Process optimization of fortified sweetened milk kefir

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Abstract: Kefir is an acidic-alcoholic fermented milk product. Regular consumption of kefir is helpful in relieving intestinal disorders, reducing flatulence, and creating healthier digestive system. An attempt was made to optimise the process to produce fortified sweetened milk kefir. Kefir was prepared using kefir grains with sugar (6, 8 and 10%), fibres (inulin, oat, and soya, each 3%) and stabilizer (0.1% pectin). Addition of 6% sugar showed significantly ($p < 0.05$) higher body and texture scores with no significant ($p > 0.05$) difference in flavour, colour and appearance when compared to kefir made with 8 and 10% sugar. Similarly, overall acceptability was highest in kefir made with 6% sugar. Firmness in kefir was highest made with 6% sugar, followed by 8 and then 10% sugar. Among the three fibers, addition of 3% inulin showed significantly ($p < 0.05$) better sensory attributes when compared to kefir added with soya and oat fibre. Acidity of inulin added kefir was lowest among soya and oat fibre added kefirs which was optimum. Kefir prepared with soya fibre showed significantly ($p < 0.05$) higher acidity than other two samples. No significant ($p > 0.05$) difference was observed in pH among all kefir samples. Firmness and consistency increased significantly ($p < 0.05$) with the addition of inulin. An acceptable quality fortified sweetened milk kefir can be prepared with 0.1% pectin, 6% sugar and 3% inulin as dietary fibre for improved health benefit.

Keywords: Colour, Dietary fibre, Flavour, Health benefit, Sweetened milk kefir

Introduction

Kefir is a fermented milk product prepared by incubating milk with either kefir grains (a group of microorganisms held together by a polysaccharide framework i.e., Kefiran) or kefir starter culture (Satir and Guzel-Seydim, 2016). Kefir differs from other fermented milk products in a way that is not a simple consequence of the metabolic action of a single microbial species but a blended microflora i.e., 66% bacilli, 16% streptococci, and 18% yeasts (Farnworth, 2003). Enthusiasm has evolved for the development, production, and utilization of kefir since its beginning, owing to its immense therapeutic benefits on the immune system, the gastrointestinal system and the digestion of cholesterol (Tamai et al. 1996). Moreover, anti-cancer, anti-bacterial and anti-fungal properties have been exhibited *in vitro*, animals or human models (De Moreno de LeBlanc et al. 2007). Additionally, kefir cultures can be linked to promote food safety by restraining coliforms and several pathogens (Van Wyk et al. 2011).

As kefir has a slightly acidic taste, yeasty and tangy flavour, sweetening of kefir will make the taste milder, improve flavour and overall acceptability which will be more relished by the Indian populace. In eastern part of India, *dahi* is used as *mistido/dahi* by adding sweetener such as jaggery which lends a caramelized flavour and colour to the *dahi*. Sweetening will make the kefir a product like *mistidahi*. Cool sweet milk kefir will be a thirst quenching and refreshing product with prickly sensation. Sweetness of the kefir will provide an exotic enrichment to the taste buds. Yet, no attempt has been made so far to prepare sweetened milk kefir and to evaluate the microbial behaviour of kefir grains in the presence of sugar.

Milk and most milk products are devoid of dietary fibre, but there is a growing awareness among consumers about the physiological benefits of fibre fortified milk products. Dietary fibres impart some basic functional characteristics like water holding capacity, viscosity, water swelling capacity, fat binding capacity, antioxidant properties etc. (Elleuch et al. 2011) and improving sensory characteristics and shelf- life of the product. Various published reports have demonstrated that there are numerous therapeutic benefits related with the consumption of dietary fibre, including lower risk of heart diseases, diabetes,

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obesity, and a few types of malignancy (Mann and Cummings 2009). Addition of inulin has shown that it can attenuate the cholesterol in blood plasma (Roberfroid, 2005) and improves calcium, magnesium, and iron absorption (Bosscher et al. 2003). Addition of oat fibres is linked with depletion of post-prandial blood glucose and insulin responses (Wood et al. 1994), higher transport of bile acids towards lower portions of the gut and higher discharges of bile acids (Dongowski et al. 2005) or reduction of serum cholesterol levels (Hecker et al. 1998). Yogurt fortified with soya is advantageous in reducing cholesterol levels (Crouse et al. 1999), blood pressure and to alleviate indications of menopause and osteoporosis (Messina et al. 2004). To know the beneficial effects of kefir consumption, an attempt was made to optimize the process for preparation of fortified sweetened milk kefir.

Materials and Methods

Raw materials and ingredients

Fresh whole and skim milk were procured from the Experimental Dairy Plant of ICAR- National Dairy Research Institute, Bengaluru (India). Kefir grains were obtained from U.S. Food grade High methoxy pectin obtained from M/s Hi Media laboratories Pvt. Ltd. Refined crystalline cane sugar from local market, Dietary soya fibre (*Fimbrim*® from M/s Solae Company, U.S.), oat fibre (*Vitacel*® HF- 600® from M/s J. Rettenmair and Sohne GmbH, Germany) and inulin (from M/s DKSH India Pvt. Ltd., Bangalore, India) were procured.

Activation of kefir grains

Kefir grains were activated according to the technique adopted by Angulo et al. (1993). Following each incubation, straining of the fermented product was done through nylon sieve (mesh size: 1/20 inch) to retrieve the kefir grains which were then washed with sterile distilled water prior to next inoculation. This process was repeated 4-5 times until the desirable kefir flavour was achieved.

Preparation of fortified sweetened milk kefir

Fresh raw milk was standardized to 3% fat, preheated at 50-60°C and homogenized in two stage (first stage- 2500 psi, second stage- 500 psi) homogenizer. All the additives were added on milk basis into the milk after homogenization and before final heat treatment. During optimization process, first pectin (0.1%) and sugar (6, 8 and 10%) were mixed and then added to standardized milk followed by addition of fibres (inulin, oat, and soya fibre). Milk was heated to 90-92°C for 10 minutes with intermittent stirring followed by cooling (28-30°C) and filtration to remove any coagulated particles. Kefir grains were inoculated to the milk at a level of 4 g/L of milk and incubated at 30°C for about 20-24 h in B.O.D incubator till the titratable acidity had reached to 1% lactic acid. The product was thoroughly stirred and sieved through nylon sieve (mesh size: 1/

20 inch). After retrieving the kefir grains, the products were then filled in polypropylene cups and kept for maturation for 24 h at 6-8°C. Thereafter, the product was stored in the refrigerator.

Sensory evaluation

Sensorial assessment of the milk kefir was carried out by an expert panel of minimum five judges to judge the kefir samples on a 9-point hedonic scale (9=like extremely; 1=dislike extremely) at room temperature. Duo-trio tests were used to determine a candidate's ability to detect differences among similar products with different ingredients for selection of Judges.

Physico-chemical analysis

pH

The pH of kefir samples was measured directly by inserting the electrode into the sample followed by recording of reading.

Titrateable acidity

The acidity of kefir samples was measured as per the method of AOAC947.05 (2012) for milk. The sample was well mixed, and 10 g of sample was taken in a beaker. Phenolphthalein indicator of 2-3 drops was added and titrated against 0.1 N NaOH till the first appearance of faint pink colour. The acidity was expressed as % lactic acid by weight.

Textural analysis

Textural properties such as firmness, consistency, index of viscosity and stickiness were determined using TA-XT Plus Texture Analyser (Stable Microsystem, UK) with P/25 cylindrical probe. At 6-8°C, textural properties of kefir samples were measured using 200 ml kefir in a 250 ml beaker. The probe travelled at a speed of 1.0 mm/s up to 10 mm distance into the kefir sample from the surface and then returned to the original position generating force-time curve. The positive peak of the curve gave firmness (Newton), the negative peak gave stickiness (Newton), the area of positive peak gave consistency (Newton-second) and the area of negative peak gave index of viscosity (Newton-second).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using IBM SPSS statistics 23 software. Results of triplicate trials were used for statistical analysis.

Results and Discussion

Effect of sugar addition on sensory attributes of milk kefir

All sugar (6, 8 and 10%) added kefir samples scored higher for flavour as compared to control (Fig. 1 a). The most notable

Fig. 1 Effect of sugar addition on sensory attributes of milk kefir

function of sugar in food is its sweet taste. Sweet taste serves as a sensory cue for source of pleasure. Body and texture score of kefir with 6% sugar was significantly ($p < 0.05$) higher than other three kefir samples (Fig. 1 b). Sugar affected the physical characteristics of kefir to a significant degree. It provided solids which impacted the mouthfeel and texture of kefir as reported by Spillane (2006) for many fermented products. No significant ($p > 0.05$) difference was observed in colour and appearance among all kefir samples (Fig. 1 c). Overall acceptability score was maximum for kefir with 6% sugar followed by control, 8 and 10% sugar added kefir, respectively (Fig. 1 d). Akter et al. (2010) evaluated the different levels of sugar (8, 10, 12 and 14%) on qualitative characteristics of *mistidahi* (Sweet Yoghurt) and found that 10 and 12% sugar levels obtained more organoleptic score than those of 8 and 14%. So, the concentration of sugar affects the characteristics of the final fermented product - higher amount is detrimental to lactic acid bacteria and lower one does not change the original sour flavour. Similarly, in the present study an optimum 6% sugar produced the best kefir when compared to control, 8% and 10% sugar.

Effect of sugar addition on pH and acidity of milk kefir

No significant ($p > 0.05$) difference in pH and acidity was observed between the control and 6% sugar added kefir. Whereas kefir with 8 and 10% sugar showed significantly ($p < 0.05$) lower acidity and higher pH than the control and kefir with 6% sugar (Table 1), making them less preferred to other kefir samples. Sugar acts as a substrate for lactic acid bacteria and yeast species in kefir grains and is responsible for development of acidity during the fermentation. The amount of acidity present depends upon the concentration of sugar and incubation time. Yeasts are recognized in playing a key role in the preparation of fermented dairy products, where they provide essential growth nutrients such as amino acids and vitamins, alter pH, secrete ethanol, and produce CO_2 (Farnworth, 2003). Hence, lower acidity was observed in 8 and 10% sugar added kefir. Ghosh and Rajorhia (1990) also found similar results in *mistidahi* and stated that higher concentration of sugar showed inhibitory effect in the *mistidahi*. The activity of the *Lactobacillus* cultures was greatly affected with the increase in sucrose concentration which alters morphology by elongating and distorting the cell.

Fig. 2 Effect of sugar addition on textural properties of milk kefir

Effect of sugar addition on textural properties of milk kefir

Firmness was significantly ($p < 0.05$) higher for kefir with 6% sugar as compared to other kefir samples (Fig. 2 a). Kefir with 6% sugar showed highest firmness, followed by 8 and 10% sugar added kefir and then control kefir (Fig. 2 a). Kefir with 6% sugar showed the highest consistency among all kefir samples. Kefir with 8 and 10% sugar showed significantly ($p < 0.05$) higher consistency as compared to control kefir (Fig. 2 b). Similarly, kefir with 6% sugar exhibited significantly ($p < 0.05$) higher index of viscosity among the four types of kefir (Fig. 2 c). Stickiness was the highest in kefir with 6% sugar, followed by with 8, 10% sugar and control, respectively (Fig. 2 d). Textural properties of kefir were improved with the addition of sugar due to increase total solids content in sweetened milk kefir as compared to control. However, kefir with 8 and 10% sugar showed lower textural properties which may be due to higher CO_2 production in the kefir. Whey pockets and gas holes were observed with increasing sugar content in kefir. Since kefir added with 6% sugar showed the best sensorial attributes,

optimum pH and acidity and better textural properties, it was selected for further optimization of dietary fibre.

Effect of dietary fibres incorporation on sensory attributes of sweetened milk kefir

The effects of dietary fibre incorporation on the sensory attributes of kefir are displayed in Fig. 3. Six percent sugar was added in all samples. Control sample was added with sugar but without dietary fibre. Significantly ($p < 0.05$) higher flavour was observed in inulin added kefir among four samples (Fig. 3 a). Soya fibre added kefir showed significantly ($p < 0.05$) lower flavour than other kefirs (Fig. 3 a). The body and texture of kefir with inulin was the highest among all kefirs (Fig. 3 b). Inulin added kefir and control kefir showed no significant ($p > 0.05$) difference in colour and appearance (Fig. 3 c). Whereas oat fibre added kefir showed significantly ($p < 0.05$) inferior colour and appearance when compared to control but significantly ($p < 0.05$) better than soya fibre added kefir (Fig. 3 c). Overall acceptability of inulin added

Fig. 3 Effect of dietary fibres incorporation on sensory attributes of sweetened milk kefir

Table 1 Effect of sugar addition on acidity and pH of milk kefir

Attributes	Control	6% Sugar	8 % Sugar	10 % Sugar
Acidity	1.13 ± 0.01 ^b	1.07 ± 0.05 ^b	0.98 ± 0.02 ^a	0.99 ± 0.01 ^a
pH	4.43 ± 0.01 ^a	4.44 ± 0.01 ^a	4.49 ± 0.03 ^b	4.48 ± 0.02 ^b

Mean ± S.D; means with different superscripts in a row differ significantly ($p < 0.05$) ($n=3$)

kefir was maximum followed by control and then kefir added with oat and soya fibre, respectively (Fig. 3 d). The better sensory attributes of inulin added kefir might be because of additional sweetening effect of inulin which might have masked the acidic taste of kefir and improved the flavour (Allgeyer et al. 2010). No significant ($p > 0.05$) difference was found in terms of taste and consistency when inulin was added to kefir made with skim milk powder (Glibowski and Kowalska 2012). Similarly, Ertekin and Guzel- Seydim (2010) found that odour and taste of kefir samples were indifferent with or without inulin. Loss of flavour, colour and appearance in soya milk kefir fortified with soya fibre was also reported by Baú, et al. (2014). Although soya fibre is relatively

soluble as compared to oat fibre, but sensory attributes were least in kefir added with soya fibre. The lower sensory attributes of kefir with oat fibre were due to settling of fibres at the bottom of container during incubation which resulted in an unacceptable appearance.

Effect of dietary fibres incorporation on pH and acidity of sweetened milk kefir

Soya fibre added kefir showed significantly ($p < 0.05$) higher acidity as compared to other samples. No significant ($p > 0.05$) difference was observed in pH among all types of kefir (Table 2). Typically,

Fig. 4 Effect of dietary fibres incorporation on textural properties of sweetened milk kefir

Table 2 Effect of dietary fibres incorporation on acidity and pH of sweetened milk kefir

Attributes	Control kefir (6%S)	Soya fibre	Oat fibre	Inulin
Acidity	1.07 ± 0.12 ^a	1.21 ± 0.03 ^c	1.12 ± 0.06 ^{ab}	1.09 ± 0.03 ^{ab}
pH	4.45 ± 0.04 ^a	4.34 ± 0.12 ^a	4.42 ± 0.04 ^a	4.44 ± 0.02 ^a

Mean ± S.D; means with different superscripts in a row differ significantly ($p < 0.05$) ($n=3$)

pH of kefirs made in dairy industry ranged between pH 4.3 and 4.4, and similar pH values were observed in the present study, where addition of different fibers did not affect pH and acidity significantly ($p > 0.05$) except that higher acidity in soya fiber added kefir was observed. Baú et al. (2014) in the study with soya milk kefir prepared with soya fibre (3% w/w) showed higher acidity and lower pH as compared to kefir without soya fibre and they concluded that some fibre may stimulate the metabolism of the kefir grains resulting in higher acidity. Ertekin and Guzel-Seydim (2010) studied the effect of 2% inulin on physicochemical properties of kefir and pH was found to be between 4.29 and 4.40. They concluded that neither high performance nor native inulin did affect the pH changes in kefirs significantly ($p > 0.05$). Although in the present study, there were no significant ($p > 0.05$) differences in pH and acidity of different fibre added kefirs, but kefir added

with inulin showed optimum pH and acidity and obtained better sensorial and textural properties.

Effect of dietary fibre incorporation on textural properties of sweetened milk kefir

Firmness was significantly ($p < 0.05$) increased after addition of inulin in the preparation of kefir (Fig. 4 a). It was highest for inulin added kefir, followed by oat and soya fibre added kefir and then control kefir (Fig. 4 a). Consistency was significantly ($p < 0.05$) better in inulin added kefir (Fig. 4 b). Kefir added with soya and oat fibre also showed significantly ($p < 0.05$) higher consistency than control kefir (Fig. 4 b). It was witnessed that index of viscosity of inulin added kefir was maximum among the four samples (Fig. 4 c). Similarly, stickiness of kefir was enhanced after inulin

addition as compared to control kefir (Fig. 4 d). Soluble fibers like inulin formed viscous gel and modified the product textural properties. Sendra et al. (2010) have also reported increased firmness and viscosity in fermented milk products with the addition of different fibres. In the present study inulin added kefir showed significantly ($p < 0.05$) higher textural properties as compared to control, oat and soya fibre added kefir which may be ascribed to stable gel formation being soluble fibre as compared to relatively less soluble oat and soya fibre which subsequently settled in the bottom. Moreover, the increased viscosity in inulin added kefir may be attributed due to increased total solids content and due to the higher molecular weight of inulin (Buriti et al. 2010). Increased production of polysaccharide due to the prebiotic effect of inulin must have also indirectly decreased the syneresis, increased the viscosity and firmness during the product storage (Jolly et al. 2002).

Conclusions

An attempt was made to develop fibre fortified sweetened milk kefir as a potential healthy alternative to *mistidohi* and yogurt. Sensory evaluation of developed sweetened milk kefir revealed that addition of 6% sugar made its taste more palatable which was liked by the taste of the Indian populace. Inulin addition of 3% improved the textural properties of sweetened kefir and contributed to its acceptance. An acceptable quality fortified sweetened milk kefir can be prepared with 0.1% pectin, 6% sugar and 3% Inulin fortification.

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