



Development of Yogurt Analogue by Blending Soy-Maize Milk

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Abstract

The aim of this research was to develop yogurt analogue by blending soy-maize milk and to study its sensory and keeping quality. D-optimal mixture design was employed for the formulation of soy-maize yogurt analogue. Five different formulations containing soymilk and maize milk in the ratio of 100:0, 85:15, 70:30, 55:45 and 40:60 were prepared. Sugar (6 g per 100 g milk base), xanthan gum (0.005 g per 100 g milk base) and culture (2U per 20 l) were mixed in all formulations. The prepared yogurts were subjected to sensory evaluation for consumer acceptability. The sensory analysis revealed that soymilk (85%) and maize milk (15%) was of acceptable quality. There was significant difference ($p < 0.05$) between the formulation in terms of color and appearance, flavor, body and texture and overall acceptance. The soy and soy-maize yogurt (optimized) were evaluated for fermentation kinetics (pH, acidity, total plate count and *Streptococcus thermophilus* count) at the interval of 2 h for 6 h during incubation; and during storage (refrigeration temperature 4-5°C) at the interval of 3 days for 12 days. The physicochemical analysis showed that there was significant difference ($p < 0.05$) in terms of protein, carbohydrate, acidity and syneresis and not significant ($p > 0.05$) in regards of moisture content, fat, ash, and pH between soy and soy-maize yogurt. Also, there was significant difference ($p < 0.05$) in pH, acidity, total plate count and *Streptococcus thermophilus* count with respect to incubation and storage time for soy and soy-maize yogurt. The yeast and molds count were observed at the 12th day of storage only. The syneresis of soy and soy-maize yogurt was also significantly different ($p < 0.05$) with respect to storage time.

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Introduction

Yogurt is a product resulting from milk by fermentation with a mixed starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* (Tamime and Robinson, 2007). Milk is considered as the only food containing all the essential substances for human health. However, some milk constituents and common contaminants such as pesticides, estrogen and insulin-like growth factor might be responsible for adverse reactions on the consumer's health. As animal milks are not favored by lactose intolerant people, they are shifting towards plant-based milks (Davoodi et al., 2013).

Soybean (*Glycine max*) is one of the most important legumes of tropics with high quality

protein and fat. Soybean contains many anti-nutritional factors such as trypsin inhibitors, hemagglutinin, estrogenic factor, saponins, phytic acid and flatus factors but recently these components are of interest because of their prebiotic properties, anti-carcinogenic and other medicinal importance (Wang, 2008). Maize (*Zea mays* L.) is one of the most cultivated cereal grains and typically contains protein (8-10%), lipid (4-5%), starch (70-75%) and ash (1-4%) (Arendt and Zannini, 2013). The ease of handling and processing of soybean and maize allows development of numerous food items, with each being characterized by its own technological characteristics and qualities. Many food producers aim to address the present consumer demands and environmental concerns by developing and working in sustainable alternative food products (Mahony et al., 2020). Soymilk support simultaneous growth of *Bifidobacteria* and *Lactobacillus acidophilus* or *Streptococcus thermophilus*, but *Bifidobacterium infantis* and *Bifidobacterium longum* both had a detrimental effect on the growth of *Lactobacillus bulgaricus* in soymilk. The bifidobacteria and lactic acid bacteria count showed no marked change in cultured soymilk with or without sucrose during storage at 5°C for 10 days (Chou et al., 2002).

Vegetarian concerns of health and environment have led a demand for plant-based milk alternatives and their products. Soy and maize milk are water extracts that can contribute affordable and plentiful supply of protein and calories. Milk analogue such as soymilk, oat milk, coconut milk and cocoa milk dominate the market (Kumar et al., 2019). The objective of this study was to evaluate the effect of incubation and storage time on the sensory, physicochemical and microbial characteristics.

Materials and Methods

Preparation of soymilk and maize milk

Glycine max (white variety), *Zea mays* (yellow variety), and sugar were purchased from the local market of Dharan. Starter culture (ST-600) manufactured by Tropilite Foods Pvt. Ltd., Madhya Pradesh, India was purchased from Itahari. The soybeans were sorted and dried in hot air oven at 100°C for 20 min. The dried soybean was soaked for 16 h and bleached with 0.5% sodium bicarbonate solution at 80-85°C for 20 min and dehulled.

The dehulled soybeans were ground with hot water 80-85°C (soybean: water at 1: 6) and the slurry was filtered through muslin cloth to obtain soymilk. Soymilk was heated at 90-95°C for 15 min and stored in refrigeration temperature (4°C) till further use (Yu et al., 2017).

Maize milk was prepared from green field maize at eating stage. The husk and silk were removed and cobs washed with potable water. Maize kernels were removed from the cobs and ground with water in grinder maintaining the ratio of maize kernel and water ratio at 1:4. The slurry was filtered through muslin cloth and maize milk was heated at 80°C for 10 min. The heated maize milk was stored in refrigeration temperature (4°C) (Geetha et al., 2018).

Manufacture of soy-maize milk yogurt analogue

The soy-maize milk yogurt analogues were prepared under different sets of soymilk and maize milk ratios: 100:0 (A), 85:15 (B), 70:30 (C), 55:45 (D), and 40:60 (E) These formulations were obtained from D-optimal mixture design in Design Expert software (Version 12). Soymilk with or without maize milk were pre-heated to 45°C and sugar (6 g per 100 g milk) and xanthan gum (0.005 g per 100 g milk) were added. Then the mixture was heated to 85°C for 15 min and cooled to 42-43°C. The milk was inoculated with starter culture at the rate of 2 U/20 L and incubated at 42°C for 6 h. The prepared soy-maize yogurt analogue was stored at refrigeration 4-5°C.

Determination of physicochemical parameters

The protein, ash, and moisture contents of yogurt analogue were determined as described by Ranganna (1986). The fat, total carbohydrate, pH, titratable acidity and total solids were determined according to Shrestha and Waldhauer (2001). Syneresis of yogurt analogue was determined by using the drainage method with slight modification as described by Shah et al. (2006). A cup of yogurt was taken out from the refrigerator and the whey on the surface was siphoned. The gel weighing about 30 g was cut with the help of stainless-steel ladle and the gel was weighed and drained on a filter paper for 2 h at room temperature. The whey was weighed and the

syneresis was expressed as the percent whey separation from the gel over the initial weight of the gel.

Microbial analysis

The soy yogurt (control) and yogurt analogue were evaluated for fermentation kinetics (pH, acidity, total plate count and *Streptococcus thermophilus* count) during incubation at the interval of 2 h for 6 h and during storage at the interval of 3 days for 12 days. One ml of yogurt was taken and serial dilutions were prepared in sterile distilled water. The total plate count was performed using total plate count agar. Appropriate dilutions were plated to total plate count agar and incubated at 30°C for 72 h. The yeast and mold count were done using potato dextrose agar. The inoculated plates were incubated for 48 h at 25°C (Shrestha and Waldhauer, 2001). *Streptococcus thermophilus* agar was used to enumerate *Streptococcus thermophilus* under aerobic condition and incubation at 37°C for 24 h (Shah and Ashraf, 2011).

Sensory analysis

Ten semi-trained panelists were selected from the teachers of Central Department of Food Technology, Dharan who were familiar and regular consumer of yogurt. A 9-point hedonic rating scale (1=dislike extremely, 9=like extremely) was used to evaluate the quality attributes like color and appearance, flavor, body and texture, and overall acceptability of the yogurt analogue.

Statistical analysis

Sensory data was subjected to statistical analysis using IBM SPSS (Version 26) for Analysis of Variance (ANOVA) at 5% level of significance. Data from physicochemical and microbial analysis were subjected to one-way ANOVA. Difference among the means was analyzed using Tukey's HSD (Honestly significant difference) method.

Results and Discussion

Analysis of soymilk and maize milk

The proximate composition of soymilk and maize milk is presented in Table 1. From statistical analysis, there is significant difference ($p < 0.05$) between soymilk and

maize milk in terms of moisture, protein, fat and carbohydrate content, but not significant difference ($p > 0.05$) in terms of ash content.

Table 1

Proximate composition of soymilk and maize milk

Parameter	Soymilk	Maize milk
Moisture (%)	89.72 ± 0.06 ^a	90.26 ± 0.13 ^b
Protein (% db)	62.35 ± 2.06 ^a	12.46 ± 0.67 ^b
Fat (% db)	26.91 ± 1.49 ^a	11.70 ± 0.74 ^b
Ash (% db)	5.32 ± 0.10 ^a	5.58 ± 0.46 ^a
Carbohydrate (% db)	5.40 ± 0.52 ^a	70.22 ± 1.68 ^b

Note. Figures are the mean ± SD of triplicate. Means with different superscript on the same row are significantly different ($p < 0.05$).

The fat, carbohydrate and ash content of soymilk were similar but protein content was slightly higher than the value reported by Sayed et al. (2016). The protein and ash content of maize milk were higher, fat content lower and carbohydrate similar to the value given by Ifediba and Nwabueze (2018). The variation in composition may be due to varieties of soyabean and maize as well as treatment methods used during milk production.

Sensory evaluation

As shown in Figure 1, the concentration of maize milk higher than 15% led to decrease in sensory appeal of soy-maize yogurt. A significant difference ($p < 0.05$) in the mean score was observed for appearance, flavor, body and texture and overall acceptance.

There was a significant effect ($p < 0.05$) of maize milk variation on color and appearance as well as body and texture of soy-maize yogurt. When quantity of maize milk was increased in milk base, the yellow color got intense due to the effects of xanthophyll (yellow color). This resulted in low rating for sample C, D and E. Makanjuola (2012) reported similar results for different combination of soy corn yogurt and also concluded that there is decrease in mean score for body and texture with increase in maize milk. This result may be correlated with the decrease in amount of protein responsible for forming gel and structure in yogurt.

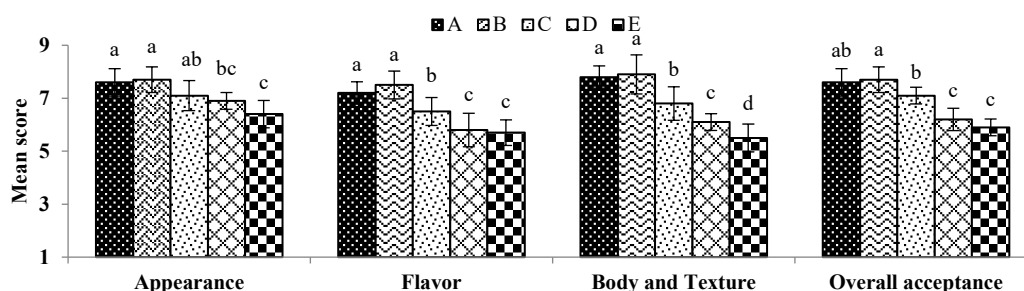


Figure 1
Sensory analysis of soy-maize yogurt analogue

Note. Bars with similar letters for any sensory attributes are not significantly different ($p < 0.05$).

The sample B was significantly different ($p < 0.05$) from other samples excluding A and had the highest mean score in terms of flavor. Raviyan et al. (2010) observed similar score for flavor in corn and cow milk yogurt. From this study, it is clear that the increase in maize milk reduce the acceptance of flavor. This may be because the maize flavor prevailed in soy-maize yogurt. On the other hand, maize contains volatile substance such as acetaldehyde which can contribute in reducing the beany flavor of soybean (Srianta et al., 2014).

The mean score for overall acceptance was significantly ($p < 0.05$) higher for sample B. In this study, the increase in the proportion of maize milk showed decrease in acceptability of soy-maize yogurt. However, from mean score of overall acceptability we can draw conclusion that acceptable soy-maize yogurt can be prepared by adding optimum quantity of maize milk. Maize milk can be used to fortify soymilk to develop quality and valuable yogurt analogue (Kolapo and Oladimeji, 2008).

Physicochemical composition of yogurt

Table 2 shows a comparison of physicochemical composition of soy yogurt and soy-maize yogurt. The statistical analysis revealed significant difference ($p < 0.05$) between soy and soy-maize yogurt in terms of protein, carbohydrate, acidity and syneresis but not difference in other parameters like moisture content, fat, ash, and pH. The protein, fat and carbohydrate content of soy and soy-maize yogurt were 35.11 and 30.14, 19.95 and 18.61 and 41.61 and 47.94 respectively. The addition of maize milk reduced the protein and fat content but increased the carbohydrate content of soy-maize yogurt in comparison to soy yogurt. The

syneresis value was higher for soy yogurt (28.14 %).

The chemical composition of soy and soy-maize yogurt analogue was consistent with that of Trindade et al. (2001). The fat and protein content of soy-maize yogurt was higher. This is not in an agreement with the study of Amanze and Amanze (2011), where they reported the fat and protein value on dry basis (db) as 15.53 and 24.13 respectively. In the study of Han et al. (2010) similar result was obtained for pH content but the acidity of soy yogurt was lower. They prepared soy yogurt with 2% starter culture and fermented at 37°C for 36 h.

Table 2
Physicochemical composition of soy and soy-maize yogurt analogue

Parameters	Control	Optimized
Moisture content (%)	83.46 ± 0.29 ^a	83.93 ± 0.20 ^a
Protein (% db)	35.11 ± 1.07 ^a	30.14 ± 1.19 ^b
Fat (% db)	19.95 ± 1.03 ^a	18.61 ± 0.94 ^a
Carbohydrate (% db)	41.61 ± 2.98 ^a	47.94 ± 2.31 ^b
Ash (% db)	3.35 ± 0.10 ^a	3.30 ± 0.16 ^a
pH	4.66 ± 0.05 ^a	4.60 ± 0.07 ^a
Acidity (as lactic acid) %	0.37 ± 0.01 ^a	0.50 ± 0.02 ^b
Syneresis (% whey separation)	28.14 ± 0.41 ^a	26.02 ± 0.70 ^b

Note. Figures are the mean ± SD of triplicate. Similar superscript on means in the same row indicate not significant difference ($p > 0.05$) and different superscript on means in the same row indicate significant difference ($p < 0.05$).

pH and titratable acidity

The pH of soy and soy-maize yogurt decreased with increase in acidity. The statistical analysis showed that there is significant difference ($p < 0.05$) in pH and acidity of soy and soy-maize yogurt with incubation time and storage days. The change in pH and acidity of soy and soy-maize yogurt during incubation is presented in Table 3. Gutiérrez and Azuero (2018) reported sharp decrease in pH and increased in acidity of yogurt prepared from reconstituted skim milk powder (13%) in distilled water, sachu inchi seeds (4%), sucrose (7.5%), different composition of β -glucans from *Ganoderma lucidum* (0-1.5%) and incubated at $43 \pm 1^\circ\text{C}$ for 5 h. They reported the pH and acidity of yogurt as 4.6 and 0.90-1.30 respectively. *Streptococcus thermophilus* alone produced most acid than other lactic acid in soy yogurt (Chou et al., 2002).

Table 4 shows the value of pH and acidity during storage for soy and soy-maize yogurt analogue. Falade et al. (2014) obtained similar trend of decrease in pH and increase in acidity of plain soy and plain bambara yogurt during storage (9 days) with added 1% glucose. However, acidity of plain soy and plain bambara yogurt were relatively higher i.e., 1.63-2.02% and 1.53-1.94% respectively. In a recent study, Igbadul et al. (2018) reported similar results for pH and acidity for yogurt prepared from the mixture of bambara nut, soybean and *Moringa oleifera* seed milks which were stored for 14 days and pH and acidity of yogurt was observed at the interval of 7 days. The lower buffering capacity of the plant-based milk may be the cause of higher reduction in pH and lower acidity development during storage (Raviyan et al., 2010).

Table 3

Effects of incubation time on pH and acidity of soy and soy-maize yogurt

Incubation time (h)	pH		Acidity	
	Soy yogurt	Soy-maize yogurt	Soy yogurt	Soy-maize yogurt
0	6.84 \pm 0.05 ^a	6.87 \pm 0.06 ^m	0.12 \pm 0.01 ^a	0.15 \pm 0.01 ^m
2	6.59 \pm 0.04 ^b	6.55 \pm 0.06 ⁿ	0.20 \pm 0.01 ^b	0.21 \pm 0.02 ⁿ
4	5.28 \pm 0.05 ^c	5.08 \pm 0.05 ^o	0.31 \pm 0.01 ^c	0.32 \pm 0.01 ^o
6	4.66 \pm 0.05 ^d	4.60 \pm 0.07 ^p	0.37 \pm 0.01 ^d	0.50 \pm 0.02 ^p

Note. Figures are the mean of triplicate and values after \pm are standard deviation. Mean values within same column with different superscripts are significantly different ($p < 0.05$).

Table 4

Effects of storage time on pH and acidity of soy and soy-maize yogurt

Storage time (days)	pH		Acidity	
	Soy yogurt	Soy-maize yogurt	Soy yogurt	Soy-maize yogurt
0	4.66 \pm 0.05 ^a	4.60 \pm 0.07 ^m	0.37 \pm 0.01 ^a	0.50 \pm 0.02 ^m
3	4.52 \pm 0.06 ^b	4.40 \pm 0.06 ⁿ	0.44 \pm 0.05 ^{ab}	0.56 \pm 0.02 ^{mm}
6	4.43 \pm 0.07 ^{bc}	4.30 \pm 0.06 ⁿ	0.46 \pm 0.02 ^b	0.58 \pm 0.05 ^{no}
9	4.35 \pm 0.03 ^c	4.29 \pm 0.06 ⁿ	0.46 \pm 0.03 ^b	0.63 \pm 0.02 ^o
12	4.32 \pm 0.04 ^c	4.30 \pm 0.05 ⁿ	0.50 \pm 0.04 ^b	0.60 \pm 0.02 ^{oo}

Note. Values are mean \pm SD. Values with different superscript within the same column are significantly different ($p < 0.05$).

Syneresis

Syneresis of soy and soy-maize yogurt analogue increased with storage time (Table 5) and was significantly different ($p < 0.05$) with storage days. Raviyan et al. (2010) obtained similar conclusion in corn milk yogurt prepared with added lactose (2% w/v) and gelation (0.4% w/v). They observed increased in syneresis of corn yogurt whereas no increase in syneresis of cow milk yogurt during storage for 35 days at 5°C . The syneresis of yogurt increases with increase in the proportion of corn milk in formulation (Geetha et al., 2018). Laboratory prepared xanthan gum (0.005%) when used in dairy and soy yogurt as stabilizer showed no syneresis for 10 days (El-Sayed et al., 2002). Syneresis of yogurt prepared with different variations in milk and soymilk increased with storage time (Mazloomi et al., 2013).

Microbial analysis

The total plate count (TPC) and *Streptococcus thermophilus* count increased during incubation and storage. The statistical analysis showed significant difference ($p < 0.05$) in total plate count and *Streptococcus thermophilus* count of soy and soy-maize yogurt. Table 6

shows the pattern of total plate count and *Streptococcus thermophilus* count during incubation.

Table 5

Effects of storage time on syneresis of soy and soy-maize yogurt analogue

Storage time (days)	Syneresis (% whey separation)	
	Soy yogurt	Soy-maize yogurt
0	28.14 ± 0.41 ^a	26.03 ± 0.70 ^m
3	30.21 ± 0.75 ^b	28.10 ± 0.63 ⁿ
6	32.19 ± 0.63 ^c	30.03 ± 0.69 ^o
9	35.70 ± 0.62 ^d	33.08 ± 0.67 ^p
12	38.64 ± 0.55 ^e	35.73 ± 0.45 ^q

Note. Values are mean ± SD. Values with different superscript within the same column are significantly different (p<0.05).

Pyo and Song (2009) reported similar trend of increase in total lactic acid during incubation in soy yogurt fermented at 35°C. Srianta et al. (2014) observed similar results with higher number of lactic acid bacteria in soy and maize milk yogurt. *Streptococcus thermophilus* can grow rapidly in soymilk during the initial stage of cultivation (Chou et al., 2002). From this study, it is concluded that *Streptococcus thermophilus* grows and survives well in soy-maize milk yogurt.

Table 7 shows the profile of total plate count and *Streptococcus thermophilus* count during storage. Initially there was an increase in TPC and *Streptococcus thermophilus* count but decreased at latter part of storage. The lactic acid bacteria growth was found similar in soy and bambara yogurt stored for 9 days (Falade et al., 2014). Han et al. (2010) observed similar pattern for *Streptococcus thermophilus* growth in soymilk stored for 15 days at 4°C. The number of lactic acid bacteria decreased with storage time; this may be the results of nutrients scarcity and changing environment (Aini et al., 2018). Yeasts and molds were not observed for 9 days. On the 12th day, the yeast and molds count of soy and soy-maize yogurt was 1.33 and 2.67 CFU/ml respectively.

Table 6

Effects of incubation time (h) on total plate count and *Streptococcus thermophilus* count of soy and soy-maize yogurt

Incubation time (h)	Total plate count (log CFU/ml)		<i>Streptococcus thermophilus</i> (log CFU/ml)	
	Soy yogurt	Soy-maize yogurt	Soy yogurt	Soy-maize yogurt
0	6.78 ± 0.03 ^a	6.79 ± 0.03 ^m	6.44 ± 0.03 ^a	6.48 ± 0.03 ^m
2	7.30 ± 0.04 ^b	7.35 ± 0.10 ⁿ	7.12 ± 0.01 ^b	7.33 ± 0.06 ⁿ
4	8.45 ± 0.09 ^c	8.48 ± 0.04 ^o	8.25 ± 0.06 ^c	8.33 ± 0.03 ^o
6	8.61 ± 0.03 ^d	8.62 ± 0.03 ^o	8.38 ± 0.03 ^c	8.41 ± 0.04 ^o

Note. Values are mean ± SD. Values with different superscript within the same column are significantly different (p<0.05).

Table 7

Effects of storage time on total plate count and *Streptococcus thermophilus* count of soy and soy-maize yogurt

Storage time (days)	Total plate count (log CFU/ml)		<i>Streptococcus thermophilus</i> (log CFU/ml)	
	Soy yogurt	Soy-maize yogurt	Soy yogurt	Soy-maize yogurt
0	8.61 ± 0.03 ^a	8.62 ± 0.03 ^m	8.38 ± 0.03 ^a	8.41 ± 0.04 ^m
3	8.74 ± 0.03 ^b	8.75 ± 0.03 ⁿ	8.42 ± 0.07 ^a	8.51 ± 0.03 ^m
6	8.76 ± 0.05 ^b	8.79 ± 0.03 ^{no}	8.50 ± 0.05 ^{ab}	8.62 ± 0.04 ⁿ
9	8.63 ± 0.02 ^a	8.86 ± 0.03 ^o	8.49 ± 0.04 ^{ab}	8.75 ± 0.04 ^o
12	8.56 ± 0.04 ^a	8.81 ± 0.04 ^{no}	8.59 ± 0.06 ^b	8.76 ± 0.04 ^o

Note. Values are mean ± SD. Values with different superscript within the same column are significantly different (p<0.05).

Conclusion

Yogurt analogue with considerable quality was produced from soy (85%) and maize milk (15%). The protein, carbohydrate, acidity and syneresis were significantly different between soy and soy-maize milk yogurt whereas moisture content, fat, ash and pH were not significantly different. There was significant difference ($p < 0.05$) in pH, acidity, total plate count, *Streptococcus thermophilus* count for soy and soy-maize yogurt during incubation and storage.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare no conflict of interest.

Ethical approval

The study did not involve any inhumane animal study.

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