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Development and evaluation of sensorial and antioxidant properties of dairy products prepared from milks of soybean and Bengal gram

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Abstract
Kulfi (traditional Indian ice-cream) and rice kheer (*payesh*) are the two popular dairy desserts from the Indian sub continent, which are consumed in almost all households. In the present study, these two deserts, prepared from milks of soybean and Bengal gram, were evaluated for their sensorial and antioxidant properties vis-a-vis products prepared from cow milk as control. The effort was done to ensure provision of quality and nourishment to the masses facing economic and health constraints. Mean scores of overall acceptability of kulfi and rice kheer revealed that the products made up of a 1:1 (w/w) combination of soybean and gram was most acceptable at 6-point hedonic scale. Protein and fat content in all the products were found to be excellent as compared to control albeit the amounts were less. This indicated potential application of these products in therapeutic nutrition. Radical scavenging assays indicated that the products contain both polar and non-polar biomolecules. Overall antioxidant studies revealed that the improved antioxidant properties might be due to greater flavonoid contents of the samples as compared to control.

Keywords: kulfi, rice kheer, soybean, Bengal gram, antioxidants

1. Introduction
Kulfi is a popular frozen dairy dessert from the Indian sub continent. It is often described as traditional Indian ice-cream [1]. Kulfi is also reported to contain higher total solids per unit volume in comparison to ice-cream [2]. The word 'kulfi' was derived from the Persian word for a covered cup. The dessert likely originated in the Mughal Empire in 16th century [3]. Kulfi is traditionally prepared by evaporating, sweetened, flavored milk, condensed heavy. Then flavorings, dried fruits, cardamom etc. are added. The mixture is then cooled, put in moulds and frozen. Rice kheer (*Payesh* in Bengali) is a popular dairy dessert from Eastern India. It is a carbohydrate rich dish. The rice is soaked in a bowl with a little water for around half an hour. After that, the milk is boiled with the cardamom and leaves till it reduces a little (whole /full cream milk works best). It is stirred till the rice gets cooked completely. At last, the sugar is added according to taste as it releases water. Kulfi or rice kheer are important supplements, especially for children and other age groups. But it is treated as a junk food and deleterious for heart patients due to high calorie and cholesterol. Recent studies revealed development to improve the nutritional qualities of the dairy products. Stevia was reported as a replacement of sugar in Kulfi [4].
The present study was conducted to develop a delicious, health beneficial and nutritious kulfi and rice kheer without using cow milk that could be replaced with vegetable milks like soybean milk or Bengal gram milk. This is because rising cost of the dairy products and growing awareness among the consumers about cholesterol and lactose necessitated modification of the existing dairy products. Earlier study revealed that use of soy protein isolate or groundnut meal improved the nutritional quality of kulfi [5, 6]. However, use of soy milk would be better as it contains both protein as well as unsaturated fatty acids but no cholesterol, which could be beneficial. Moreover, these two products are of low calories. Phytochemical constituents and antioxidant capacities of the samples in comparison to control was also evaluated.

2. Materials and methods
2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), ABTS, were obtained from Sigma, USA. 2, 2′-Diphenyl-1-picrylhydrazyl (DPPH) and Bradford's reagent were obtained from Himedia, India. 3, 5-Dinitrosalicylic acid (DNS) was obtained from Merck, India. Analytical grades of Gallic acid, Folin-Ciocalteau's solution, sodium hydroxide, sodium carbonate, sodium nitrite and aluminium chloride were obtained from Merck, India. All other reagents and chemicals used were of analytical grade procured from local sources. Demonized distilled water was used in the entire study.

2.1 Collection of samples
Soyabean seeds and Bengal gram are procured from local market of Sodepur, Kolkata (India). The samples were kept in tight glass containers and extracted within seven days of purchase.

2.2 Preparation of seed milk
Soybean seeds milk and Bengal gram milk was prepared according to the procedure described elsewhere [7]. Preparation process involved the cleaning and dehulling of seeds. For preparation of seed's milk, seeds (100gm) were soaked in water containing 1% sodium hydroxide over night at a room temperature in the ratio of 1:4(w/v). After soaking husks were removed by rubbing with hands. Then soaked seeds were blanched for 15 minutes in boiling water. Seeds were taken out and remaining water was drained out. After that 400ml of

water was added of seeds and blended in a grinder. The resulting suspension was then filtered through a double layer muslin cloth. At last cane sugar at a calculated amount of 6% (w/v) was added in seed milk and boiled for 5 minutes with constant stirring. Soybean seed milk was designated as SM and Bengal gram milk as BM.

2.3 Preparation of Kulfi and Rice Kheer

Four variants of kulfi were prepared by incorporating 100% cow milk (control), 100% SM, 100% BM and 1:1 (v/v) mix of SM and BM and their compositions are shown in Table 1. Similarly, Four variants of rice kheer were prepared by incorporating 100% cow milk (control), 100% SM, 100% BM and 1:1 (v/v) mix of SM and BM and their compositions are shown in Table 2.

Table 1: Composition of variants of kulfi. Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 volume ratio.

Ingredients	Control	SM kulfi	BM kulfi	1_1 kulfi
Cow milk (ml)	100	-	-	-
Soya milk (ml)	-	100	-	50
Bengal gram milk (ml)	-	-	100	50
Sugar (gm)	20	20	20	20
Corn flour (gm)	3	3	3	3
Cardamom (gm)	2	2	2	2

Table 2: Composition of variants of rice kheer. Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 volume ratio.

Ingredients	Control	SM rice kheer	BM rice kheer	1_1 rice kheer
Cow milk (ml)	100	-	-	-
Soya milk (ml)	-	100	-	50
Bengal gram milk (ml)	-	-	100	50
Sugar (gm)	25	25	25	25
Rice (gm)	5	5	5	5
Cardamom (gm)	2	2	2	2

2.4 Nutrient analyses

The Bligh & Dyer extraction was performed for estimation of lipid contents according to an established procedure with some minor modification [8]. Briefly, 1g sample is homogenized with 100 ml chloroform & 200 ml methanol (monophasic system). The solution is homogenized with 100 ml chloroform, following which 100 ml of distilled water is added. After filtration is performed under suction, the final biphasic system is allowed to separate into two layers &the lower (chloroform) phase is collected. Lipid content is then determined gravimetrically after evaporating the combined phase to dryness under vacuum. Protein contents were determined by Bradford's method using coomassie Brilliant Blue G-250 [9]. Total carbohydrate was determined by the following method with some modifications [10]. Briefly, 1g of the sample to be analyzed was taken in a flask to which, and added successively 40 ml of distilled water, 1ml of 30% zinc acetate & 5ml of 2.5 N hydrochloric acid. The mixture was boiled for 3 hours in a water bath & then cooled. It is supplemented with solid sodium carbonate until the effervescence seized. The resulting solution was filtered into a flask 100 ml and the filtrate was added with distilled water up to the mark. The solution was measured for reducing sugars by DNS reagent using UV-vis spectrophotometer at 510 nm. A standard curve of maltose was prepared similarly to estimate the carbohydrate content.

2.5 Sensory evaluation

The sensory evaluation of recipes was carried out using 6-point Hedonic rating scale by 10 semi-trained panel members selected by triangle difference test. Appearance, flavour, mouth feel and overall acceptability were considered for evaluation.

2.6 DPPH radical decolorization assay

The DPPH assay was performed using a previously described procedure [11]. 1 ml DPPH solution was mixed with 0.5 ml sample extract and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). Gallic acid was used as positive control and the results were expressed as Gallic acid equivalents (µg/gm sample).

2.7 ABTS radical decolorization assay

The ABTS assay was performed using a previously described procedure [11]. $ABTS^{-\bullet}$, the oxidant, was generated by per sulfate oxidation of 2, 2′-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid. This solution was diluted with phosphate buffer (pH 7.4) until the absorbance reached 0.7 to 0.8 at 734 nm in a Systronics spectrophotometer (model – 2202). The oxidant solution was mixed with the sample extracts in such a way that total volume of the solution reached 1 ml. The absorbance was read at room temperature, 4 minutes after mixing. Gallic acid was used as positive control and the results were expressed as Gallic acid equivalents (µg/gm sample).

2.8 Estimation of total phenolic contents

Total phenolic compound contents were determined by the Folin-Ciocalteau method [12]. The sample extracts (0.5 ml) were mixed with Folin-Ciocalteau reagent (5 ml, of 1:10 diluted sample with distilled water) for 5 min and aqueous sodium carbonate (4 ml, 1 M) was then added. The absorbance of the reaction mixture was then measured at 765 nm with a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid was used as standard. The results were expressed in terms of mg gallic acid equivalent/gm sample.

2.9 Estimation of total flavonoid contents

Total flavonoid content was determined according to a published colorimetric method [13] with some modification. Briefly 0.5 ml sample extract was mixed with 2 ml of distilled water and 0.15 ml of aqueous sodium nitrite solution ($NaNO_2$, 5% w/v), allowed to stand for 6 min, 0.15 ml aqueous aluminium trichloride solution ($AlCl_3$, 10% w/v) was added and allowed to stand again for 6 min, followed by addition of 2 ml of aqueous sodium hydroxide (NaOH, 4% w/v) solution. The final volume was made up to 5 ml by distilled water. The reaction mixture was mixed thoroughly and allowed to stand

for another 15 min. The absorbance of the reaction mixture was then measured at 510 nm with a UV-Vis spectrophotometer (model – Systronics 2202). Quercetin was used as standard. The results are expressed in terms of quercetin equivalent (µg/gm sample).

2.10 Statistical analysis
Experimental results are expressed as mean ± SD of three individual samples. The statistical analysis was done by using the software 'SPSS Statistics 17.0' (IBM Corporation, USA).

3. Results & Discussion
Fat content of SM kulfi was found to be 3.22±0.15 gm/100g which was slightly lower than control that contained 4.14±0.21 gm/100g fat. BM kulfi contained 5.50±0.34 gm/100g fat which was higher than the control. 1:1 combined kulfi contained 4.56±0.29 gm/100g fat. Since soya seed contains 13-25% oil while Bengal gram contains fatty acids including palmitic, stearic, oleic, linoleic acids, the fatty acid content was appreciable although cholesterol level was nil in the test samples. Similar results were found in rice kheer. Results are depicted in Fig. 1.

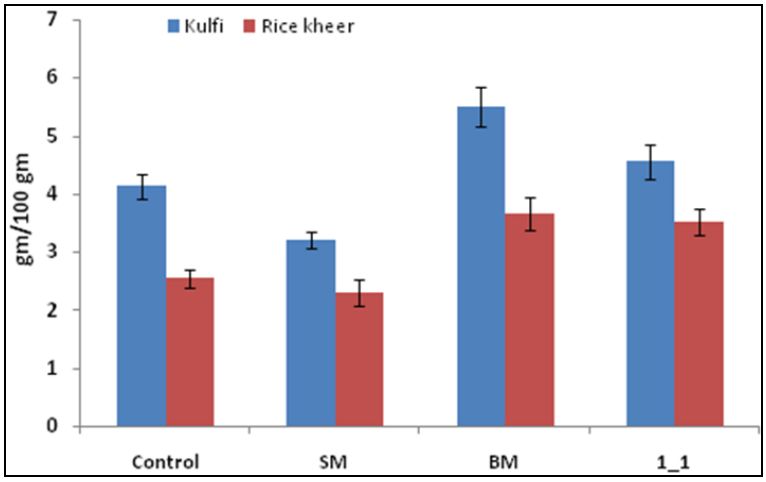

Fig 1: Fat contents of different kulfi and rice kheer samples. Data are Mean ± SD (*n*=4), Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 weight ratio.

Protein content of SM kulfi was found to be 4.23±0.25 gm/100g which was higher than the control that contained 3.50±0.16 gm/100g protein. BM kulfi contained 3.78±0.22 gm/100 gm and 1:1 combined kulfi contained 3.89±0.23 gm/100gm. There was a significant difference in protein content of soya milk in comparison to standard cow's milk. Since soya seeds contain 40% protein and its protein and oil content are not only high in quantity but also rated as best in quality too, milks prepared from those would certainly improve the quality of the final product. Similar results were found in rice kheer. Results are depicted in Fig. 2.

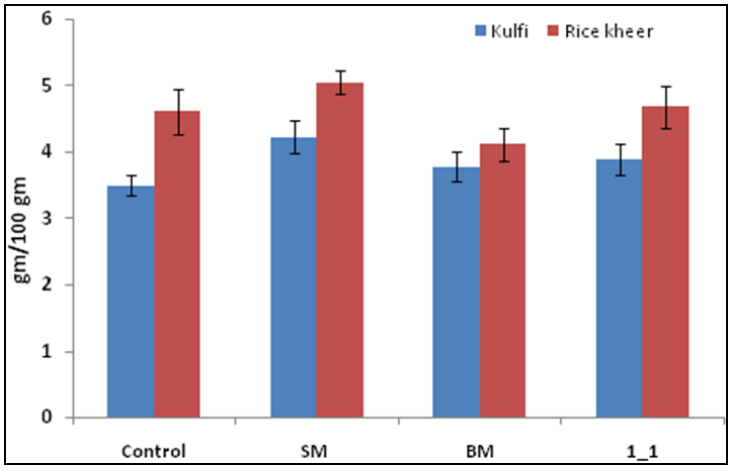

Fig 2: Protein contents of different kulfi and rice kheer samples. Data are Mean ± SD (*n*=4), Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 weight ratio.

Total carbohydrate content of SM kulfi was found to be 11.25±0.68 gm/100g which was higher than the control, which contained 10.64±0.78 gm/100g and BM kulfi (10.82±0.88 gm/100 gm). 1:1 combined kulfi contained 12.34±0.91 gm/100gm. There was a significant difference in carbohydrate content of soya milk in comparison to standard cow's milk. Similar results were found in rice kheer. Results are depicted in Fig. 3.

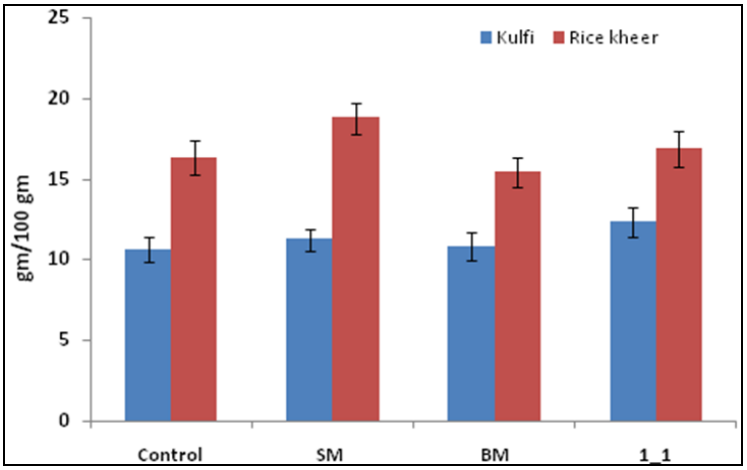

Fig 3: Total carbohydrate contents of different kulfi and rice kheer samples. Data are Mean ± SD (*n*=4), Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 weight ratio.

Sensory attributes of the products were furnished in Table 3 for the kulfi samples and in Table 4 for the rice kheer samples. Mean scores of the SM Kulfi made up of soya milk was found slightly varied at all attributes like appearance, flavour, mouth feel and overall acceptability as compared to control. The variation was more in BM kulfi. Between the two, BM kulfi was liked more by the panellists, although the values were much lower than the control. However, kulfi made up of two vegetable milks in 1:1 ratio was the most acceptable kulfi as reported by the panel members on the 6-point hedonic scale.

Table 3: Mean scores of sensory attributes of kulfi samples. Data are Mean ± SD (*n*=10), Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 weight ratio.

Attributes	Control	SM kulfi	BM kulfi	1_1 kulfi
Appearance	5.55±0.30	4.48±0.58	4.77± 0.42	4.92±0.62
Flavor	5.88±0.47	4.25±0.41	4.80±0.75	4.80±0.80
Mouth feel	5.25±0.55	4.39±0.52	4.72±0.75	4.86±0.52
Overall acceptability	5.43±0.29	4.42±0.84	4.75±0.60	4.75±0.55

There were no significant differences observed in the case of rice kheers prepared by different methods.

Table 4: Mean scores of sensory attributes of kulfi samples. Data are Mean ± SD (*n*=10), Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 weight ratio.

Attributes	Control	SM rice kheer	BM rice kheer	1_1 rice kheer
Appearance	5.63±0.76	5.72±0.44	5.66±0.66	5.77±0.36
Flavor	5.29±0.42	5.44±0.60	5.22±0.75	5.88±0.38
Mouth feel	5.38±0.62	5.38±0.80	4.88±0.81	5.42±0.64
Overall acceptability	5.64±0.51	5.52±0.34	5.28±0.45	5.58±0.88

The *in vitro* radical scavenging activities like ABTS and DPPH assay are generally used to indicate antioxidant potential of plant extracts. DPPH assay is based on non-aqueous less polar medium [14], which was suitable for estimation of antioxidant capacities of the mostly non-polar bioactives. On the contrary, ABTS assay was suitable for more polar bioactives as the assay is performed in aqueous medium [15]. The results of these radical scavenging assays showed that kulfi prepared with soya milk was the best in ABTS assay (Fig. 4) whereas kulfi prepared with Bengal gram milk was best in DPPH assay (Fig. 5), although the betterment was non-significant with respect to soya milk product. Rice kheer prepared from the vegetable milks showed similar trends. The results suggested that soya milk products were rich in polar bioactives whereas Bengal gram milk products were rich in non-polar bioactives.

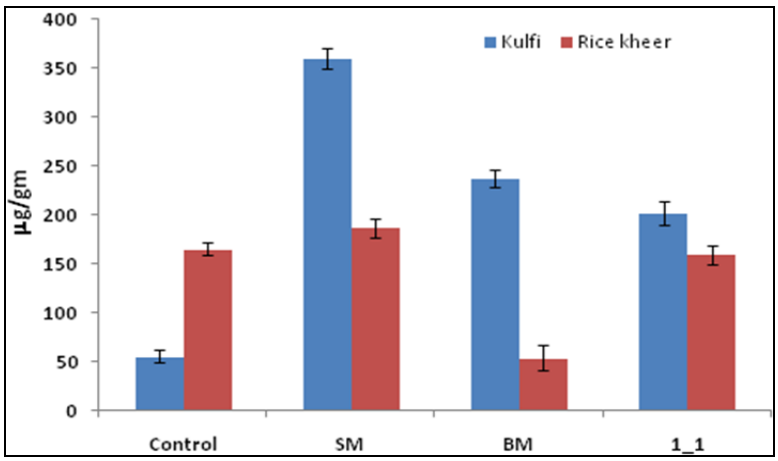

Fig 4: ABTS radical decolorization potential of different kulfi and rice kheer samples. Results are expressed in gallic acid equivalents. Data are Mean ± SD (*n*=4), Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 weight ratio.

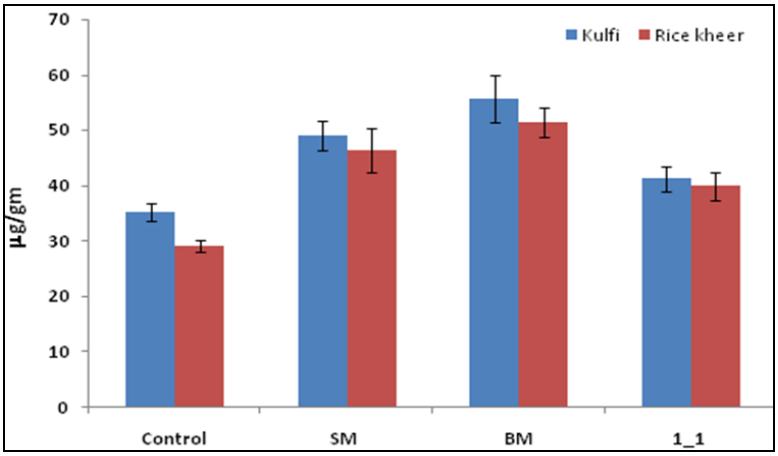

Fig 5: DPPH radical decolorization potential of different kulfi and rice kheer samples. Results are expressed in gallic acid equivalents. Data are Mean ± SD (*n*=4), Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 weight ratio.

Flavonoid contents were found to be greater in kulfi prepared with soya milk (Fig. 6). This was in accordance with the fact that soya extracts are usually enriched with flavonoids, notably soy isoflavones. However, relative levels became less in rice kheer products, where products prepared with Bengal gram showed higher content. This might be due to the fact that preparation of rice kheers needed treatment at higher temperature, which might degrade the levels of the flavonoids in soy products.

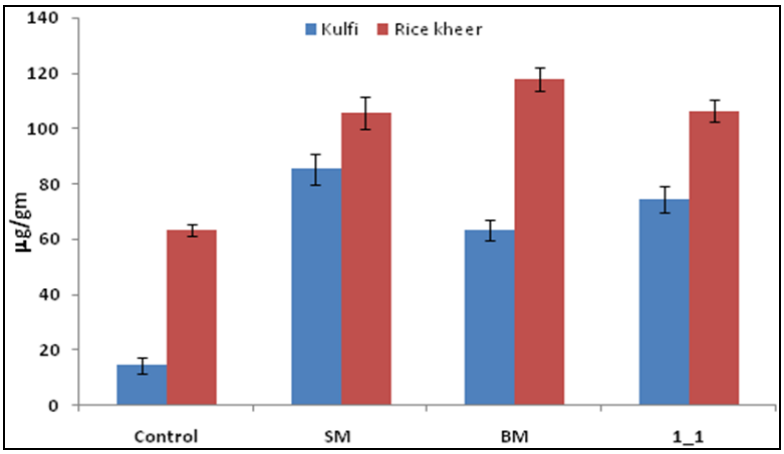

Fig 6: Total flavonoid contents of different kulfi and rice kheer samples. Results are expressed in quercetin equivalents. Data are Mean ± SD (*n*=4), Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 weight ratio.

Total phenolic contents were higher in the products prepared with soy milk (Fig. 7). This indicated that dairy products prepared with soy extracts might be a better option as functional foods.

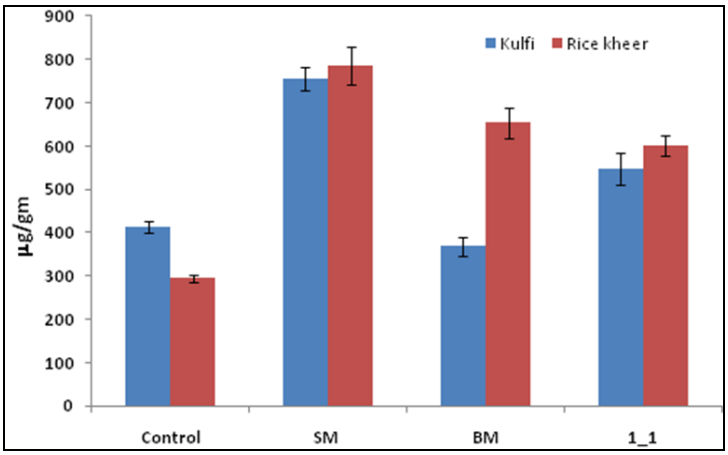

Fig 7: Total phenolics contents of different kulfi and rice kheer samples. Results are expressed in gallic acid equivalents. Data are Mean ± SD (*n*=4), Control - preparations with cow milk, SM - preparations with Soya milk, BM - preparations with Bengal gram milk, 1_1 - preparations with Soya and Bengal gram milk in 1:1 weight ratio.

A plethora of literature have been accounted in the past few years where a number of alternatives have been used to produce dairy products like kulfi with better nutritional values [16, 17]. However, most of them used milk as one of the components which remained a problem for complete removal of cholesterol from the products. In the present study, the products were devoid of any milk components, which lent credence to their potential application in the field of functional foods.

4. Conclusions

Four different nutritious kulfi and rice kheer products were prepared from cow milk (control), soy milk, Bengal gram milk and a combination of two vegetable milks. This effort was done to ensure provision of quality of nourishment to the masses facing economic and health constraints. Mean scores of overall acceptability of kulfi & rice kheer reveals that among all samples, kulfi made with the combination of the two experimental milks (50% proportions each) were most acceptable in 6-point hedonic scale. However, there were no significant differences in the sensorial attributes of rice kheers prepared by different methods. Nutrient analysis of most acceptable kulfi & rice kheer indicated that protein and fat content were found to be excellent as compared to control albeit the amounts were less than cow milk. This indicated potential application of these products in therapeutic nutrition. Kulfi made out of soy milk and Bengal gram milk can be easily used in community as these are highly acceptable and free from beanie flavour. The results of the present study emphasised on the fact that the kulfi and rice kheer prepared with the vegetable milks were not only rich in protein but also rich in antioxidants and devoid of cholesterol.

5. Acknowledgments

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