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Utilization of Moringa Pod Powder as a Value Added Ingredient in *Lassi*

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Abstract

The present study was conducted to formulate a recipe and develop a method for manufacture of Moringa lassi containing Moringa pod powder (MPP) as a value added ingredient. Moringa lassi was prepared from dahi made from standardized milk (4.5% fat/9.5% MSNF). The effect of varying level of total milk solids in lassi (6 to 10% w/w of lassi), acidity of dahi (0.6 to 0.9% LA) and level of MPP (1.25 to 2.00% w/w of lassi) and their interactions on physico-chemical and sensory parameters of the product were assessed using Response Surface Methodology. Based on surface responses, the optimal levels were: 10.0% TMS in lassi, 0.8% LA acidity of dahi and 1.63% MPP. Using the optimized levels, the developed product was found to be an "excellent source of calcium" providing 25% DV and "good source of iron and potassium and protein" providing 12, 10 and 10% DV respectively. Moreover, the product was found to contain considerable amount of Vitamin C (7% DV) and fibre (5% DV). These nutrients could be supplied by one serving size (250 g) of Moringa lassi.

Keywords: *Moringa, drumstick pod, lassi, dahi, iron, calcium, potassium*

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INTRODUCTION

In India, *dahi* and *dahi*-like products occupy a focal position in the daily diet of people. About 6.9% of total milk produced in India is utilized for making *dahi* and *dahi*-like products intended for direct consumption. The volume of fermented products was reported to be 6.0 MMT valued 120 billion rupees in the market [1]. The demand for fermented milk products is increasing and it has been estimated that about 10% of total milk produced in India is used for preparation of traditional fermented milk products among all the traditional products, *lassi* is one of the most widely consumed fermented milk. It resembles Western stirred yogurt in most characteristics. *Lassi* is a white to creamy-white viscous liquid, with a sweetish, rich aroma and mild to high acidic taste. *Lassi* has valuable therapeutic properties and aids in curing gastrointestinal disorders. The composition of *lassi* depends on composition of milk used and the extent of dilution. On an average, *lassi* may contain fat ranging from 3.0–3.5%, total solids ranging from 16.0–18.0% and acidity varying from 0.75–0.88% LA [2]. *Lassi* contains appreciable amounts of milk proteins and phospholipids and nutritive value of fermented milk products is derived from the nutrients among various metabolites produced by lactic acid bacteria during fermentation besides the nutrients available from milk. A slight increase in CLA content was observed during manufacture of *lassi* [3].

In India, the manufacture of *lassi* was confined to household levels but recently things have changed. In February 2007, Amul was the first company to launch *lassi* on commercial level. Recently, other dairies like Mother dairy which has the largest milk plants in Asia launched various probiotic products like b-Activ Probiotic *Dahi*, b-Activ Probiotic *Lassi*, b-Activ Curd and Nutrifit (Strawberry and Mango). Other such companies are Go, Danone, and Nestle has launched different types of *lassi*. Most of the commercially available *lassi* are seasoned with essences of fruits like mango, strawberry or rose.

The demands of consumers change from time to time. This can be due to demands for improving food safety, improved shelf life, demands for foods having special characteristics in terms of nutritional value, convenience and improved taste. Health is a major concern of consumers; therefore, manufacturers are finding new ways to incorporate natural and innovative ingredients such as vegetables into dairy products for health benefits. A range of innovative applications are known to enhance nutritional quality of food including novel materials and nutrient delivery mechanisms [4]. To further improve the therapeutic and nutritional value of *lassi*, it is necessary to incorporate natural and innovative ingredients such as vegetable products.

In India, Moringa pod is known as *munga, saragwa* or *saragwe* and is often referred to as Moringa in generic name [5]. *Moringa oleifera* has numerous medicinal uses, which have long been recognized in the Ayurvedic and Unani systems of medicine [6]. Fruits pods of Moringa are rich sources of minerals like calcium, iron and good sources of vitamins A, B, C and protein including fair amounts of sulfur containing amino acids [7]. Moringa pods are rich source of calcium, iron and fiber; out of which, 40% is soluble dietary fiber. Nutritionally, Moringa pods are of great value as sources of calcium, phosphorus and Vitamin C. Edible portion of Moringa pods are rich in calcium (30 mg/100 g), phosphorus (110 mg/100 g), iron (5.3 mg/100 g) Vitamin C (120 mg/100 g) [8]. Dehydrated Moringa powder is an integral part of Indian cuisine and is extensively used in many food and curry preparations since it gives a distinct palatable taste and is rich source of glutamic acid [9].

Although Moringa is sometimes used in the traditional foods, information on the use in commercial products is limited. Besides, astringency, aftertaste bitterness and dark green colour are also serious limitations for the use of Moringa leaf in food formulations [10]. Apilado *et al.* reported that Moringa leaf powder cannot be added to cream cheese from buffalo’s milk at the levels greater than 0.5% [11]. Use of higher levels adversely affected its acceptability. In order that a product be labeled as a "good source of nutrients", incorporation of Moringa would be required at higher levels. At present, there is no research work carried out in literature on utilization of any part of Moringa in *lassi*. Use of *lassi* as a carrier for incorporating Moringa products is expected to have the following benefits: *lassi* like products are widely consumed regularly in predictable amounts by people of all age groups, cost is affordable by target population, addition of nutrients such as Moringa is expected to cause minimum change in sensory characteristics of *lassi* like product because of its acidic nature. It was envisaged that incorporation of Moringa in *lassi* would result in elevating its nutritional and functional value by increasing its iron, vitamin C, calcium, potassium, zinc and fiber content. Therefore, the present study was planned to produce a value added *lassi* using pods of *Moringa oleifera*.

MATERIALS AND METHODS

Standardized milk having an average fat 4.5±0.2% and average MSNF content of 8.5±0.05% and Christen Hansen Exact *Dahi* 2-Mat no. 706272 (Freeze-dried lactic culture for Direct Vat, Denmark) culture was used for preparation of *dahi*. This culture consists of mesophilic/thermophilic culture. The culture was selected based on its ability to achieve desired acidity i.e. 0.7% LA within 5 h of incubation. Moringa pod powder (PKM1 variety) was procured from Pushpam Foods, Kunjrao. The composition of MPP was 1.7% fat, 16.1% protein, 7.6% ash, 29.21% crude fibre and 2.1% moisture. Other basic ingredients like cane sugar, common salt, ginger powder, black pepper and cumin powder were procured from the local market. Low Methoxy Pectin pure of Loba chemical, Mumbai and Iota Carrageenan of Himedia Laboratories Pvt. Ltd., Mumbai brand was used as a stabilizer.

Preparation of *Lassi*

Fresh, sweet, good quality milk, standardized to 4.5% fat/8.5% MSNF was preheated to 35–40°C, filtered through a muslin cloth, heated to 90°C for 5 min and cooled to 40±2°C. Curd was prepared using DVS starter culture which was added @ 7 g/100 l milk. The inoculated milk was transferred to a sanitized stainless steel vessel and incubated at 40±2°C till slightly less (0.05% LA less) than desired acidity in the *dahi* (0.7 to 1.0% LA) was obtained. In another vessel, calculated quantity of dry ingredients viz. stabilizer, sugar @ 4% (w/w of *lassi*), salt @ 0.4% (w/w of *lassi*), MPP @ 1.25 to 2.00% (w/w of *lassi*) and flavoring materials (mixture of equal quantity of roasted cumin powder, black pepper, ginger powder) @ 0.5% (w/w of *lassi*) were mixed with pasteurized water and heated to 90°C for 1 min. The quantity of water was calculated on the basis of final TMS in *lassi* (i.e. between 6.00 and 10.00%). The ingredients were blended thoroughly by stirring at 2000 rpm (Yorko Micro Tissue Homogenizer, New Delhi) to prepare the Moringa base. Finally, the base was blended with *dahi*. The product was subjected to thermization at 65°C for 5 min and hot filled in pre-sterilized PET bottles (250 ml), screw capped and stored at refrigeration temperatures (i.e. 7±2°C).

Experimental Design

The central composite rotatable design was used for designing the experimental combinations. The experiment was designed using software Design Expert version 8.0.10. The variables used were TMS (% w/w of *lassi*), acidity of *dahi* (% LA) and MPP (% w/w of *lassi*) and level of these variables along with experimental plan consisting of three variables at five levels and six replicates at center point. The variables were standardized to simplify computation and to deduce the relative effect of variables on response. The magnitude of the coefficients in second order polynomial shows the effect of that variable on the response. The experimental plan consisted of a set of 20 experiments.

ANALYSIS

Fat content of *lassi* was determined using Mojonnier method as described in BIS Handbook [12]. Titratable acidity of the *lassi* was measured according to the procedure mentioned in BIS Handbook for milk [12]. The total nitrogen was determined using semi-micro Kjeldahl method [13]. Ash and Total solids (TS) content were determined by procedure described in BIS handbook [12]. The pH values of samples were measured by use of Electronic pH meter (M/s. Mettler Toledo AG, Schwerzenbach, Model CH-8603). Viscosity of product was determined by using 'Brook field' viscometer (DV II + Pro Viscometer, Model- LVDV-II + P, USA) at 20±2°C. Iron content of MPP and *lassi* was estimated by atomic absorption spectrophotometry (Spectrophotometer-Perkin Elmer model 3110), λ=248.3 nm. Vitamin A content of pod powder and *lassi* were analyzed by using the standard procedure of AOAC [14]. The calcium content of product was measured as per method given by BIS (SP: 18 (Part XI) 1981) [12]. Potassium content of pod powder and *lassi* was analyzed by using the standard procedure of AOAC (2004). Estimation of vitamin C content was carried out by procedure described as per BIS (1089) [15]. The crude fibre content of pod and *lassi* was determined by the method described in EC No. 152/2009.

Microbiological Analysis

For microbiological analysis (for lactobacilli, SPC, yeast and mold and coliforms), 11 g of sample was diluted in 99 ml phosphate buffer according to method described in IS: 1479 (1961) [16]. Further serial dilutions were prepared in 9 ml phosphate buffer. 1 ml each from two or three suitable dilutions was poured with either acidified MRS agar. The plates were allowed to solidify and then layered with 6–7 ml of the same agar. Number of colonies developed were counted after incubating plates at 37°C for 48 h and expressed as cfu of *lactobacilli*/g. The methods of plating, incubation and counting for the enumeration of SPC, yeasts and molds as well as coliforms were followed as described by IS:1989 (Part XI) [17].

Sensory Evaluation

The product was subjected to the sensory evaluation by semi-expert panel of 10 judges using a 9-point hedonic scale scorecard. The sensory panel (n=10) was composed of staff members and post graduate student working in the institution. The selection criterion was that the subject had to be familiar with the product as well as show similar behavior between sensory evaluation sessions. *Lassi* (50 ml) were served in odorless disposable polystyrene cups with lids. The samples were tempered to ±2°C. The cups were labeled with random 3-digit codes. The order of presentation of samples was randomized across judges. Subjects judged a maximum of five samples in one session. Sensory analysis was conducted in isolated booths illuminated with incandescent light in a sensory evaluation lab maintained at 23±2°C. Panelists were instructed to use lukewarm water as a rinsing agent as and when required.

Statistical Analysis

The data obtained during different phases of this study was analysed using completely randomized design and response surface methodology. The experiment was designed and responses were analyzed using software Design Expert version 8.0.10.

RESULTS AND DISCUSSION

Various forms of Moringa viz. fresh pulp and dried pod powder were studied for feasibility and suitability for use in Moringa *lassi* keeping commercialization in the view. Pulp was found to be unsuitable as inefficient mechanical extraction and lower in nutrients as the husk/skin was removed resulting in loss of fibers and vitamins. Therefore it was decided to add dried pod powder. MPP was procured from seven sources and based on preliminary screening, the most acceptable brand of MPP was selected.

Selection of Mode of Addition of Moringa in *Lassi*

Considering the manufacturing process for *lassi* was envisaged that there are three possible ways to add Moringa in to *lassi* as: (a) Before heat treatment of milk; (b) After heat treatment of milk but before fermentation and (c) Directly into the *lassi* after fermentation. To select the mode of addition of MPP in *lassi*, *Lassi* was manufactured according to the procedure standardized by Kumar *et al*. and MPP was added @ 1.5% in *lassi* [18]. In the preliminary trials during investigation of the three stages, it was observed that addition of MPP before heat treatment of milk and before fermentation resulted in adverse effect sensory characteristics of the product. Therefore, addition of MPP before heat treatment of milk was not considered. To find out the effect of addition of Moringa before and after fermentation, calculated amount of MPP was added at two different stages. Before addition, MPP was mixed with potable water at 40°C (about 10 times the wt. of MPP) and heated to 90°C for 1 min for proper dissolution. In one experiment the dissolved MPP was directly added to milk after heat treatment but before addition of starter culture and in the other, it was directly incorporated into the *lassi*. Seven replications of this experiment were conducted. The results presented in Table 1 represent the influence of stage of addition of MPP on the sensory attributes of *lassi*. It can be seen from Table 1 that addition of MPP before fermentation in *dahi* resulted in a significantly ($P<0.05$) lower flavour, color and appearance, body and overall acceptability scores compared to when added after fermentation. It was found that addition of MPP before fermentation resulted in a product with very harsh astringent flavour and uneven body and texture with pronounced whey separation. Therefore, it was decided to add the dissolved MPP directly into the *lassi*.

OPTIMIZATION OF PARAMETERS FOR MORINGA POD *LASSI*

The *lassi* prepared with different levels of Moringa powder, with different TMS and acidity levels are as shown in design matrix of three factor CCRD deign consisting of 20 experiments as shown in Table 1 and Table 2 were evaluated for sensory and physico-chemical characteristics.

Table 1: *Effect of Stage of Addition of Moringa on the Sensory Quality of Lassi.*

Stage of Addition	Flavour Score	Color and Appearance Score	Body (Consistency) Score	Overall Acceptability Score
Before Fermentation	$6.01±0.23^{b}$	$6.07±0.22^{b}$	$6.96±0.12^{a}$	$6.35±0.27^{b}$
After Fermentation	$6.32±0.19^{a}$	$6.35±0.21^{a}$	$6.77±0.16^{b}$	$6.79±0.21^{a}$
CD (0.05)	0.19	0.23	0.14	0.23

Each observation is a mean ± SD of seven replicate experiments (n=7);
$^{a\text{-}b}$ *Superscript letters following numbers in the same column denote significant difference (P<0.05).*

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Table 2: *Experimental Design Matrix and Sensory Scores of Moringa Fortified Lassi (Pod).*

Run	A: Total Milk Solids (%)	B: Acidity of *Dahi* (% LA)	C: Moringa Pod Powder (%)	Flavour Score	Body Score	Acidity Score	Colour and Appearance Score	Overall Acceptability Score	Acidity Value (% LA)	Viscosity at 20°C (cP)
				Out of 9						
1	8.00	0.75	1.63	8.25	8.00	8.00	8.50	8.50	0.428	1175
2	10.00	0.90	2.00	6.75	7.75	7.00	7.00	6.50	0.651	1505
3	4.64	0.75	1.63	8.00	8.00	8.25	8.25	8.00	0.248	0438
4	6.00	0.60	2.00	6.00	7.25	6.75	7.00	6.25	0.250	0567
5	8.00	0.50	1.63	6.50	7.00	7.00	7.00	7.00	0.283	1184
6	10.00	0.60	2.00	6.50	7.00	7.25	7.50	6.50	0.420	1850
7	6.00	0.90	1.25	7.50	8.00	7.25	7.00	7.25	0.390	0691
8	8.00	0.75	2.26	7.00	7.75	7.00	6.50	7.00	0.428	1190
9	10.00	0.90	1.25	7.25	7.25	7.00	7.25	7.75	0.650	1608
10	6.00	0.90	2.00	8.50	8.50	8.50	8.00	8.25	0.390	1132
11	11.36	0.75	1.63	8.50	8.00	8.00	8.50	8.25	0.608	1800
12	8.00	0.75	1.63	8.25	8.50	8.25	8.25	8.25	0.428	1200
13	8.00	1.00	1.63	6.00	7.00	6.00	7.00	6.25	0.580	1184
14	8.00	0.75	1.63	8.00	8.25	8.25	8.50	8.00	0.428	1225
15	8.00	0.75	0.99	7.50	8.00	8.00	7.25	8.00	0.428	1230
16	10.00	0.60	1.25	8.00	8.25	7.50	7.50	8.00	0.420	1775
17	6.00	0.60	1.25	7.75	7.50	7.00	7.50	7.75	0.255	0681
18	8.00	0.75	1.63	8.25	8.00	8.00	8.00	8.25	0.428	1205
19	8.00	0.75	1.63	8.50	8.25	8.50	8.00	8.50	0.420	1198
20	8.00	0.75	1.63	8.25	8.00	8.25	8.25	8.25	0.428	1212

Effect of Different Levels of Moringa, TMS in *Lassi* and Acidity of *Dahi* on Sensory Properties and Physico-Chemical Attributes of Moringa *Lassi*

The flavour, body (consistency), acidity, color and appearance and overall acceptability score of product was observed in the range from 6–8.5, 7–8.5, 6–8.5, 6.5–8.5, 6.25–8.5 (out of 9) respectively. The scores for physico-chemical responses varied from 0.24 to 0.65% LA and 438 to 1850 cP. The coefficient of determination (R^2) is shown in Table 3 which reflects the proportion of variability in data explained or accounted by the model for flavour, body, acidity score, C&A and OA were 0.8939, 0.8815, 0.8311, 0.8804 and 0.8726 respectively. A larger R^2 values (>0.80) is statistically adequate for developing a model or equation. The probability value (p) showed the adequacy of the models so used to describe the effect of variables on different responses. The effect of TMS (% w/w of *lassi*), acidity of *dahi* (% LA) and MPP (% w/w of *lassi*) on the responses is shown in Table 3. The sign and magnitude of coefficients indicate the effect of the variable on the responses. The total effect of individual variable and combined effect of the variables at all levels are presented in Table 3.

Flavour Score

MPP had significant ($P \leq 0.05$) effect on the flavour profile in linear terms (Table 3). The negative value indicates that increasing level of MPP decrease the flavour score significantly ($P \leq 0.01$). The interaction effect of TMS-acidity of *dahi* and acidity of *dahi*-MPP had negative significant effect ($P \leq 0.05$) on flavour scores which indicates that by increasing the level of one factor, the level of other factor has to be decreased. While acidity of dahi-MPP has positive interaction effect which means by increasing the level of one factor, the level of other factor has to be increased. Acidity of *dahi* and MPP had negative significant changes at quadratic terms ($P \leq 0.01$) (Table 3).

When MPP was kept at center point (1.63%), increasing acidity of *dahi* increased the flavour score but at higher acidity levels it reduces the flavour score. Also, increasing the TMS content has slightly increased the flavour score. In case of keeping acidity of *dahi* at center point (0.75% LA), increasing MPP slightly increased the flavour score only up to certain levels, also flavour score was increased by increasing TMS content. When TMS level was kept constant at center point (8%), with the increase in MPP content has decreased flavour score and then increased while increasing the acidity of *dahi* up to certain level i.e. around 0.82% LA.

No reports are available on flavor scores of Moringa pod powder fortified fermented dairy products for comparison.

Body (Consistency) Score

Moringa pod powder, TMS and acidity of *dahi* didn't show any significant changes at linear terms on body scores ($P>0.05$) (Table 3). The coefficient estimates of body in interaction effect, TMS-acidity of *dahi* had significant ($P<0.05$) negative effect on body score. Acidity of *dahi*- Moringa pod powder had positive significant ($P\leq0.01$) effect in interactive effect.

When MPP was kept at center point (1.63%), increasing acidity of *dahi* did not affect the body score significantly while TMS level has increased the body score. In case of keeping acidity at center point (0.75%), increasing MPP slightly increased the body score, while increase in TMS content has not affected body significantly. When TMS level was kept constant at center point (8.0%), with the increase in MPP content, body score decreased while increasing the acidity of *dahi* showed increase in body score up to certain level then decreased at higher levels.

These results are in agreement with those obtained by Salem *et al.*, who reported that organoleptic properties of Labneh revealed that addition of DLMO (Dried leaves of *Moringa oleifera*) had significant effect on flavour, appearance, body (consistency) and total score [4]. As the concentration of DLMO was increased in fortified Labneh, the score of flavour, body (consistency), appearance and total score was decreased. No data are reported in the literature on effect of total milk solids and acidity of *dahi* on body of Moringa *lassi*. According to Badmos *et al*., the control cheese was poorest in colour, taste and texture rating ($p<0.05$) and cheese with leaf extracted in ethanol has higher scores [19]. Salem *et al.* also reported that sour cream made with moringa leaf extract had acceptable flavor, body and texture and appearance scores [20]. However, no reports are available on consistency scores of Moringa pod powder fortified fermented dairy products for comparison.

Acidity Score

Moringa pod powder, TMS and acidity of *dahi* did not show any significant changes at linear terms on acidity scores ($P>0.05$) (Table 3). The coefficient estimates of acidity score in interaction effect, TMS-acidity of *dahi* had significant ($P<0.05$) negative effect on acidity score.

MPP was kept at center point (1.63%), increasing acidity of *dahi* increased the acidity score up to around 0.82% LA and then decreased. Whereas TMS level has increased the acidity score. In case of keeping acidity of *dahi* at center point (0.75%), increasing MPP and TMS slightly increased the acidity score but not significantly. When TMS level was kept constant at center point (8.0%), with the increase in MPP content, acidity score increased then decreased at higher levels. While increasing the acidity of *dahi* showed increase in acidity score up to certain level (0.82% LA). No reports are available on acidity scores of Moringa fortified fermented dairy products. But Salem *et al*. (2013) [4] reported the higher titratable acidity of Moringa fortified labneh than control which resulted in lower acceptability of the product.

Color and Appearance Score

Moringa pod powder, TMS and acidity of *dahi* did not show any significant changes on color and appearance ($P>0.05$) at linear terms as well as in interactive effect (Table 3). At quadratic levels, acidity of *dahi* and MPP has negative effect on color and appearance of the product ($P<0.01$).

When MPP was kept at center point (1.63%), increasing acidity of *dahi* up to around 0.82%, LA increased the color and appearance and then decreased. Whereas TMS level has increased the color and appearance. In case of keeping acidity of *dahi* at center point (0.75%), increasing MPP level showed increase in color and appearance then decreased at higher levels. TMS slightly increased the color and appearance but not significantly. When TMS level was kept constant at center point (8.0%), with the increase in MPP content and TMS level, color and appearance increased then decreased at higher levels.

Nadeem *et al.* also reported addition of LEMO up to 600 ppm level did not have any negative effect on flavor scores [21]; when the addition level was increased to 800 ppm, the sensory scores of these parameters significantly (P>0.05) decreased. Some of the panelists criticized the 800 ppm sample for having phenolic smell and taste, which resulted in lower smell and taste scores. The reason for the low color score of 800 ppm sample was due to the dark green colour of LEMO which has a slight but definite effect on this parameter. These results are agreement with Badoms *et al*. [19]. They reported that cheese samples preserved with ethanol extracts of Moringa leaves were most acceptable to consumers. However, no reports are available on colour and appearance scores of Moringa pod powder fortified fermented dairy products for comparison.

Overall Acceptability Score

The values presented in Table 3 reveal that the level of MPP has significant effect in linear terms. The negative sign indicates the negative effect of level of MPP on the overall acceptability score. This indicates that the increase in the MPP decreases the overall acceptability score significantly (P<0.01). Also, at extremely higher levels of MPP, overall acceptability scores decreased significantly (P<0.05). TMS had a non-significant effect on overall acceptability score at linear and quadratic level. Acidity of *dahi* had a non-significant effect on overall acceptability score and at quadratic level it has a significant negative effect (P<0.01). TMS-MPP (AC) has non-significant effect on overall acceptability of Moringa pod *lassi*. Acidity of *dahi*-MPP has positive significant effect on overall acceptability.

A Multiple Regression equation generated to predict the overall acceptability as affected by different factors in terms of actual factors is shown below where, A, B and C refer to TMS, Acidity of *dahi* and MPP respectively.

$$\text{Overall acceptability} = -13.8109 + 1.4493 \times A + 36.3393 \times B + 4.3239 \times C - 0.7291 \times A \times B - 0.3750 \times A \times C + 6.1111 \times B \times C - 0.0190 \times A^2 - 26.9613 \times B^2 - 2.1139 \times C^2$$

The response surface plot for the values obtained is shown in Figure 1 which is based on the above model with varying levels of the two variables studied within the experimental range. These observations suggest that TMS was kept constant (8.0%), increase in MPP had makeable decrease in overall acceptability; whereas acidity of *dahi* showed increase in overall acceptability up to certain level i.e. 0.8% LA and then after, decrease in overall score was found. When MPP was kept constant (1.63%), increasing acidity of *dahi* increase the overall acceptability the effect being higher but negative on increased additions. Higher TMS affects the overall acceptability score by increasing it. Keeping acidity of *dahi* at center point, increase in MPP has decreased overall acceptability but only at higher levels; while TMS has increased it significantly.

Ibrahim *et al*. (2015) [22] reported that 0.5% level of Arjuna and 1% level of Moringa were best and highest in sensory evaluation scores. As the concentration of DLMO was increased in fortified Labneh, the decrease in total score was found by Salem *et al*. [4]. The similar trend was found in sour cream and West African soft cheese also by Salem *et al*. and Badmos *et al*. respectively [19, 20]. However, no reports are available on overall acceptability scores of Moringa pod powder fortified fermented dairy products for comparison.

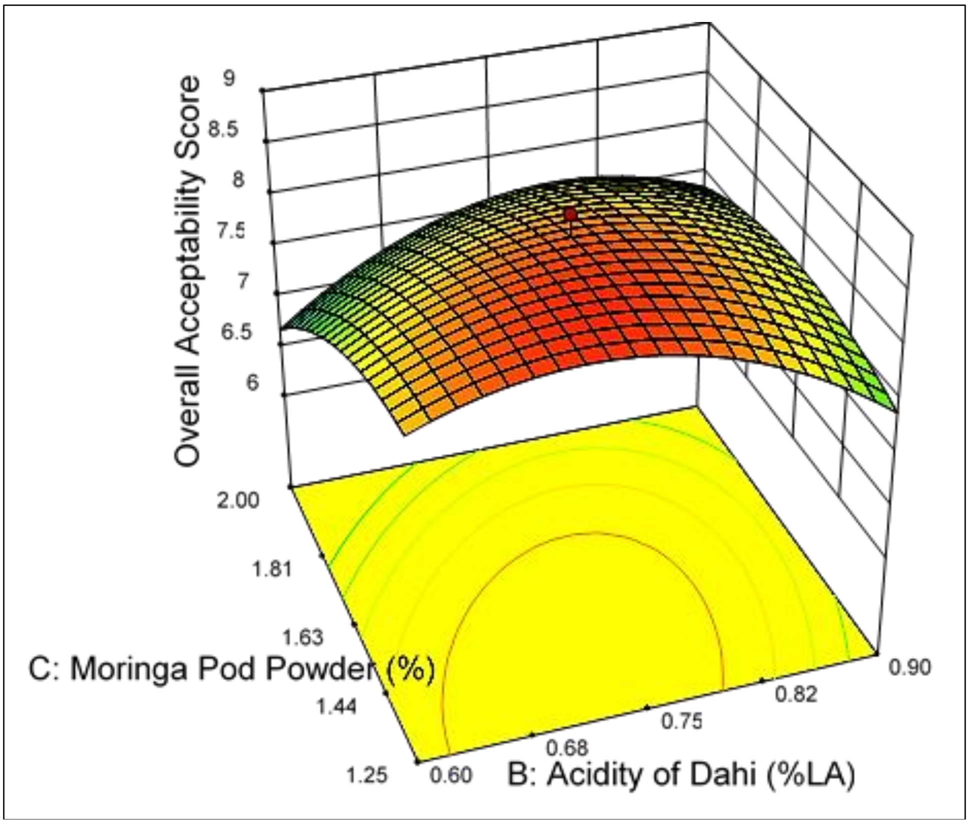

Fig. 1: *Influence of Moringa Pod Powder and Acidity of Dahi on Overall Acceptability Scores of Moringa Lassi.*

Acidity Value/Titratable Acidity

TMS and acidity of *dahi* showed significant changes at linear terms on acidity value ($P<0.01$) (Table 3). MPP has non-significant effect on acidity value of the product. The coefficient estimates of acidity value in interaction effect, TMS-acidity of *dahi* had significant ($P<0.01$) positive effect. At very high levels, acidity of *dahi* showed positive significant effect on acidity of product ($P<0.05$).

When MPP was kept at center point (1.63%), increasing acidity of *dahi* and TMS increased the acidity value. In case of keeping acidity of *dahi* at center point (0.75%), increasing TMS has increased the acidity score. But MPP has no effect on acidity value of the product. When TMS level was kept constant at center point (8.0%), with the increase in TMS content, acidity value increased; while increasing MPP shown no effect.

But Salem *et al*. reported the higher titratable acidity of Moringa fortified labneh than control [4]. This can be correlated to the results obtained but here the titratable acidity of all the products was different from the initial levels which was suggested by RSM.

Viscosity

TMS shows significant positive effect on viscosity ($P<0.01$). Moringa pod powder and acidity of *dahi* did not show any significant changes at linear terms on body scores ($P>0.05$) (Table 3). The coefficient estimates of viscosity in interaction effect, only TMS-acidity of *dahi* (AB) had significant ($P<0.01$) negative effect on viscosity.

When MPP was kept at center point (1.63%), increasing acidity of *dahi* did not affect the viscosity significantly while TMS level has increased the viscosity. In case of keeping acidity at center point (0.75%), increasing MPP slightly increased the viscosity, while increase in TMS content shows significant increase in viscosity. When TMS level was kept constant at center point (8.0%), with the increase in MPP and acidity of *dahi* showed no effect on viscosity. No reports available on viscosity of Moringa pod fortified fermented dairy products for comparison.

Table 3: *Coefficient of the Full Second Order Polynomial Model for Coded Sensory Responses to Different Levels of Ingredients of Moringa Fortified Lassi.*

Terms		Flavour Score	Body Score	Acidity Score	Color and Appearance Score	Overall Acceptability Score	Acidity Value	Viscosity
		(Out of 9)					(% LA)	At 20°C (Cp)
Intercept		8.2470	8.1636	8.2097	8.2507	8.2943	0.4285	1200.6600
Linear Level	A: Total Milk Solids	–0.0300	–0.0732	–0.0857	0.0125	–0.0241	0.1075*	436.2353*
	B: Acidity of *Dahi*	0.0666	0.1098	–0.0316	–0.0183	–0.0008	0.0892*	4.6131
	C: Moringa Pod Powder	–0.2629#	–0.0674	–0.0682	–0.0741	–0.3611*	0.0000	16.9679
Interactive Effect	A × B	–0.3438#	–0.2500#	–0.3438#	–0.1563	–0.2188	0.0226*	–135.875*
	A × C	–0.1563	–0.1250	–0.1563	–0.0937	–0.2813	0.0000	–44.3750
	B × C	0.4688	0.3125	0.2188	0.1563	0.3438#	0.0000	47.1250
Quadratic Level	A^2	0.0194	–0.0388	–0.0386	0.0395	–0.0763	0.0003	–17.4858
	B^2	–0.6877	–0.3924	–0.6131	–0.4467	–0.6066	0.0013#	5.4951
	C^2	–0.3342	–0.0830	–0.2595#	–0.4909	–0.2973#	0.0003	14.6876
R^2		0.8939	0.8816	0.8311	0.8805	0.8726	0.9999	0.9666
Model F-value		9.3700	8.2700	5.4700	8.1800	7.6100	1130.1100	32.1100
APV		8.2289	9.7170	6.5082	7.8060	7.3474	347.7789	21.107
Suggested Model		Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic

**: P≤0.01; #: P≤0.05; ns: non-significant; APV= Adequate Precision Value; R^2= Coefficient of determination.*

OPTIMIZATION OF PRODUCT FORMULATION FOR MORINGA *LASSI*

Process optimization for the manufacture of Moringa *lassi* was carried out with the objective of determining the best possible combination(s) of different levels of factors viz. TMS (A) Acidity of *dahi* (B) and MPP (C) that would lead to the most acceptable product in terms of sensory scores, compositional attributes and other characteristics. The goals for all sensory responses were set to be maximized and overall acceptability was given higher importance level i.e. 4 than other responses.

Considering the parameters and their limits, the RSM suggested the one most suited solution. Suggested solution from RSM analysis for Moringa *lassi* is 10.00% TMS in *lassi* (w/w), 0.80% LA, and 1.63% MPP (w/w) having desirability of 0.664. The predicted sensory scores, physico-chemical parameters viz. flavour, overall acceptability, acidity of product, viscosity from RSM analysis are depicted in Table 4.

The final product was manufactured employing this suggested formulation and the actual results were obtained from the Moringa *lassi* manufacture. The predicted values of the criteria/responses selected for process optimization under study were compared with the actual values of the selected responses. The results obtained confirm that the selected combination is the best one in terms of the sensory, compositional and textural responses delineated at the beginning of the study. The results are also validated statistically by 't' test. The calculated 't-value' for all the parameters are reported in Table 4. The values for 't' test being less than the table values, it is inferred that there is no significant (P>0.05) difference between the predicted and actual values of responses as shown in Table 4.

Standardized Method for Manufacture of Moringa *Lassi*

Based on the results obtained above, a method was standardized for manufacture of Moringa *lassi*. In this method, standardized milk (4.5% fat/SNF 8.5%) is taken after checking its quality criteria and pre-heated to 35–40°C, filtered followed by heating to 90°C for 5 min, cooled to 40±2°C and inoculated with DVS lactic mesophilic/thermophilic *dahi* culture at 0.7 g/10 l milk and blended for 5 min before

Table 4: *Comparison of Predicted vs. Actual Values of Responses Used for Process Optimization of Moringa Lassi Manufacture.*

Response	P Value	Predicted Value*	Actual Value @	Cal. t-Value#	Level of Significance
Flavour	0.9789	8.01	8.06	0.0282	NS
Body Score	0.5104	7.95	7.83	0.7217	NS
Acidity Score	0.8772	7.87	7.85	0.1647	NS
Colour **and** Appearance	0.6799	8.16	8.10	0.4441	NS
Overall Acceptability	0.8934	7.94	7.92	0.1427	NS
Acidity	0.5375	0.57	0.55	0.6735	NS
Viscosity	0.8915	1580.82	1582.73	0.1452	NS

***:** *Predicted values of Design Expert 8.0.3 package;*
***@:** Actual values are average of five trials for optimized product;*
***#:** t-values found non-significant at 5 % level of significance;*
***NS=** Non Significant; and*
Tabulated t-value =2.776 (cal. t-value less than tabulated value).

incubation at 40±2°C till acidity of 0.75% LA is achieved. The *dahi* is then transferred to refrigerator (7±2°C) where the final acidity of 0.8% LA is achieved upon cooling.

For preparing processed Moringa base, the required amount of dry ingredients viz. MPP @ 1.63%, pectin @ 0.12%, carrageenan @ 0.03%, sugar @ 4.0%, salt @ 0.4%, flavoring mixture @ 0.5% are taken. All the dry ingredients are calculated on the basis of w/w of *lassi*. Calculated amount of potable water (45 to 50°C) to lower down TMS of *dahi* from 13.0 to 10% is taken in a SS vessel. The dry ingredients were added to the water and blended at 2000 rpm in high speed blender for 2 min and heated to 90°C/1 min followed by immediate cooling to room temperature (35–40°C) for complete hydration.

Finally, the Moringa base as obtained above is blended with the *dahi* in a mixture at low speed 30 s. The product is then subjected to thermization at 65°C for 5 min and filled in pre-sterilized PET bottles and stored at 7±2°C.

Evaluation of the Standardized Product for Its Nutritive Value

The standardized Moringa *lassi* was compared with *lassi* prepared using the method standardized by Kumar *et al.*, since most of the commercially available *lassi* have composition which is almost similar to that reported by Kumar *et al.* [18]. The Moringa *lassi* was analyzed for proximate composition, selected vitamins and minerals and microbiological quality parameters using standard methods. The energy values of both the *lassi* samples viz. Moringa *lassi* was also calculated and compared with control.

According to guidelines provided by FDA (2013) [23] on food labeling, the product label must include % daily value (% DV), to designate both the daily reference value (DRV) and recommended daily intake (RDI). According to Code of Federal Regulations (21 CFR 101.12) reference amount customarily consumed (RACC) for beverages is 240 ml. Thus, DV (%) of *lassi* (250 g serving size) was calculated based upon the obtained values in this study for its macronutrients and micronutrients. The proximate composition of daily value (%) of the moringa lassi and control for various nutrients are presented in Table 5. It can be seen from the table that the standardized product contains higher levels of Vitamin A, Vitamin C, iron, fiber and potassium compared to control.

It can be seen from Table 5 that one serving size (250 g) per day of product could be an "excellent source of calcium" having 25% DV. Moringa *lassi* had the higher content of iron and potassium (providing 12 and 10% DV respectively) than control. The DV of protein is also 10%. In order to make a "good source of micronutrient", the finished product must ideally contain 10 to 19% of DV per serving, since the iron and potassium content were in this range, the product can be labeled as "good source of iron and good source of potassium and protein".

The product could also provide a considerable amount for Vitamin C, fiber and Vitamin A (7, 5 and 3% DV respectively).

Table 5: *Comparison of Proximate Composition and % DV of Moringa and Control Lassi Samples.*

Components (Unit)	Control@	% DV	Moringa *lassi*	% DV
Total solids (g/100 g)	17.27		15.48	
Fat (g/100 g)	2.1		3.45	
Protein (g/100 g)	1.8	8.0	2.42	10.0
Ash (g/100 g)	0.57		1.35	
Total Carbohydrate (g/100 g)	12.8		8.25	
Added Sugar (g/100 g)	12		3.75	
Total Dietary Fiber (g/100 g)	0	0	0.467	5.0
Vitamin C (mg/100 g)	1.03	4.0	1.54	7.0
Vitamin A (mcg/100 g)	60	3.0	53.33	3.0
Calcium (mg/100 g)	74	18.0	100	25.0
Iron (mg/100 g)	0.11	1.5	0.84	12.0
Potassium (mg/100 g)	90	6.5	130	10.0
Energy (kcal/100 g)	77.3		73.73	

#: Values represents mean of duplicates;
@: Lassi prepared using method standardized by Kumar et al. [18];
** %DV (250 g serving size) calculated based upon a caloric intake of 2,000 cal (8400 kJ), for adults and children four or more years of age.*

It is well known that milk and milk products are not a good source of Vitamin C, iron and fiber. Therefore this study was successful in formulating a composite fermented *lassi* which can be labeled as "good source of iron, potassium and protein", with considerable amount of Vitamin C and fiber.

Based upon the results, conclusively, Moringa *lassi* may be characterized as excellent source of calcium and good source of protein, potassium and iron. The product can be consumed with breakfast or meals and designated as ready to drink (RTD) beverage.

Consumer Response

The commercial success of any new product developed depends on the consumer response. Consumer response studies play a key role in launching a newly developed product in the market. Local preference of the consumers is very important. Therefore, the Moringa *lassi* sample manufactured using the recipe formulated in the present study was evaluated through a consumer survey conducted by selecting randomly 120 consumers representing different segments of the society. For consumer acceptance trial in the present project, the product was manufactured as per the procedure developed in this study and packed in 100 ml PET bottles. The products were distributed to 120 probable consumers and their comments were recorded. The consumers were asked to indicate whether they like the product or not, and if yes, to what level i.e. “Excellent”, “Very Good” or “Good”. None of the consumers disliked the product. Out of 120 consumers who judged the product, 23% consumers rated it as excellent, 35% consumers rated it as very good and 19% rated it as good. This indicates that the product has a good potential for marketing.

CONCLUSION

Thus it can be concluded on the basis of this study that formulation and method for manufacture of Moringa *lassi* using Moringa pod powder was successfully developed. The standardized product was found to contain higher levels of Vitamin A, Vitamin C, iron, fiber and potassium compared to *lassi* prepared without addition of Moringa. Based upon the results, conclusively, MPL may be characterized as excellent source of calcium and good source of iron, potassium and protein. Consumer response studies indicated that the product has a good potential for marketing.

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