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Development of Protein Enriched Peanut Milk based Misti Doi

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ABSTRACT: The study was undertaken to evaluate the feasibility of incorporating peanut milk along with cow milk for the production of nutrient-rich Misti Doi. The peanut milk was extracted by, roasting peanuts for 5 mins, soaking for 3 hours followed by grinding with the water (1:4 ratio). The Misti Doi was prepared in five different proportions of cow milk and peanut milk *viz.*, Control (100% cow milk), T_1 (90% cow milk: 10% peanut milk), T_2 (80% cow milk: 20% peanut milk), T_3 (70% cow milk: 30% peanut milk), T_4 (60% cow milk: 40% peanut milk), T_5 (50% cow milk: 50% peanut milk) using NCDC- 159 as an inoculum. Among the various concentration, 70% cow milk and 30% peanut milk recorded the highest overall acceptability and was chosen for further evaluation. The Misti Doi was evaluated for organoleptic characteristics (colour, flavour, taste, texture and overall acceptability), physico-chemical (pH, acidity, TSS, Total Solids, syneresis, Viscosity), nutritional composition (moisture, protein, fat, ash, lactose, crude fibre, carbohydrate and energy value. The results of chemical test includes pH, viscosity, Total solids, Fat, Protein, Crude fibre, energy value, TSS increased significantly whereas Acidity, lactose, Ash, moisture, carbohydrate, Syneresis decreased significantly due to the addition of Peanut milk. The results of the present study indicates that 30% replacement of Cow milk with Peanut milk could be used successfully which would produce the quality of Misti Doi nearly similar to the Doi made purely from the Cow milk. It was concluded that peanut milk substituted Misti Doi was affordable and accessible to people with lower income.

Keywords: Peanut milk, Cow milk, Peanut, Misti Doi, Protein, syneresis.

INTRODUCTION

Milk has played a major role in the human diet across the world. India is the World's largest producer of Milk with an annual production of 146.3 million tones. In India peanut production increased dramatically to meet the demand of increased population who need both foods crop as well as oil seed. Peanut is world's fourth largest produced oilseed crop and second largest produced legume crop among worldwide population (Shilman *et al.*, 2011). Peanut is an annual commercial cash crop and belongs to the fabaceae family or leguminacaea. It was considered as a universal legume and most nutritious oil seed crops. Peanut was originated in South America in the area of Bolivia and Argentina (Sharma and Bhatnagar-Mathur 2006). Peanut contains mostly Mono Unsaturated Fatty Acids (MUFA) and Ply Unsaturated Fatty Acids (PUFA) which cannot be synthesized in our body and it will increase the high density lipoprotein level in the body and prevents heart disease (Settaluri *et al.*, 2012). In India, peanuts were produced at high rates, but consumption was low. Therefore, sufficient research may be required in India to process groundnuts or develop new food products related to peanuts. This will help to increase peanut consumption, which will help to prevent malnutrition among children and infants (Yadachi *et al.*, 2012).

Over the last 50 years, researchers have developed a variety of techniques for making peanut milk (Diarra *et al.*, 2005). The peanut milk was produced by soaking the raw roasted peanuts in water, grinding the peanut

with water and then filtered through double layered muslin cloth (Lee and Beuchat 1992). Then it was homogenized and double pasteurized in the same way as fresh milk. Peanut milk has been converted successfully into low cost edible products with high nutritional value. Peanut milk is a healthy beverage as it contains good quality proteins that are necessary for muscle development. Peanut milk contains no lactose, it is recommended for people with lactose intolerance or milk allergy. Peanut milk and its products have nutritional benefits for young and old people because of richness in protein, minerals and essential fatty acids such as linoleic and oleic acids (Isanga and Zhang 2009). On the other hand, cow milk also contains higher protein which is suitable for making fermented products with better consistency and hardness. Production of Misti Doi with the combination of animal and plant milk has been investigated by many researchers to understand the quality characteristics inherent to raw milk in the final product. The mixture of cow milk and peanut milk is of technological practice which would overcome the problems associated with rheological parameters such as weaker gel formation and sensory issues like nutty flavour in the peanut. Hexanal is the compound responsible for the nutty flavour in the peanut. It was destroyed through the fermentation process. In India, about 9% of the total milk produced is converted into fermented milk products (Singh, 2007). Dahi is the oldest and most popular fermented milk product. It is quite analogue to yogurt. Dahi is well-known for its distinctive flavour, semi-solid consistency, and high nutritional and therapeutic value. It can be consumed either as a part of our daily diet or as a refreshing beverage (Singh *et al*., 2015). The traditional fermented dairy product, Dahi has been extended to a dessert by sweetening it. The sweetened variety of dahi is known as Misti Dahi. Misti Dahi is also known as Misti Doi, Payodhi and Lal dahi. It is a traditional sweetened fermented milk product popular in the eastern part of India, mostly West Bengal, Bihar and Assam. It is prepared by lactic acid fermentation of sweetened milk. In India Misti Doi is characterized by its creamish to light brown colour, firm consistency, smooth texture and pleasant aroma (Raju and Pal 2011). However to our knowledge no research have been undertaken to prepare the Misti Doi from the mixture of cow milk and peanut milk. Misti Doi produced from cow milk and peanut milk together could provide a product with enhanced functional attributes which eventually would give more health benefits to consumer.

MATERIALS AND METHODS

Raw materials and starter culture. Fresh standardized cow milk (4.5% Fat & 8.5 % SNF) and Skim milk powder (SMP) were procured from Aavin Milk parlour, Red hills, Chennai. The Raw shelled, mature, mould free peanut varieties were procured from the local market (koyambedu), Chennai. The Doi starter's *Lactobacillus* spp (NCDC- 159) was purchased from NDRI, Karnal. Sugar and cardamom were purchased from the local departmental stores of Chennai city.

Culture preparation and maintenance. The freeze-dried cultures of *Lactobacillus spp* (NCDC- 159) revived as per the standard protocol and sub-cultured in skim milk before use.

Preparation of peanut milk. Peanut milk was prepared using the process modified in the study of Salunkhe and Kadam (1989). 100 g of local peanut variety were roasted for 5 minutes in open pan roasting method by constant stirring. After cooling, they were soaked in water for 3 hours at room temperature. The soaked peanut seeds were drained, washed with tap water and grinded with water (1:4 ratio kernels to water). The slurry was filtered through the double layered muslin cloth to obtain the peanut milk and then homogenized. Peanut milk was then double pasteurized at 85ºC for 15 mins, cooled and then stored in the bottle.

Preparation of different types of Misti Doi. Six treatments of Misti Dahi were prepared in this experiment. Out of six samples, one was prepared only from standardized cow milk (control) and other five were prepared by replacing standardized cow milk with peanut milk at the concentrations of 10, 20, 30, 40 and 50% respectively. The following blends of cow milk with peanut milk were made for the preparation of Misti Doi.

Control - 100% cow milk
T_1 -90% cow milk: 10% peanut milk
T_2 -80% cow milk: 20% peanut milk
$_{T3}$ -70% cow milk: 30% peanut milk
T_4 - 60% cow milk: 40% peanut milk
T_5 -50% cow milk: 50% peanut milk

Preparation of Misti Doi from cow milk (Control). Fresh milk is obtained, boiled and then condensed. Caramelized sugar was added to the condensed milk. The milk is then boiled to 45° C and culture was added. The inoculated milk was poured into the earthen pots and covered with aluminum foil. And the earthen pots were placed in the incubator at 37±1° C for 8 hrs. After incubation, the earthen pots were kept in the refrigerator for 2 hrs to cool it.

The flowchart as standardized by Ghosh and Rajorhia (1990) for the preparation of Misti Doi was given in (Fig. 1).

Preparation of Misti Doi from the admixture of cow milk and peanut milk. Cow milk- peanut milk blends were heated to 90-95°C for 5 mins with continuous stirring and then allowed to cool down at 40°C. Caramelized sugar was added to the blended milk and heated to 45°C and then cooled. Each blend were inoculated with 2% of dahi starter, poured into the earthen pots and covered with aluminum foil. And the

earthen pots are placed in the incubator at 37±1° C for 8 hrs. After incubation, the earthen pots were kept in the refrigerator for 2hrs to cool it and to carry out further analysis.

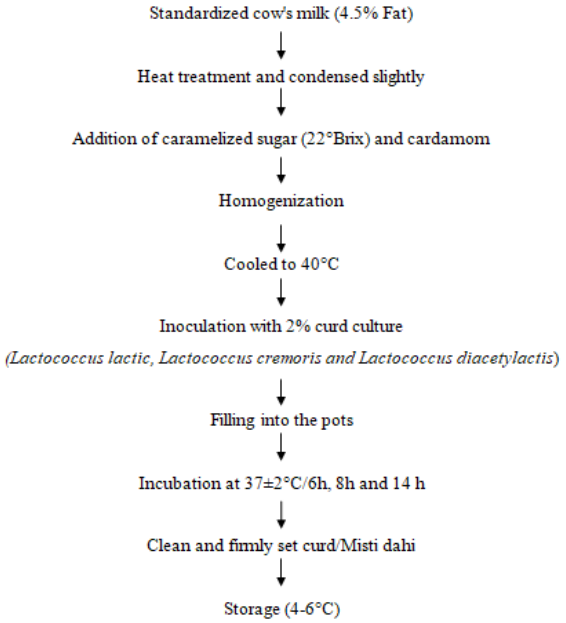

Fig. 1. Standardization of Misti Doi.

Physicochemical analysis of Misti Doi. The Misti Doi was evaluated for its physico-chemical parameters such as pH, acidity, TSS, Syneresis, viscosity and Total Solids.

— **pH:** The pH of the samples was estimated by the method described by (Hart and Fisher 1971).

— **Acidity:** The titratable acidity, expressed in terms of per cent of lactic acid in the Misti Doi was estimated as per the procedure of (IS: 1479, 1960). Misti Doi (9g) was diluted with distilled water and titrated against 0.1N sodium hydroxide using phenolphthalein as an indicator to measure the titratable acidity.

— **TSS:** Total Soluble Solids of the sample was measured using a Hand Refractometer (0 to 32° Brix). The sample was placed on the surface of the Hand Refractometer and the reading was noted down.

— **Syneresis:** Thirty ml of inoculated sample was taken in the centrifuge tube and allowed to incubate. Then they centrifuge at the 3000 rpm for five minutes. The water loosen out is separated and measured using the measuring cylinder.

$$\text{Syneresis} = \frac{\text{Volume obtained}}{\text{Sample taken}} \times 100$$

— **Viscosity:** The viscosity of the samples was analyzed using Brooke field viscometer. Brooke field viscometer instrument was calibrated and the torque was set to zero and the viscosity (centipoise) was measured at a minimum of 100rpm.

— **Total solids:** Total solids content of samples were measure by method described in (AOAC, 1990).

Nutritional composition of Misti Doi. The Misti Doi was evaluated for its nutritional parameters such as moisture, fat, protein, carbohydrate, energy value, lactose and crude fibre.

— **Moisture content:** Moisture content of the samples was determined by the gravimetric method as described in (AOAC, 2000).

— **Protein content:** The protein content of the samples was determined by Kjeldahl method using Kjeltron protein analyzer as described in (AOAC, 1990).

— **Fat Content:** The Fat content of samples was determined using the standard extraction method (AOAC, 2000).

— **Lactose content:** The lactose content of the samples was determined as prescribed in (AOAC, 2000).

— **Ash Content:** The ash content of samples was estimated using standard method of (AOAC, 1990).

— **Crude Fibre:** Fibre content of the samples was determined by method suggested by (Sadasivam, 1996). Fat free sample was taken in a pre weighed glass crucible (W_1) and it was placed in the crucible holder with glass extractor. 150 ml of 1.25% H_2SO_4 was preheated and it was added in the extractor and the contents were boiled for 30 mins at 500°C and 30 minutes for 400°C. Then the residue was drained out from extractor through fibra flow system. Then the residue was washed with distilled water. Then 150 ml of 1.25% NaOH was preheated and digested for 30 minutes at 500 °C and 30 minutes at 400 °C. Again the residue was washed with distilled water and dried for 2-4 hrs in hot air oven, cooled and weighed.

$$\text{Fibre (\%)} = \frac{(W3 - W2) \times 100}{W1}$$

W_1= Weight of sample
W_2 = Weight of crucible
W_3 = Weight of residue with crucible

Microbial analysis of Misti Doi. The microbial quality of the Misti Doi (sweetened curd) was enumerated by the method described by (Istavankiss, 1984). The microbiological enumerations of Bacteria, Yeast and mould, coliforms were recorded based on the procedure given below.

One gram of the Misti Doi was serially diluted and used for microbial enumeration. Dilution factor of 10^{-6} was used for bacterial count and 10^{-3} dilution factor was used for yeast and fungi count respectively. Autoclave was used to sterilize the petriplates, media and test tubes. One ml of the serially diluted sample was taken in a petriplate and appropriate media was poured into the sample. Total plate count agar media is used for bacterial enumeration and yeast extract malt agar media was used for fungi and yeast. Violet Red Bile agar was used for the enumeration of coliforms present in the sample. Plates were incubated at room temperature for 48 hrs for coliforms, 24hrs for bacteria and 48 hrs for yeast and mould. The colonies were counted and calculated by below mentioned formula. The values

were recorded by Log CFU (Frazier and Westhoff 2003).

$$\text{Total plate count (N)} = \frac{\Sigma_c}{(N1 + 0.1\,N2)\,D}$$

Where

Σc- The sum of the colonies counted in dishes retained

N1- The number of dishes retained in the first dilution

N2- The number of dishes retained in the second dilution

D- Dilution factor corresponding to first dilution

Sensory evaluation of Misti Doi. The sensory evaluations of the samples were evaluated for its organoleptic parameters (color & appearance, flavor, taste, texture and overall acceptability) by a panel of 20 semi-trained panels using 9 point hedonic rating score card.

Statistical analysis. All parameters were conducted in six replications and an analysis of variance was performed using IBM SPSS® 20.0 for Windows® software as per the standard procedure of Snedecor and Cochran, (1989). Results were expressed as mean or mean log ± standard error.

RESULTS AND DISCUSSION

Physico-chemical analysis of Misti Doi. The pH, acidity (%), syneresis (ml/100ml), TSS (°Brix), Viscosity (cp) and Total Solids (%) of Doi were represented in the Table 1. The pH of the Misti Doi was in the range of 4.42±0.01 (control) to 4.53±0.01 (T_3) (P<0.01). Maximum pH values were recorded for control Misti Doi followed by T_3 (70% cow milk: 30% peanut milk). It was observed that the pH of peanut milk enriched Misti Doi was increased as the concentration of peanut milk increased. The similar pH values were observed by Matin *et al*. (2020). The acidity of Misti Doi was varied significantly in the range of 0.80±0.02 (control) to 0.78±0.02 % (T_3) (P<0.01). It was observed that the acidity of peanut milk enriched Misti Doi was decreased as the concentration of peanut milk increased. The similar findings were reported by Pratap *et al*. (2018) who prepared flavoured yogurt with different proportion of cattle milk and soy milk. This might be due to the absence of lactose in the peanut milk results in the poor acid production.

The Syneresis of Misti Doi varied from 28.16±0.08 (control) to 26.84±0.10 ml/100ml (T_3) (P<0.01). It was observed that the syneresis of peanut milk enriched Misti Doi was decreased as the concentration of peanut milk increased. This might be due to higher Total solid content of peanut milk compared to cow milk. Gamli and Atasoy (2018) stated that dahi samples containing lower fat tend to have a higher degree of syneresis compared with samples containing higher fat. The present study results on par with the finding of Younus *et al*. (2002). The TSS of Misti Doi was in the range of 25.78±0.01 (control) to 26.32±0.01 °Brix (T_3) (P<0.01). The Total soluble Solids of Misti Doi made from 30% inclusion of peanut milk was higher than the Control. It might be due to the low moisture content in the peanut milk. Similar results were observed by Paul *et al*. (2016). The viscosity of control and peanut milk blended Misti Doi was documented in the range of 175.31±0.53 (control) to 207.58±0.52 (cp) (T_3) (P<0.01). Gamli and Atasoy (2018) stated that high total solids in the groundnut milk resulted in the higher viscosity. The current research findings were correlated with the results of (Isanga and Zhang 2009).

Table 1: Physiochemical analysis of control and optimized samples prepared from the admixture of cow milk and peanut milk.

Parameters	Control	T_3	t-value
pH	4.42±0.01	4.53±0.01	**8.051****
Titratable acidity(%Lactic acid)	0.80±0.02	0.78±0.02	**8.266****
TSS (°Brix)	25.78±0.01	26.32±0.01	**-39.523****
Viscosity (cp)	175.31±0.53	207.58±0.52	**42.893****
Syneresis (ml/100ml)	28.16±0.08	26.84±0.10	**9.614****
Total solids (%)	13.22±0.01	14.76±0.01	**-112.716****

@ Average of six trials, ** Highly significant (P<0.01); Control - 100% cow milk, T_3 -70% cow milk: 30% peanut milk

Table 2: Nutritional composition of control and optimized samples prepared from the admixture of cow milk and peanut milk.

Parameters	Control	T_3	t-value
Moisture (%)	86.83±0.09	84.78±0.09	**14.873****
Fat (g/100g)	4.77±0.09	5.65±0.08	**-6.678****
Protein (g/100g)	3.7±0.09	4.9±0.09	**-8.783****
Carbohydrate (g/100g)	4.34±0.09	3.21±0.09	**1.700^{NS}**
Energy value (Kcal)	310.86±0.77	360.35±0.98	**39.618****
Ash (g/100g)	0.85±0.01	0.74±0.01	**8.051****
Lactose (g/100g)	4.14±0.09	3.92±0.09	**8.535****
Fibre (g/100g)	0.00±0.00	1.3±0.01	**-13.456****

@ Average of six trials; ** Highly significant (P<0.01); NS – Non-Significant (P>0.05); Control - 100% cow milk, T_3 -70% cow milk: 30% peanut milk

The significant difference observed in the Total solids Content of control and peanut milk enriched Misti Doi varied from 13.22±0.01 (control) to 14.76±0.01 (%) (T_3) (P<0.01). Ghosh and Rajorhia (1987) reported that total solids content of dahi varied from 26.92 to 43.04. The findings of the present study are in concordance with the results of Saha *et al*. (2002).

Nutritional composition of Misti Doi. The Moisture (%), Fat (g/100g), Protein (g/100g), carbohydrate (g/100g), Energy value (Kcal), ash (g/100g), lactose (g/100g) and crude fibre (g/100g) of Misti Doi was represented in the Table 2. The Moisture content of Misti Doi varied significantly from 86.83±0.09 (control) to 84.78±0.09 (%) (T_3) (P<0.01). This might be due to the higher moisture content of cow milk compared to peanut milk. The findings of this study were on par with Saha *et al*. (2002). The fat content of the Misti Doi was in the range of 4.77±0.09 (control) to 5.65±0.08 (T_3) (g/100g) (P<0.01). Ghosh and Rajorhia (1987) stated that fat content of plain misti dahi varied from 4.3 to 8.8 with an average of 3.78 per cent. The present study results on par with the findings of Matin *et al*. (2020). Higher protein content was documented for the combination T_3 than Control. The protein content of peanut milk enriched Misti Doi was in the range of 4.9±0.09 (T_3) to 3.7±0.09 (control) (g/100g) (P<0.01). This might be due to the higher amount of protein in the peanut milk. The similar findings are in accordance with the results of Sultana *et al*. (2006) who studied the quality of dahi from buffalo milk and soy milk. There was no significant difference in carbohydrate value of control and peanut milk enriched Misti Doi. The results obtained in the investigation are in conformity with the findings of Islam *et al*. (2015). Based on the results of Table 2, there was a highly significant difference between the control and peanut milk enriched Misti Doi in terms of energy value. The similar results were obtained by Isanga and Zhang (2009) reported that peanut milk yogurt contains higher calorific value than cow milk yogurt. The lactose content of the Misti Doi was in the range of 4.14±0.09 (control) to 3.92±0.09 (T_3) (g/100g) (P<0.01). This might be due to the higher lactose in the cow milk whereas peanut milk contains no lactose. The current research findings are in agreement with (Davis and Mclachlan 2001). Higher crude fibre content was documented for the combination T_3 than Control. The crude fibre content of peanut milk enriched Misti Doi was in the range of 1.3±0.01 (T_3) to 0.00±0.00 (control) (g/100g) (P<0.01). The results obtained in the investigation are in conformity with the findings of Islam *et al*. (2015).

Table 3: Microbial analysis of control and optimized samples prepared from the admixture of cow milk and peanut milk.

Parameters	Control	T_3	t-value
Total plate count (log_{10} cfu/g)	5.78±0.07	5.83±0.09	**-0.505**^{NS}
Yeast and mould count (log_{10} cfu/g)	ND	ND	ND
Coliforms count (log_{10} cfu/g)	ND	ND	ND

@ Average of six trials; NS – Non-Significant (P>0.05); Control - 100% cow milk; T_3 -70% cow milk: 30% peanut milk

Table 4: Sensory evaluation of control and optimized samples prepared from the admixture of cow milk and peanut milk.

Samples	Colour & appearance	Flavour	Taste	texture	Over all acceptability
Control	8.33±0.18	8.40±0.19	8.60±0.13	8.20±0.20	8.40±0.13
T_3	8.33±0.18	8.46±0.16	8.66±0.12	8.60±0.13	8.93±0.06
t- value	**0.000** ^{NS}	**-0.264** ^{NS}	**-0.366** ^{NS}	**-1.673** ^{NS}	**-3.629****

@ Average of six trials, NS – Non-Significant (P>0.05), ** Highly significant (P<0.01); Control - 100% cow milk, T_3 -70% cow milk: 30% peanut milk

Microbial analysis of Misti Doi. Microbial parameters such as Total Viable Count, Yeast and Mould and coliforms were presented in the Table 3. From the Table 3, there was no significant difference in total viable count observed between control and peanut milk enriched Misti Doi. The result shows that lowest level of total bacterial count was observed in both the samples. The present investigation supports the results of Sultana *et al*. (2006) disclosed no significant difference between the samples. There is no yeast and mould count observed between the control and control and peanut milk enriched Misti Doi. The results obtain on par with the findings of Jitender *et al*. (2016) reported there is no yeast and mould colony detected in any product. There is no coliforms observed between the control and peanut milk enriched Misti Doi. The results obtained in the investigation are in accordance with the findings of Akgun *et al*. (2016).

Sensory evaluation of Misti Doi. Sensory evaluation is an essential component of consumer preferences. Sensory characteristics of peanut milk enriched Misti Doi was presented based on the mean score of colour, flavour, taste, texture and overall acceptability are presented in the Table 4. It was observed that mean scores for colour, flavour, taste, texture and overall acceptability was in the range of 6.47-8.33, 5.80-8.47, 5.73-8.67, 5.67-8.60 and 5.93-8.40 respectively. The Misti Doi prepared by blending of 30% peanut milk

with 70% cow milk obtained the higher scores based on the sensory evaluation using 9 point hedonic scale by panel of 20 semi-trained judges. Misti Doi prepared with admixtures T_3 (70% cow milk: 30% peanut milk) gained the highest score followed by T_1 (90% cow milk: 10% peanut milk), T_2 (80% cow milk: 20% peanut milk), T_4 (40% toned milk: 60% peanut milk), T_5 (50% cow milk: 50% peanut milk) and control (100% cow milk). As the concentration of peanut milk increased the colour value of different combinations of peanut milk blended Misti Doi decreased. Thus the overall acceptability of peanut milk enriched Misti Doi significantly increased, as the proportion of peanut milk increased. In this study, we observed that Misti Doi prepared from 70% cow milk: 30% peanut milk (T_3) was close similar to that made from 100% cow milk (Control). The results of the current findings are in concordance with the results of Pratap *et al*. (2018) who reported 30% replacement of cattle milk with soy milk for the dahi preparation was highly acceptable and the dahi made from this combination exhibit highest score in terms of colour, flavour, body texture and overall acceptability. From this experiment, it was concluded that 70% cow milk and 30% peanut milk was highly acceptable for its organoleptic characteristics. It was found that increased peanut milk concentration is associated with the lower organoleptic scores due to the nutty flavour of peanut milk.

CONCLUSIONS

From the results of the present study, it can be concluded that 30% level of peanut milk incorporated misti dahi was found to be ideal based on the sensory evaluation. This exhibits similar physico-chemical (pH, acidity, TSS, Syneresis, viscosity and Total Solids) and nutritional composition (moisture, protein, fat, CHO, energy value, lactose and crude fibre) to the Doi prepared from 100% cow milk. This study encourage industries for commercial production of Misti Doi with 30% cow milk replaced with peanut milk which will be widely acceptable by overall population. It was concluded that peanut milk substituted Misti Doi was affordable and accessible to people with lower income without affecting the palatability and acceptability of Doi.

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Conflict of Interest. None.

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