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Development of functional buttermilk utilizing freeze-dried *M. oleifera* leaf powder

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Abstract
The present investigation was aimed at formulating a fermented beverage with incorporation of freeze dried *Moringa oleifera* leaf powder (MLP) to develop therapeutic buttermilk. Buttermilk was prepared with four different concentrations of freeze dried *Moringa oleifera* leaf powder. Sensory characteristics of *Moringa oleifera* leaf powder-based buttermilk samples were also evaluated such as color and appearance, body and mouthfeel, flavor and overall acceptability level. The developed buttermilk with *Moringa oleifera* leaf powder showed significant changes in sensory properties. Samples with 0.5% *Moringa oleifera* leaf powder obtained the highest scores in the sensory evaluation. *Moringa oleifera* leaf powder at 0.5% level in buttermilk improved nutritive values and desirable sensory characteristics.

Keywords: *Moringa oleifera* leaf powder, chhash, buttermilk, fermented beverage

1. Introduction
Buttermilk is traditionally known as "Chhash", sour buttermilk, which is very popular in several parts of the world. It has a mild pleasing flavor with uniform thick consistency and smooth texture (Rao *et al.*, 2003) [9]. The colour of the buttermilk varies from yellowish creamy white to creamy white (Chandan *et al.*, 2006) [5]. Now-a-days, most of the dairy products are fortified with spices, herbs and condiments in order to enhance the nutritive properties and shelf life of the foods.
Moringa oleifera Lam. is a multi-purpose tropical tree which belongs to *Moringaceae* family (Anwar *et al.*, 2006) [3]. It is an important food commodity which has received a lot of attention as the “natural nutrition of the tropics”. The leaves are widely consumed as a highly nutritious vegetable in India (Ninivaggi *et al.*, 2010) [7]. The plant leaves have low calorific value and contain all essential amino acids. It contains numerous phytochemical properties such as flavonoids and carotenoids which are used for treating various infectious diseases. They are also rich in iron, zinc, β-carotene, vitamin C, calcium, protein, potassium and acts as a good source of natural anti-oxidants.
Micronutrient deficiency is a major serious problem where pregnant women and children are more vulnerable (Das *et al.*, 2013) [6]. Saini *et al.* (2014a) [10] reported that the *M. oleifera* contain a rich source of iron (17.5 mg/100 g DW), respectively. In a bioavailability model, research study conducted on a rat model, showed that the iron from the *M. oleifera* leaf was found to be higher compared to ferric citrate, in overcoming iron deficiency. In *moringa* plant, calcium is considered as one of the important minerals for human growth. While eight ounces of milk can provide 300–400 mg, where *M. oleifera* leaves can provide 1000 mg and *M. oleifera* leaf powder can provide more than 4000 mg. The *Moringa* leaf powder can be used as a substitute for iron tablets, hence as a treatment for anemia. Research studies reported that the plant leaves contain 44% of amino acids, vitamins like beta-carotene of vitamin A, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E (17.3 mg/100 g) (Saini *et al.*, 2014b) [11].
Teixeira *et al.* (2014) [13] recorded the proximate studies of the leaf powder contain 44.4% carbohydrate, 28.7% crude protein, 10.9% ash, 7.1% fat, 103.1 mg/100 g iron, and 3.0 mg/100 g calcium. Also, the protein profile showed 70% insoluble proteins, 3.5% glutelin, 3% albumin, 2.2% prolamin, and 0.3% globulins. Antinutritional compounds, such as tannins, trypsin inhibitor, nitrates, and oxalic acids, respectively.

Quercetin and kaempferol glycosides (glucosides, rutinosides and malonyl glucosides) are the most common flavonoids present in the *moringa* leaves. The amount of quercetin and kaempferol are found in the concentration range of 0.46–16.64 and 0.16–3.92 mg/g dry weight, respectively (Bennett *et al.*, 2003; Siddhuraju and Becker, 2003; Amaglo *et al.*, 2010) [4, 12, 2]. *Moringa* leaves contain tannins. The highest concentration of tannin found in dried leaves (20.7 mg/g) (Teixeira *et al.*, 2014) [13].

In order to improve the human health and wellness through food nutrition and functional foods is one of the priorities identified in the world. Researchers are finding a new way to incorporate natural ingredients into dairy products for human health benefits. One such plant product is *M. oleifera*, which not only has a wide range of medical applications and great nutritional value but may also help treat micronutrient deficiencies. Therefore, it was envisaged that incorporation of *M. oleifera* leaf powder in buttermilk to elevate its nutritional and functional properties.

The objective of the present research study was to develop a unique buttermilk by incorporation of freeze-dried *M. oleifera* leaf powder that result in elevating its nutritional properties and accessing the shelf- life properties of the developed product. *M. oleifera* leaf powder in buttermilk can also be used as a vehicle for delivery of bioactive components and development of functional foods.

2. Materials and Methods

2.1 Materials

Standardized milk was used as the base material for preparation of buttermilk. This culture containing a mixed strain of thermophilic and mesophilic homo-fermentative bacterial culture was obtained from the Department of Food Safety and Quality Assurance, CFDT. The culture was stored at -18 °C until used. *M. oleifera* was procured from the local market. They were cleaned by tap water and shade dried with good ventilation for almost two weeks. The dried *M. oleifera* leaf samples were blended and passed through a sieve (20 mesh). The powdered leaf samples *M. oleifera* were kept in sealed air-tight containers and stored in darkness until their treatment.

2.2 Conventional solid-liquid extraction

Conventional solid-liquid extraction was carried out as described by (Abubakkar and Haque, 2020) [1] with some modifications. First, 2g of dried powdered leaves of *M. oleifera* was extracted with distilled water (100%). The maceration was carried out for 3× 24h at room temperature (28±2 ºC) with occasional shaking. The process is repeated 3 times. The extracts were centrifuged for 15min at 5000 rpm to remove solids. Next, the solvent was evaporated using rotary evaporator under vacuum at 40 ºC. The final extracts were freeze dried and used for further analysis.

2.3 Preparation of *Moringa* leaf powder-based buttermilk

Buttermilk was prepared as per the procedure of (Patel *et al.*, 2017) [8] with some modifications. Buttermilk sample was prepared using standardized milk. All the glassware used in this study were pre-sterilized. The contact surface of the blender and incubator were alcohol sanitized. The standardized milk was heated to 90 ºC for 5 min. The milk was inoculated with starter culture and incubated at 40±2 ºC, desired acidity in the curd was obtained. After that, curd was stirred using a laboratory blender and immediately cooled to 7±2 ºC. The period spent for stirring cooling for about 25 to 30 min was enough to give final desired acidity in the curd. *M. oleifera* leaf powder was prepared. The amount of water was calculated on the basis of Total Milk Solids (TMS) in buttermilk (i.e. between 4.00 to 6.00%). Finally, the *M. oleifera* leaf powder was blended with stirred curd with four different concentrations. The product was subjected to thermization at 65 ºC for 5 min and cooled immediately to 10±2 ºC and filled in clean and sanitized in PET bottles and stored at 7±2 ºC. All the buttermilk samples were prepared in triplicate and the results were expressed as mean.

2.4 Sensory evaluation

Sensory evaluation of control and *M. oleifera* leaf powder-based buttermilk samples was carried out using 9-point hedonic scale. Twenty panellists were selected includes students and staff of our college who were regular consumers of buttermilk. Color and appearance, body and mouthfeel, flavor and overall acceptability were the sensory parameters evaluated by sensory panellists. All the buttermilk samples were taken out from the refrigerator. Each sample was presented in a plastic bottle filled with 50 ml buttermilk sample and labelled. The presentation of samples order was randomized. The evaluation was divided into 3 sections as visual characteristics (color and appearance), flavor and texture (body and mouthfeel) evaluations. Visual analysis of *M. oleifera* leaf powder-based buttermilk was followed by texture and flavor evaluations.

2.5 Statistical Analysis

One-way analyses of variance (ANOVA) followed by a Duncan post hoc test were applicable and used to analyze the level of statistical significance of the growing regions on the levels of total phenolic, total flavonoid and antioxidant content. $p<0.05$ were considered statistically significant.

3. Results and Discussion

3.1 Sensory evaluation

Results of sensory evaluation of control and *M. oleifera* leaf powder-based buttermilk samples are presented in Table 1. The overall sensory characteristics of freeze-dried *M. oleifera* leaf powder-based buttermilk increased upto 2%, beyond this the level sensory acceptability of the buttermilk was decreased. Color & appearance, body, mouthfeel, flavor of control and *M. oleifera* leaf powder-based buttermilk sample showed significant difference and overall acceptability was recorded. Body and mouthfeel acceptability of the buttermilk fortified with 0.5% *M. oleifera* leaf powder by the sensory panel members were highest among all samples.

Table 1: Sensory characteristics of control and *M. oleifera* leaf powder-based buttermilk

Sr. no	Concentration	Sensory attributes of *M. oleifera* buttermilk				
		Appearance	Body	Color	Flavor	Overall Acceptability
1.	T_1	8.6 ± 0.10^{a}	8.3 ± 0.21^{a}	8.3 ± 0.15^{a}	8.3 ± 0.22^{a}	8.3 ± 0.09^{b}
2.	T_2	8.7 ± 0.19^{a}	8.0 ± 0.18^{a}	8.4 ± 0.15^{a}	8.3 ± 0.19^{a}	8.8 ± 0.14^{a}
3.	T_3	6.7 ± 0.20^{b}	6.2 ± 0.20^{b}	6.1 ± 0.23^{b}	6.4 ± 0.24^{b}	6.0 ± 0.21^{c}
4.	T_4	5.3 ± 0.33^{c}	5.8 ± 0.27^{bc}	5.7 ± 0.21^{b}	5.9 ± 0.27^{b}	4.9 ± 0.17^{d}
5.	T_5	5.8 ± 0.18^{c}	5.5 ± 0.29^{b}	5.8 ± 0.19^{b}	6.0 ± 0.28^{b}	5.6 ± 0.22^{c}
6.	F value	45.768^{**}	29.619^{**}	51.859^{**}	24.599^{**}	95.710^{**}

Values followed by different letters are significantly different at $p<0.05$, T1= Control, T2=0.5% MO, T3=1% MO, T4=1.5% MO, T5= 2% MO

Beyond 2% *M. oleifera* leaf powder fortification level, there was a decrease in body and mouthfeel characteristics of buttermilk samples. The flavor acceptability of buttermilk samples decreased as dominance of bland flavor and bitterness smell of *M. oleifera* leaf powder which was not accepted by panellist at higher level of *M. oleifera* leaf powder fortification. Sensory evaluation as well as overall acceptability results of the buttermilk samples showed that T_2 buttermilk sample (0.5% *M. oleifera* leaf powder) was the most acceptable sample as it scored highest among all samples with respect to body, mouthfeel, flavor and overall acceptability.

4. Conclusion

A method was standardized for manufacture of buttermilk containing *M. oleifera* leaf powder as an ingredient. It makes it a complete food as buttermilk is naturally devoid of vitamin C, iron and dietary fibre like milk and milk products. *M. oleifera* leaf powder being a good source of iron and dietary fibre can be utilized in buttermilk. Preparation of buttermilk with *M. oleifera* leaf powder 0.5% level produced buttermilk with improved nutritive and desirable sensory characteristics. Sensory evaluation results revealed that T_2 buttermilk sample fortified with 0.5% *M. oleifera* leaf powder had highest acceptability. It is concluded that 0.5% *M. oleifera* leaf powder-based buttermilk would produce an acceptable product with improved nutrient profile with respect to iron and dietary fibre.

5. Conflict of interest statement

There is no conflict of interest related to this research work.

6. Acknowledgements

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