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Effects of Soluble Soybean Polysaccharides on Properties of Kefir Produced from Cow and Buffalo Milks

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Abstract

Background and objective: Fermented dairy products are considerably known due to several benefits including high nutritional values, immunity stimulations, antimicrobial and cancer suppressing effects. Kefir is a fermented dairy product with acidic-alcoholic flavors made from various sources of milk with various characteristics. The aim of this study was to investigate impact of soluble soybean polysaccharides on properties of kefir produced from cow and buffalo milk.

Materials and methods: Soluble soybean polysaccharides at concentrations of 0 (control), 0.5, 1 and 1.5% (w v^{-1}) were added to kefir samples produced from cow and buffalo milks and the physicochemical, sensory and microbiological characteristics as well as fatty acid profile analysis of the kefir samples were compared during one month of cold storage.

Results and conclusion: Results showed that soluble soybean polysaccharides ($P \leq 0.05$) had significant effects on kefir properties. By increasing concentration of soluble soybean polysaccharides and storage time of the kefir, some properties including acidity, viscosity, sensory score and counts of the lactic acid bacteria and yeasts were increased. The fatty acid analysis revealed that unsaturated fatty acids of cow and buffalo kefirs were more than cow and buffalo milks while these were reverse for saturated fatty acids. The best microbial and sensory properties of kefir were observed by adding 0.5 to 2% (w v^{-1}) soluble soybean polysaccharides on day 30 of storage.

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1. Introduction

Kefir is a fermented dairy beverage originating from Caucasus Mountains, traditionally produced from small, irregularly shaped, gelatinous yellowish grains that contain a complex flora of lactic acid bacteria (LAB), yeasts and sometimes acetic acid bacteria [1,2]. Its popularity is majorly based on its nutritive contents and health benefits. Kefir includes numerous benefits for the human health such as improvement of lactose tolerance in adults as well as antimicrobial, antitumoral, antioxidant, antimutagenic and antiapoptotic effects [2]. Kefir can be made from various milks of animal and plant origins. Several studies have been carried out to assess effects of the milk type on kefir properties [3,4]. It has been shown that changing the milk type such as bovine, caprine, ovine, buffalo [3], camel [5] and plant milks [1,4] (particularly soy, rice and coconut milks) includes substantial effects on kefir properties. Recently, buffalo milk and its products have received much attention, particularly for their nutritional values such as nutritional importance and bioactive properties. The buffalo milk includes special taste and high contents of calcium, fat, protein, lactose, mineral and vitamin with low contents of cholesterol, compared to that the cow milk does. Furthermore, kefir is a good source of conjugated linoleic acids for humans [2,6]. Compared to cow milk, buffalo milk is even further appropriate for the production of traditional and industrial dairy products, especially mozzarella cheese and fermented dairy products such as kefir. For example, Gul et al. reported that flavor and aroma of kefir produced from buffalo milk were further preferred than those produced from cow milk [2]. In recent years, various

compounds such as inulin, thistle, sugar and xanthan have been added to kefirs for the improvement of kefir taste, quality, biological values and health benefits [1,7-9]. Soluble soybean polysaccharides (SSPS) are water-soluble polysaccharides, including a protein fraction that is extracted and refined from soybeans. The SSPS consists of D-galactose, L-arabinose, D-galacturonic acid and L-rhamnose [10]. Although pectin is a frequently used stabilizer, SSPS may prevent protein coagulation without substantial enhancing of the viscosity and hence protecting the product quality [11]. Therefore, SSPS is used as stabilizer in acidified milk drinks, beverages, puddings and low-fat ice creams [12-14]. Furthermore, SSPS forms strong intermediary films [15], prevents oxidation of oils and includes good thermal stability and emulsifying properties [16]. These allow kefirs to be used in foods such as baked goods, dairy products and dressings [11]. Moreover, prebiotic properties of the SSPS have recently been verified [17]. It has been shown that SSPS includes capability to form gel networks inside the human digestive system allowing SSPS to prevent food degradation and entrap glucose molecules and hence lowering the rate of sugar release after food consumption [13,17]. Prebiotics are food supplements called as functional foods, which are foods play significant roles in avoidance and lessening of risk factors of numerous diseases and are proficient of improving certain imperative physiological roles [18]. Prebiotics are non-viable food components that confer health benefits in hosts, associated with modulation of the intestinal microbiota [7]. To the best of the authors' knowledge, no data are available on effects of added SSPS in kefirs.

So, the aim of this study was to assess the effects of addition of SSPS on physiochemical (acidity and viscosity) and sensory properties as well as microbial quality (yeast and LAB counts) of kefirs produced with cow and buffalo milk during one month of cold storage.

2. Materials and methods

The cow milk and buffalo milk were provided from the Animal Husbandry Research Station of Agricultural Sciences and Natural Resources University of Khuzestan, Southwest-ern Iran (Mollasani, Khuzestan, Iran). The edible SSPS was provided by Fuji Oil Chemical Co., Ltd. (Osaka, Japan).

2.1 Activation of kefir grains

The kefir grains were prepared in the Laboratory of Food microbiology of agricultural sciences and natural resources university of Khuzestan. These were preserved in Pasteurized milk at 4ºC. For activation, kefir grains were incubated in an incubator (TOBGVD-45 Binder, Germany) at 25ºC for 18-24 h and then used as kefir culture.

2.2 Production of kefir drinks

Fat contents of the buffalo milk were equally adjusted to fat contents of the cow milk (3.25%) through separating the fraction of milk fat using laboratory fat separator (Hermle Labortechnik GmbH Z 206, Germany). Briefly, 5 lit of cow milk and buffalo milk were used for the production of each kefir treatment. After heat processing (90ºC for 5 min), temperature of milk samples was reached to 70ºC and SPSS at concentrations of 0 (control), 0.5, 1 and 1.5% (w v^{-1}) were added to the samples and stirred gently for 10 min [19]. Then, temperature was quickly adjusted to 25ºC and kefir grains (3% w v^{-1}) were added to each treatment and fermented for 24 h. After fermentation, milk solids and salt of the cow and buffalo kefir samples were adjusted to 5 and 0.5%, respectively. Kefir samples were stored at 4°C and assessed for physic-chemical, microbial and sensory properties on Days 1, 10, 20 and 30 of storage.

2.3 Physicochemical properties analysis

Acidity (percentage of lactic acid), ash, fat, protein and dry matter of milks were assessed based on the methods of the Association of Official Analytical Chemists (AOAC) [20]. Viscosity of the kefir samples were assessed using Ostwald Viscometer DV2T Extra Touch Screen, (Ostwald, Brookfield, USA) and spindle No. 61 at 50 rpm. Color assessment was carried out using Minolta Colorimeter Model CR-400 (Konica Minolta, Osaka, Japan) and CIE L*a*b* value scales; where, L* indicated lightness, including values in a range of 0 (black) to 100 (white), *a** included positive values for reddish colors and negative for the greenish colors and b* included positive values for yellowish colors and negative values for bluish ones.

2.4 Fat extraction and free fatty acid (FFA) profiling

Fat extraction and FFA profiling were carried out as previously described with some modifications [21]. After milk fat separation by centrifugation at 6,000 ×g for 15 min in 4°C (Eppendorf AG 22331, Germany), trans-methylation was achieved using boron trifluoride in methanol. The FFA assessment was carried out using gas chromatograph (GC; Unicam 4600, Unicam, Cambridge, UK) equipped with a flame ionization detector (FID) and a fused-silica capillary column (BPX70; SGE, Melbourne, Australia) with 30 m × 0.25 mm × 0.22 m film thickness. Detector and injector were held at 300 and 250ºC, respectively. Helium was used as carrier gas. Results were expressed as percentage of each FFA with respect to the total FFA.

2.5 Sensory evaluation

Sensory evaluation (overall acceptance) of the kefir samples was carried out by ten trained panelists aged 24-45 (six females and four males). For each product, panelists were asked to indicate a mark on a 9-point hedonic scale based on the overall quality. Grades of the scale included awfully dislike (1), very dislike (2), moderately dislike (3),

somewhat dislike (4), not dislike nor like (5), slightly like (6), moderately like (7), very like (8) and extremely like (9). Overall acceptability of the kefir samples was evaluated after kefir temperature reached to ambient temperature (20°C). Kefir drinks were gently mixed and poured into 100-ml transparent plastic cups (approximately 20 g) set in white plastic dishes and offered to the panelists. All treatments were encoded randomly. Bottled water was provided to clean the mouth between the sample evaluations. Panelists were asked to describe their own comments/suggestions on the assessment questionnaires.

2.6 Microbial analysis

Total number of the LAB was enumerated on MRS agar (Liofilchem, Italy) and incubated under anaerobic condition at 37°C for 72 h using pour plate technique. Yeast count was carried out using potato dextrose agar (Merck, Germany) and surface plate technique. After sterilization of potato dextrose agar at 121°C for 15 min, 10 mg l^{-1} of the tetracycline hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) were added to the media to inhibit the growth of bacteria. Proper dilutions of the kefir samples were prepared and aliquots of each dilution was inoculated onto the culture media. Incubation of yeast was carried out at 28°C under aerobic conditions for 3-5 days. Viable cell counts of the LAB and yeasts were calculated and expressed as log CFU ml^{-1}.

2.7 Statistical analysis

Experiments were carried out using completely randomized factorial design with three replications. One-way analysis of variance test was carried out using SPSS Software v.20.0 (SPSS Science, Chicago, IL, USA). Duncan's multiple range test was used to show significant differences of the mean values at $P \leq 0.05$.

3. Results and discussion

3.1 Physicochemical characteristics of the cow and buffalo milks

Table 1 shows chemical composition of the cow milk and buffalo milk used for kefir production. The buffalo milk in equivalent fat content (3.25%) included higher total solid, protein, lactose and ash contents but lower protein/MSNF ratio, compared to that the cow milk did. In fact, buffalo milk is further whitish, yellowish and less greenish, compared to that the cow milk is. Specific color characteristics of the buffalo milk are owned to milk high casein concentration and absence of β-carotene, compared to that specific color characteristics of the cow milk are [22,23]. Similar to these results, Petridis et al. reported higher L* and the lower b* values for yogurt samples incorporated with buffalo milk [24].

3.2 Fatty acid (FA) composition

The FA profile analysis of various milk and kefir samples are listed in Table 2. In general, the cow milk significantly included higher saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) and lower unsaturated fatty acid (UFA) and mono-unsaturated fatty acid (MUFA) contents, compared to that the buffalo milk did ($P \leq 0.01$). By converting cow milk to cow kefir, significant changes in FA compositions were detected while these changes were not significant in buffalo milk (Table 2). In contrast, cow kefir included lower SFA and higher UFA, MUFA and PUFA than that cow milk and buffalo milk and buffalo kefir did. Studies have shown that fermentation of dairy products by LAB may affect chemical constituents, particularly increase or decrease of their FA compositions [25]. Ghoneem et al. showed that increases in FA contents might be due to oxidative deamination and decarboxylation of the amino acids, which converted amino acids into their corresponding FAs [26]. The current results were similar to results by Kavas, who reported lower SFA and higher PUFA contents in kefir samples produced with kefir grains, compared to cow milk [5]. Yadav et al. reported that addition of probiotic *Lactobacillus (L.) acidophilus* and *L. casei* to dahi resulted in higher lipolytic activity and higher FFA, compared to routine dahi cultures [27]. Among all FAs, palmitic acid was the major SFA and oleic acid was the major MUFA in investigated kefir and milk samples. Guzel-Seydim et al. showed that kefirs included more oleic and linoleic acids, compared to that milk and yogurt did [28]. Results revealed that the long-chain UFA (C>20) in kefir samples were significantly removed and hence not detected in GC analysis. This was attributed to lipase/esterase enzymes released by kefir microorganisms during its fermentation [29]. Decreases in pentadecanoic acid (15:0) were attributed to LAB activity and their biochemical reactions. As a natural response to oxidative stresses, condition caused significant increase in FA desaturation [26].

Table 1. Physicochemical characteristics of the cow and buffalo milks used to produce kefirs (w w^{-1}) (mean ±SD)

Milk	Constituents based on	MSNF (%)	Initial Fat (%)	Protein (%)	Lactose (%)	Ash (%)	L*	a*	b*
Cow	Wet basis (%)	8.63±0.03	3.50±0.03	3.19±0.03	4.72±0.5	0.72±0.01	77.92±0.53	-3.17±0.04	2.70±0.08
	Compound/MSFN ratio			36.96	54.69	8.34			
Buffalo	Wet basis (%)	9.65±0.09	6.17±0.07	3.93±0.05	4.88±0.01	0.84±0.01	84.91±0.75	-2.72±0.04	3.37±0.15
	Compound/MSFN ratio			40.73±0.08	50.57	8.70			

Table 2. Fatty acid profiles of the milk and kefir samples containing 1.5% (w v^{-1}) of SSPS after 30 days of cold storage (mean ±SD)

Fatty acid (% w w^{-1})	Cow milk	Cow kefir	Buffalo milk	Buffalo kefir
Butyric acid (C4:0)	0.71±0.01	ND	1.26±0.01	ND
Caproic acid (C6:0)	0.65±0.04	0.97±0.010	0.63±0.03	1.31±0.07
Caprylic acid (C8:0)	0.44±0.01	1.31±0.01	0.38±0.01	0.83±0.01
Capric acid (C10:0)	2.29±0.05	1.99±0.06	0.96±0.04	1.35±0.02
Lauric acid (C12:0)	3.32±0.54	ND	1.56±0.02	ND
Tridecylic acid (C13:0)	0.13±0.01	ND	0.06±0.01	ND
Myristic acid (C14:0)	12.43±0.61	9.12±0.08	9.96±0.03	9.13±0.67
Myristoleic acid (C14:1)	1.33±0.01	1.36±0.04	0.98±0.01	0.41±0.01
Pentadecylic acid (C15:0)	0.75±0.04	ND	0.80±0.04	ND
Palmitic acid (C16:0)	36.75±2.05	31.94±1.20	34.03±0.01	33.12±1.12
Palmitoleic acid (C16:1)	1.97±0.06	2.25±0.03	1.85±0.03	2.13±0.05
Margaric acid (C17:0)	2.06±0.02	0.73±0.03	1.37±0.01	0.70±0.03
Stearic acid (C18:0)	8.93±0.63	10.89±0.56	15.07±0.03	16.27±0.93
Oleic acid (C18:1)	22.67±1.01	32.48±2.06	28.09±0.04	28.55±1.34
Linoleic acid (C18:2)	4.12±0.08	5.17±0.09	2.13±0.01	4.72±0.00
Linolenic acid(C18:3)	0.86±0.01	0.29±0.00	1.37±0.03	ND
Arachidic acid (C20:0)	0.32±0.07	1.14±0.00	0.14±0.02	0.41±0.03
Gondoic acid (C20:1)	0.15±0.07	ND	0.16±0.04	ND
Eicosenoic acid (C20:2)	0.04±0.01	ND	0.05±0.01	ND
Arachidonic acid (C20:4)	0.06±0.01	ND	0.09±0.01	ND
Eicosapentaenoic acid (C20:5)	0.02±0.06	ND	0.03±0.03	ND
Docosahexaenoic acid (C22:6)	0.01±0.04	ND	0.04±0.02	ND
ΣSFA	69.09	58.09	65.21	64.38
ΣUFA	31.23	41.55	34.79	35.81
ΣMUFA	26.12	36.09	31.08	31.09
ΣPUFA	5.11	5.46	3.71	4.72

SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; ND, not detected

3.3 Acidity

Effects of various SPSS concentrations on kefir acidity during the storage period (30 days) are shown in Table 3. Results showed that increased concentrations of SPSS up to 1% increased acidities of cow and buffalo kefir significantly (P≤0.01). Furthermore, cow kefir samples included significantly a higher titratable acidity, compared to that buffalo kefir samples did (P≤0.05). By extending storage time, acidity of all kefir samples was extended significantly (P≤0.01). The highest acidity was recorded in cow kefir samples containing 1.5% (w v^{-1}) of SPSS at the end of storage (0.95% acidity) and the lowest was recorded in buffalo kefir samples (containing 0% of SSPS) at the first day of cold storage (0.32%). Possible reasons for the increased acidity of kefir beverages at the end of storage period are linked to activity of kefir microorganisms, conversion of food elements to organic acids and missing ability of yeasts to decompose organic acids produced by LAB [7]. Similar changes in acidity of kefir samples during storage have been reported in other studies [7,8,30,31]. In contrast, Kok-Tas et al. reported that acidity of kefir samples did not change during the storage period [32].

Table 3. Effects of milk type and SSPS concentration on the titratable acidity of kefir samples (based on percentage of lactic acid) during storage at 4°C (mean ±SD)

Milk	SSPS (% w v^{-1})	Storage period (Day)			
		1	10	20	30
Cow	0	$0.41±0.01^{Ac}$	$0.63±0.01^{ABb}$	$0.84±0.01^{BCa}$	$0.84±0.03^{Ca}$
	0.5	$0.44± 0.02^{Ac}$	$0.63±0.02^{ABb}$	$0.85±0.06^{Ba}$	$0.86±.05^{BCa}$
	1	$0.49±0.05^{Ac}$	$0.65±0.03^{Ab}$	$0.88±0.07^{ABa}$	$0.89±0.03^{ABa}$
	1.5	$0.51±0.06^{Ac}$	$0.66±0.04^{Ab}$	$0.93 ±0.06^{Aa}$	$0.95 ±0.08^{Aa}$
Buffalo	0	$0.32±0.01^{Ec}$	$0.53±0.02^{Db}$	$0.67±0.01^{Fa}$	$0.69±0.05^{Ea}$
	0.5	$0.33±0.02^{Ec}$	$0.55±0.03^{Db}$	$0.72±0.03^{Ea}$	$0.73 ±0.04^{DEa}$
	1	$0.35±0.03^{Dc}$	$0.57±0.03^{CDb}$	$0.76±0.01^{DEa}$	$0.77±0.04^{Da}$
	1.5	$0.37±0.01^{Cc}$	$0.60±0.05^{BCb}$	$0.78±0.04^{CDa}$	$0.79±0.05^{CDa}$

Means shown with different capital and small letters in the same columns and rows represent significant differences, respectively (P≤0.05)

3-4 Viscosity

Table 4 shows effects of various SSPS concentrations and storage time on viscosity of the kefir samples. Results showed that samples with a higher SSPS concentration included a higher viscosity with significant differences ($P \leq 0.05$). Chen et al. demonstrated that SSPS included a great flexibility with low-level molecular interactions in solutions, resulting in low viscosities, compared to other hydrocolloids [13]. Furthermore, Fabek and Goff demonstrated that SSPS addition to protein-starch solutions distinctly decreased starch hydrolysis, resulting in decreased glucose releases in digestive system through the inhibition of gastrointestinal enzymes [11]. As stated previously, SSPS may protect protein particles from coagulation and hence sustain primary characteristics of the products. Therefore, addition of perceptible levels of SSPS, as favorite fibers, to fortify dairy foods is broadly suggested [10-13].

Similar to titratable acidity, milk type included significant effects on viscosity of cow milk kefirs (mean value of 52.89 cp), compared to buffalo milk kefirs (mean value of 37.41 cp). Furthermore, by extending storage time, all kefir samples were become more viscose; with changes in buffalo kefir samples were slightly more significant than changes in cow kefir samples. Viscosity of the cow kefir samples increased from 49.60 cp at the beginning of storage to its maximum level of 56.75 cp (an increase rate of 14.42%) at the end of storage while viscosity level of the buffalo kefir samples was developed during one month of storage from 34.05 cp to its maximum level 40.15 cp (an increase rate of 16.59%). In this study, increases in viscosity of the samples during storage could be attributed to the activity of kefir microorganisms, which produced a significant quantity of exopolysaccharides, particularly kefiran [3]. Nagovska et al. showed that increases in viscosity during storage could be explained by the presence of acetic microflora in kefir, which is the major cause of high viscosity of products even after expiration [8]. Similar results were reported by Temiz and Dagyıldız who reported increases in viscosity of kefirs during 20 days of storage [30]. In contrast, Sabooni et al. reported considerable decreases in viscosity of kefir samples containing transglutaminase and xanthan gum during storage ($P \leq 0.05$) [9]. Decreases in viscosity during storage are associated to the microbial enzyme activity on the matrix of casein network [33].

3.5 Total number of microorganisms

3-5-1 Total number of lactic acid bacteria

Effects of various SSPS concentrations and storage time on the count of LAB kefir samples are shown in Table 5. As the concentrations of SSPS increased in kefir samples, the LAB counts of cow and buffalo kefir samples increased significantly ($P \leq 0.05$). Although buffalo kefir samples usually included a lower LAB count, differences between these groups were not significant ($P > 0.05$). The higher extents of titratable acidity in cow kefir samples are linked to these results. The mean LAB counts in cow and buffalo kefir samples containing 1.5% of SSPS were recorded as 8.52 and 8.49 log CFU ml^{-1}, respectively. These were significantly lower, recorded as 7.96 and 7.87 log CFU ml^{-1} for cow and buffalo kefir control samples (0% of SSPS), respectively. These results were similar to results by Ying et al. [34] and Perez-Lopez et al. [17] who reported positive effects of dietary fibers on count of the probiotic bacteria.

Table 4. Effects of milk type and SSPS on the viscosity (cP: centipoise) of kefir samples during storage at 4°C (mean ±SD)

Milk	SSPS (% w v^{-1})	Storage period (Day)			
		1	10	20	30
Cow	0	46.72 ± 1.25^{Cc}	47.91 ± 1.22^{Cbc}	50.81 ± 0.95^{Cb}	54.48 ± 1.18^{Ca}
	0.5	47.04 ± 1.12^{Cc}	49.15 ± 1.57^{Cbc}	51.52 ± 1.48^{Cb}	55.52 ± 2.48^{BCa}
	1	51.02 ± 1.54^{Bc}	53.71 ± 2.60^{Bb}	54.55 ± 1.21^{Bb}	57.55 ± 1.21^{Aba}
	1.5	53.61 ± 2.03^{Ac}	55.79 ± 2.45^{Abc}	57.45 ± 2.36^{Aab}	59.45 ± 2.36^{Aa}
Buffalo	0	31.73 ± 0.82^{Fd}	33.12 ± 1.58^{Fc}	35.07 ± 0.98^{Fb}	37.79 ± 1.65^{Fa}
	0.5	32.94 ± 1.45^{EFc}	36.83 ± 1.49^{Eb}	38.23 ± 1.45^{Eab}	39.00 ± 1.52^{EFa}
	1	34.21 ± 1.11^{Ec}	36.96 ± 1.27^{Eb}	40.48 ± 0.37^{Da}	41.81 ± 1.38^{DEa}
	1.5	37.31 ± 1.65^{Dc}	39.08 ± 1.67^{Db}	41.89 ± 1.79^{Da}	42.00 ± 0.45^{Da}

Means shown with different capital and small letters in the same columns and rows represent significant differences, respectively ($P \leq 0.05$)

Table 5. Effects of milk type and SSPS concentration on LAB counts (log CFU ml^{-1}) in kefir samples during storage at 4°C (mean ±SD)

Kefir	SSPS (% w v^{-1})	Storage period (Day)			
		1	10	20	30
Cow	0	7.82 ± 0.05^{DEb}	7.96 ± 0.12^{Cab}	8.13 ± 0.02^{CDa}	7.92 ± 0.44^{Cab}
	0.5	7.95 ± 0.18^{CDb}	8.31 ± 0.13^{Ba}	8.42 ± 0.01^{ABa}	8.24 ± 0.16^{Ba}
	1	$8.15\pm0.06A^{BCb}$	8.53 ± 0.10^{Aa}	8.56 ± 0.12^{Aa}	8.53 ± 0.20^{Aa}
	1.5	8.36 ± 0.04^{Ab}	8.55 ± 0.06^{Aab}	8.61 ± 0.11^{Aa}	8.55 ± 0.21^{Aab}
Buffalo	0	7.71 ± 0.08^{Eb}	7.97 ± 0.03^{Ca}	7.98 ± 0.03^{Da}	7.83 ± 0.06^{Cab}
	0.5	7.85 ± 0.08^{DEb}	8.28 ± 0.11^{Ba}	8.30 ± 0.14^{BCa}	8.21 ± 0.09^{Ba}
	1	8.10 ± 0.07^{BCb}	8.52 ± 0.07^{Aa}	8.50 ± 0.08^{ABa}	8.48 ± 0.13^{Aa}
	1.5	8.33 ± 0.05^{ABb}	8.55 ± 0.07^{Aa}	8.58 ± 0.10^{Aa}	8.53 ± 0.10^{Aa}

Means shown with different capital and small letters in the same columns and rows represent significant differences, respectively (P≤0.05)

As seen in Table 5, storage time included statistically significant effects on the bacterial population. Through the storage, significant increases in LAB count of all kefir samples were recorded, with a slightly higher rate in buffalo kefir samples. However, no significant differences were recorded between the LAB counts of various kefir samples during storage from day 10 to the last day. The primary means of LAB count at the first day of storage (8.03 log CFU ml^{-1}) increased significantly on day 10 (8.33 log CFU ml^{-1}) and then mildly increased on Day 20 (8.38 log CFU ml^{-1}) but then slightly decreased (8.29 log CFU ml^{-1}) on day 30 of storage. This might be due to depletion of substrates for bacterial growth. Guzel-Seydim et al. reported increased number of total LAB, lactobacilli and lactococci in non-polysaccharide added kefir samples at the initial stages of storage [35]. They reported mild decreases in number of lactobacilli and lactococci at the end of Day 21 of storage. Temiz and Dagyıldız reported that the lactobacilli count in kefir beverages with no additional polysaccharides decreased progressively from 7.66 to 5.54 log CFU ml^{-1} through 30 days of storage [30]. Decreases in LAB count, particularly at the end of cold storage, have been attributed to the production of significant quantities of organic acids [36] and enhancement of hydrogen peroxide concentrations [37] caused by the metabolic activity of LAB.

3.5.2 Total number of yeasts

In general, kefir grains are mixed starter cultures of three microbial groups of LAB (*Lactobacillus*, *Lactococcus*, *Streptococcus*, *Enterococcus* and *Leuconostoc* sp.), yeasts (*Kluyveromyces*, *Candida*, *Saccharomyces*, *Pichia* and *Rhodotorula* sp.) and acetic acid bacteria (*Acetobacter* and *Gluconobacter* sp.) in a hetero-polysaccharides matrix known as kefiran. Effects of SSPS concentration and milk type on yeast population of cow and buffalo kefir samples during 30 days of cold storage are presented in Table 6. The yeast count in all kefir beverages (6.72 log CFU ml^{-1}) was lower than the LAB content (8.26 log CFU ml^{-1}). Similar results were reported by Guzel-Seydim et al. who reported a less count (6.28 log CFU ml^{-1}) in compare to LAB count (9.04 log CFU ml^{-1}) through 21 days of storage [35]. As the incorporation of SSPS in kefir samples increased from 0 to 1.5%, the count of yeasts increased progressively from 6.57 to 6.88 log CFU ml^{-1}. Similar to the LAB count, milk type included no significant effects on the yeast count of kefir samples produced from cow milk and buffalo milk (6.75 instead of 6.69 log CFU ml^{-1}, respectively). Low difference in yeast population of the cow and buffalo kefir samples could be resulted from differences in the milk components [2].

Table 6. Effects of milk type and SSPS concentration on the population of yeasts (log CFU ml^{-1}) in kefir samples during storage at 4°C (mean ±SD)

Milk	SSPS (% w v^{-1})	Storage period (Day)			
		1	10	20	30
Cow	0	6.34 ± 0.20^{Ab}	6.48 ± 0.08^{BCab}	6.61 ± 0.20^{BCab}	6.77 ± 0.17^{Da}
	0.5	6.38 ± 0.24^{Ac}	6.65 ± 0.20^{ABCbc}	6.83 ± 0.16^{ABab}	7.01 ± 0.36^{ABCDa}
	1	6.36 ± 0.19^{Ac}	6.78 ± 0.26^{ABb}	6.99 ± 0.21^{Aab}	7.16 ± 0.11^{ABCa}
	1.5	6.39 ± 0.23^{Ac}	6.90 ± 0.27^{Ab}	7.07 ± 0.14^{Aab}	7.29 ± 0.32^{Aa}
Buffalo	0	6.30 ± 0.19^{Ab}	6.43 ± 0.28^{Cb}	6.54 ± 0.14^{Cb}	6.83 ± 0.28^{CDa}
	0.5	6.43 ± 0.13^{Ab}	6.58 ± 0.10^{BCb}	6.68 ± 0.24^{BCab}	6.88 ± 0.17^{BCDa}
	1	6.44 ± 0.12^{Ab}	6.66 ± 0.18^{ABCab}	6.82 ± 0.22^{ABa}	6.97 ± 0.33^{ABCDa}
	1.5	6.49 ± 0.23^{Ac}	6.72 ± 0.30^{ABCbc}	6.99 ± 0.27^{Aab}	7.22 ± 0.16^{Aba}

Means shown with different capital and small letters in the same columns and rows represent significant differences, respectively (P≤0.05)

The storage time included significant effects on the yeast population since the initial count of 6.39 log CFU ml^{-1} at the first day steadily increased to 7.02 log CFU ml^{-1} at the end of 30 days of storage. As presented in Table 6, a direct relationship exists between the numbers of yeasts and LAB through the storage. Acidification by LAB enhances growth of yeasts and production of amino acids and vitamin B6 by yeasts stimulates growth of lactobacilli. Montanuci et al. have shown that increased count of the yeasts during storage was due to the bacterial growth in environment and use of bacterial produced compounds (such as organic acids) by yeasts [7]. Increases in yeast growth have correlated to increases in ethanol concentration during storage [38]. Similar to the present results, Guzel-Seydim et al. reported that the means of yeast counts for the kefir samples continuously increased from 6.28 to 6.56 log CFU ml^{-1} during 21 days of storage [35]. Increases of yeasts during storage have been reported by other studies [34, 39-40].

3.6 Sensory evaluation

Sensory attributes of various fermented dairy products such as yoghurt and kefir depend on their physicochemical characteristics, especially titratable acidity, serum separation and consistency/viscosity parameters [41]. Figure 1 shows the overall acceptance of kefir samples during 30 days of storage. Results showed that similar to other evaluated parameters, the total acceptance of kefir samples were significantly affected by the addition of SSPS and usually a direct relationship is seen between the SSPS addition and sensory scores. By using polysaccharides in beverages, rheological behaviors of the products can be modified and its stability may be improved. However, the perceived flavor strength of products may be prevented. This is possibly due to inadequate dispersing/dissolving of polysaccharides in drink solutions with a substantial quantity of polymer entrapments [42]. Therefore, since SSPS is incapable of enhancing viscosity of the beverages largely, it cannot be used to improve rheological properties and expand flavor characteristics of the products. In a similar study, Sabooni et al. reported that adding xanthan gum could improve the rheological properties of kefirs [9]. In a study by Chen et al., the flavor acceptability of ice creams decreased as the SSPS concentration increased, scoring from “like slightly” for 2% to “neither like nor dislike” for 4% SSPS concentrations [13].

In this study, buffalo kefir samples included higher overall sensory scores than that cow kefir samples did. However, these differences were not significant ($P>0.05$). As previously highlighted, cow kefir samples included higher quantities of MUFA and PUFA, compared to that buffalo kefir samples did. Degradation of these compounds during fermentation results in odor enhancement. Furthermore, cow milk fat contains higher quantities of β-ketoglycerides (approximately two folds) and methyl ketones than that buffalo milk fat does [43]. These compounds include critical effects on sensory attributes of the milk and its products. However, due to superior characteristics of buffalo milk (e.g. a further whitish color and a lower acidity, compared to cow milk), no significant differences were found between the overall acceptability of cow and buffalo kefir samples. In the other words, buffalo kefir samples received higher scores of color and the moderate acidity but lower scores of odor and other taste characteristics (results are not shown). The overall acceptance for cow kefir samples containing 1 and 1.5% (w v^{-1}) of SSPS included 8.09 and 8.21 points, respectively. These for buffalo kefir samples containing 1 and 1.5% (w v^{-1}) of SSPS included 7.96 and 8.11 points, respectively.

By increasing storage time up to 20 days, a significant increase ($P\leq0.05$) in overall acceptability was observed. However, this decreased significantly up to the end of storage time (Figure 1). Increased sensory scores during storage are significantly linked to the yeast activity. Yeasts use metabolites derived from the bacterial growth in kefirs and produce various compounds such as acetaldehyde, alcohol and carbon dioxide, which provide appropriate organoleptic properties to the final products [7]. The lower sensory scores at the end of storage could be associated to loss of carbonyl compounds [30] and over developed acidity (Table 3). The highest score (8.61 points) was linked to cow kefir samples containing 1.5% of SSPS on day 20 and the lowest score (7.32 points) was associated to buffalo kefir control samples containing 0% of SSPS on day 1 of storage.

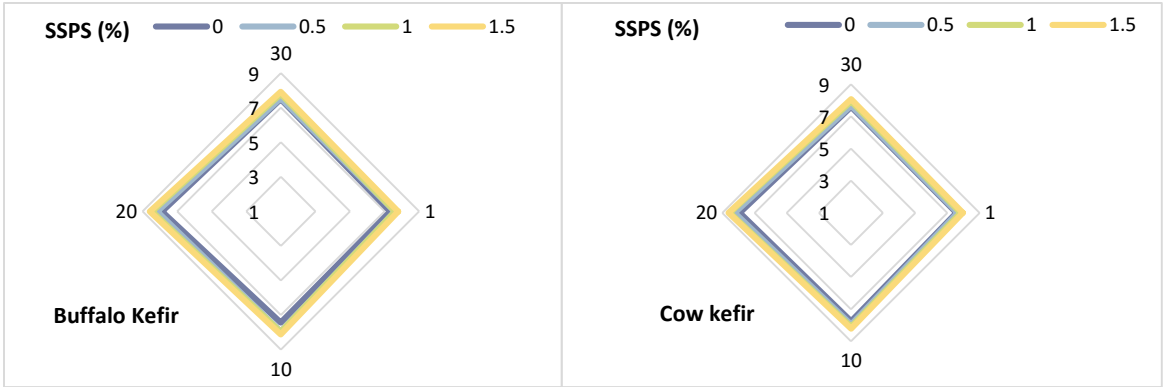

Figure 1. Effects of milk type and SSPS concentration on the overall acceptance of kefir samples during 30 days of storage at 4°C

4. Conclusion

In general, addition of SSPS to kefir formulations stimulated kefir cultures. As the SSPS concentrations increased, the acidity, viscosity and sensory scores and LAB and yeast counts increased significantly. Similar results were found in all parameters over the storage time. Based on the sensory evaluation, the highest scores were recorded for the kefir samples containing higher levels of SSPS (1 and 1.5%), while no significant differences were seen between the kefir types. Counts of LAB and yeasts were significant, particularly at the end of storage time, with more than 10^6 and 10^8 log CFU ml^{-1}, respectively. These microorganisms are categorized as probiotics and may include beneficial effects on the human therapeutic methods. The current findings revealed that cow and buffalo kefir included higher quantities of UFA, compared to cow milk and buffalo milk. Therefore, kefirs could be regarded as further valuable and healthier dairies than their original milks due to the changes in FA profiles of the kefir samples. Since SSPS is not digestible by the human digestive system and since a direct relationship exists between the kefir microbial count and the SSPS concentration, SSPS in kefirs can potentially include prebiotic effects in the gut of consumers. Regarding beneficial health effects of the additive SSPS, FA compositions of the kefirs (compared to milks and kefirs without SSPS), considerable counts of the probiotic microbiota and nutritional values of the cow milk and buffalo milk constituents, the produced kefirs can be regarded as functional foods and their consumption recommended to promote health and performance of the human organs. In conclusion, microorganisms of the kefir grains included potentials to use 1-1.5% (w v^{-1}) of SSPS as a prebiotic compound for their growth and the best overall acceptance of kefirs was seen on Day 30 of storage.

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6. Conflict of interest

The authors report no conflicts of interest.

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تأثیر پلی‌ساکارید محلول در آب سویا بر خصوصیات کفیر تولید شده از شیر گاو و گاومیش

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چکیده

سابقه و هدف: فرآورده‌های تخمیری لبنی به‌طور قابل توجهی به دلیل فواید زیادی مانند ارزش غذایی بالا، تحریک سیستم ایمنی بدن، اثرات ضد میکروبی و خواص ضد سرطانی شناخته شده‌اند. کفیر نوعی نوشیدنی تخمیری شیری با طعم اسیدی-الکلی است که از منابع مختلف شیر با خصوصیاتی متفاوت تولید می‌گردد. هدف از این مطالعه بررسی اثرات افزودن پلی‌ساکاریدهای محلول در آب سویا بر ویژگی‌های کفیر تولید شده از شیر گاو و گاومیش بود.

مواد و روش ها: پلی‌ساکاریدهای محلول در آب سویا در غلظت‌های ۰(نمونه شاهد)، ۰/۵، ۱و ۱/۵ درصد (وزنی/حجمی) به نمونه‌های کفیر تهیه شده از شیر گاو و گاومیش اضافه و خصوصیات فیزیکوشیمیایی، حسی و میکروبی آن‌ها به همراه پروفایل اسیدهای چرب در مدت یک ماه نگهداری سرد مورد مقایسه قرار گرفت.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد که پلی‌ساکاریدهای محلول در آب سویا تأثیر معنی‌داری ($p<۰/۰۵$) بر خواص کفیر داشت. با افزایش غلظت پلی‌ساکاریدهای محلول در آب سویا و زمان نگهداری، اسیدیته، گرانروی، امتیاز حسی و تعداد باکتری های اسید لاکتیک و مخمرها افزایش یافت. آزمایش آنالیز اسیدهای چرب نشان داد که مقادیر اسید چرب غیراشباع در هر دو نوشیدنی کفیر گاو و گاومیش، بیشتر از شیرهای آن‌ها بود، در حالی‌که این تغییرات برای اسیدهای چرب اشباع برعکس بود. بهترین خواص حسی و میکروبی کفیرها با افزودن ۰/۵ تا ۲ درصد پلی‌ساکاریدهای محلول در آب سویا در روز سی ام نگهداری مشاهده شد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- باکتری‌های لاکتیک اسید
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- آب‌اندازی

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