(control)	8.31^{aA}	6.30^{dB}	6.22^{eC}	6.1^{dD}
T3P	8.31^{aC}	9.67^{aB}	9.71^{aA}	9.63^{aB}
T3S	8.31^{aD}	9.30^{bB}	9.37^{bA}	9.17^{bC}
T3A	8.31^{aC}	8.82^{cB}	8.90^{cA}	8.83^{cB}
T3K	8.31^{aC}	8.77^{cB}	8.80^{dA}	75.8^{cB}

**T3: Treatment 50:50-ABY-1, T3A: T3 flavored with apricot, T3K: T3 flavored with kiwi ,*
T3P: T3 flavored with pear and T3S: T3 flavored with strawberry

pH value. In fact, an increase in titrable acidity and amount of acetic and lactic acids due to the addition of fruits led to decrease the viability of *Bifdobacterium* (*Champagne* and *Gurdner* 2008; *Saarela et al*. 2006 [36]. *Vinderola et al*. (2002) [37] understood that fruit juice additives, namely coloring and flavoring agents, could be related to a decrease in probiotic viability. *Puupponenpinia et al*. (2001) reported that the growth of selected harmful bacteria in the gastrointestinal tract was inhibited by phenolic berry extracts, but it had no effect against beneficial lactic acid bacteria [38]. *Bedani et al.* showed that fruit pulp had no effect on probiotic viabilities. However, the fruit pulps reduced probiotic survival significantly to simulated gastrointestinal stress [39]. Furthermore, they observed that strawberry's bioactive compounds inhibited pathogenic bacterial strains. Also, there was no significant difference in the content of lactic and acetic acid up to 14 days of storage, but after that, they increased significantly. Growth of *L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus* during fermentation and storage increased organic acid content caused a reduction in viability of probiotics in yogurt, especially a significant decrease in the population of *Bifdobacterium* at the end of storage. In fact, the highest content of lactic acid produced during fermentation and storage was owing to the addition of lactose in milk. Favaro (1980) observed that by adding glucose and galactose to culture composition, the content of organic acid was increased, which justified the increase of organic acid content in this research [1].

Sensory evaluation during refrigerated storage

According to the sensory evaluation (Table 3) 50:50/ABY-1 with strawberry flavoring and after that, 50:50/ABY-1 with apricot flavoring had the highest acceptability because fruit pulp improved product color and taste. In fact, the relatively high concentrations of sugar in fruit concentrates decreased and vanished the astringent and beany off-flavor of soy milk as well as increased the probiotic's viability [40]. Therefore, the total acceptability of soy fruit yogurt was notably greater than plain soy yogurt. The acceptance and tendency of soy yogurt with mango pulp were higher than in the control sample and soybean yogurt with pulp guava was previously reported in the study of *Bedani et al.* (2014) [39]. Additionally, results demonstrated that although adding fruit pulp led to greater acceptance of the product, it may reduce probiotic viability.

CONCLUSIONS

The results showed that the kind of commercial probiotic starter culture and proportion of cow's milk to soymilk significantly ($p<0.05$) influenced both viabilities of probiotics and the sensory attributes of products. *L. acidophilus* LA-5 and *B. lactis* BB-12 in commercial starter culture ABY-1 in the ratio of 50:50 (cow's milk:soymilk) showed more viability in comparison with other treatments. Also, it was observed that all probiotic flavored soymilk-based yogurts included more than 10^8 cfu/mL probiotic at the end of 21-day cold storage, and there was a considerable reduction in syneresis of fruity probiotic soy-yogurt during the cold storage. The sensory evaluation indicated that the flavored treatment with apricot and strawberry had the highest acceptance at the end of cold storage and the treatment 50:50/ABY-1 with apricot was found as the best formula for industrial production, regarding all aspects (probiotic viability, functional property of soy proteins, and sensory attributes).

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