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Development and characterization of functional yoghurt prepared with *Moringa oleifera* leaf extract

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Abstract
Nowadays there is an increasing demand for low calorie foods with high therapeutic properties. The development of dairy foods with the addition of medicinal herbs and probiotics is the need of the hour. Therefore, the present study was aimed to develop functional yoghurt using *Moringa oleifera* leaf extract (ME) as a source of all kinds of vitamins, trace minerals and fibre content, and to assess the sensory, physico-chemical, textural and microbial properties of the developed functional product. Different levels of ME (0.5, 1, 1.5, 2 and 2.5 per cent) were added to the yoghurt to assess the optimum level of inclusion. The plain yoghurt samples prepared without ME was taken as the control. Fermentation time was significantly reduced in the ME yoghurt when compared with the control yoghurt. The pH value of control and treatments ranged from 4.51 to 4.67. No variation was found in the fat and total solids content of the control and treatment yoghurt samples. The textural properties like firmness and consistency of control and treatments exhibited a significant ($p < 0.05$) difference among them. As the ME inclusion level increased, the firmness and consistency scores of the treatments were also increased. The coliform and yeast and mould count of control and treatments exhibited no significant difference among them. Increasing the level of ME significantly ($p < 0.05$) increased the level of probiotic bacteria in yoghurt and the maximum probiotic viability was shown by the yoghurt with highest ME concentration. The developed functional yoghurt was also subjected to organoleptic evaluation. Accordingly, *Moringa oleifera* leaf extract at the rate of 1.5 per cent was found to be superior in terms of sensory and textural qualities for inclusion in the functional yoghurt that was found to be more beneficial with numerous therapeutic properties and good probiotic viability.

Keywords: Yoghurt, *Moringa oleifera*, probiotic viability, sensory and textural properties

1. Introduction

Yoghurt is derived from the Turkish word 'Jugurt' describing any fermented food with an acidic taste. Its manufacture involves the use of specific symbiotic/mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Kon, 1972) [1]. Singh (1979) [2] defined yoghurt as a product made from milk that is heat-treated and fermented by lactic cultures to bring about flavour and aroma development with an acidity of at least 0.7g of lactic acid per 100g of yoghurt. Yoghurt is rich in nutrients like potassium, calcium, protein and vitamin B and has beneficial effects on human health by supplying prebiotic and probiotic bacteria. It helps to strengthen the immune system and improves lactose digestion and gastrointestinal conditions including lactose intolerance, constipation, diarrhoea, colon cancer, inflammatory bowel disease and allergies (Fitzgerald *et al*., 2004) [3].

Although many healthy and nutritious impacts are well established, milk and its products are generally not regarded as a rich source for vitamin c and bioactive ingredients such as polyphenols and antioxidants (Achi and Asamudo, 2019) [4]. In addition, like any other dairy products, yoghurt also lacks dietary fibre. Thus, the formulation of novel dairy products using medicinal herbs or their extracts has got more attention to meet the demand of health conscious consumers (Jamshidi-Kia *et al*., 2018) [5]. In this regard, inclusion of dietary fibre, antioxidant and vitamin C rich *Moringa oleifera* is expected to enhance the nutritional and therapeutic values of the yoghurt.

The resultant product is anticipated to have particular vital response such as anti-inflammatory, antioxidant, anti-carcinogenic, antihypertensive, anti-spasmodic, anti-diabetic, anti-epileptic, anti-arthritis, diuretic, cholesterol lowering and hepato-protective activities. It is also proposed for different diseases and conditions including anaemia, anxiety, bronchitis, chest congestion and cholera (Razis *et al*., 2014) [6]. Therefore, by considering the valuable aspects of *Moringa oleifera*, the present study was aimed to develop functional yoghurt using *Moringa oleifera* leaf extract (ME) as a source of all kinds of vitamins, trace minerals and fibre content, and to assess the sensory, physico-chemical, textural and microbial properties of the developed functional product.

2. Materials and Methods

Fresh cow milk was purchased from Livestock Farm Complex, Veterinary College and Research Institute, Namakkal. Spray-dried skim milk powder (Aavin Dairy, Erode) was used to adjust the solids-not-fat content in yoghurt. Ampoules of freeze-dried culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were obtained from National Dairy Research Institute, Karnal, Haryana. Commercially available cane sugar and locally available fresh *Moringa oleifera* leaves were used in this experiment.

2.1 Experimental design

Different treatments of functional yoghurt were designed as follows:

Treatments	Details
Control	Plain yoghurt
T_1	Yoghurt with 0.5% *Moringa oleifera* leaf extract
T_2	Yoghurt with 1.0% *Moringa oleifera* leaf extract
T_3	Yoghurt with 1.5% *Moringa oleifera* leaf extract
T_4	Yoghurt with 2.0% *Moringa oleifera* leaf extract
T_5	Yoghurt with 2.5% *Moringa oleifera* leaf extract

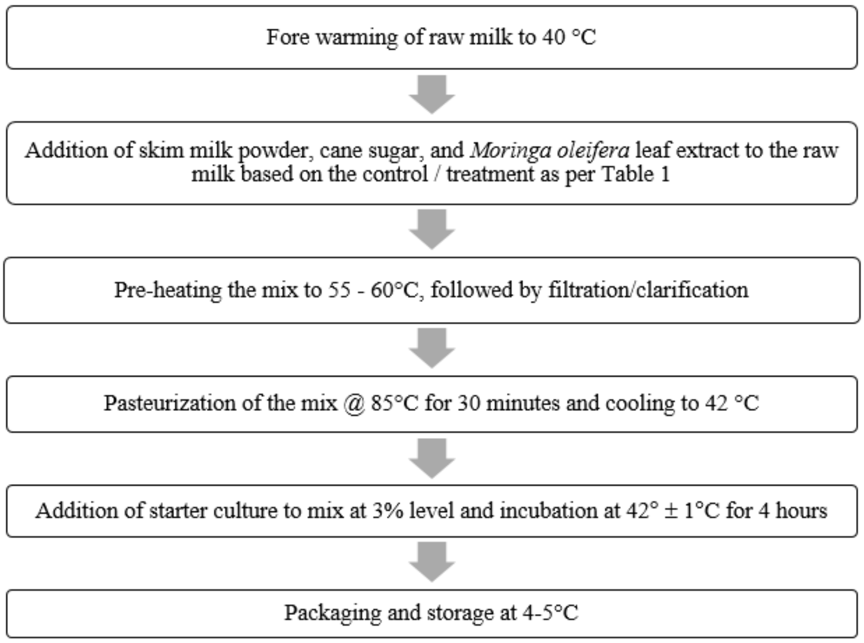
2.2 Preparation of functional yoghurt using *Moringa oleifera* leaf extract

Plain yoghurt was prepared as per De (2004) [7]. Yoghurt mixes were prepared by incorporating cow milk, skim milk powder and cane sugar, and the *Moringa oleifera* leaf extract (ME) was added to the mix at five different levels *viz.*, 0.5, 1.0, 1.5, 2.0 and 2.5 per cent based on the treatment. The yoghurt mixes, after filtration/clarification at 55-60°C, was pasteurized at 85°C for 30 minutes and then it was cooled to 42°C. Yoghurt starter culture containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus* was added at the rate of 3 per cent, and incubated at 42 ± 1 °C for 4 hours until a firm coagulum of yoghurt was formed, which is then packed and stored at 4-5 °C.

Table 1: Ingredients for 100 grams of functional yoghurt mixes

Ingredients (grams)	Control	T_1	T_2	T_3	T_4	T_5
Cow milk	87.5	87.0	86.5	86.0	85.5	85.0
Skim milk powder	6.5	6.5	6.5	6.5	6.5	6.5
Sugar	6.0	6.0	6.0	6.0	6.0	6.0
Moringa oleifera leaf extract	0	0.5	1.0	1.5	2.0	2.5
Total	100.0	100.0	100.0	100.0	100.0	100.0

2.3 Process flow chart for the preparation of functional yoghurt



2.4 Physicochemical analysis of yoghurt

pH was estimated using a digital pH meter. The total solids content was determined according to AOAC (1990), 15th edition. Fat was estimated as per the procedure described in IS:SP:18 (Part XI) – 1981.

2.5 Texture analysis of yoghurt

The firmness and consistency of functional yoghurt were characterized using the Instron Texture Analyser (Model: TA.XT Plus, Stable Microsystems) and Texture Expert Software. A Back Extrusion Cell (A/BE) with a 35mm disc and extension bar using a 5kg load cell was used to measure the firmness and consistency of the developed functional yoghurt. Six measurements for each sample were recorded using a 5 mm diameter and 150 mm long stainless steel probe adapter attached to a 5kg load cell. The penetration depth at the geometrical centre of the samples contained in a standard size back extrusion container (50mm diameter) was 30 mm and the penetration speed was set at 1.0 mm/s. The firmness of the samples was determined as the peak compression force during penetration. The maximum negative force is taken as the indication of consistency/resistance to flow off the disc during back extrusion. All determinations were carried out at 15 °C.

2.6 Organoleptic evaluation

Yoghurt samples were evaluated for appearance, flavour, body and texture and total sensory by a panel using a 9-point Hedonic scale (Dubey *et al.*, 2011) [8]. All the samples were appropriately coded before being subjected to sensory evaluation.

2.7 Statistical analysis
The data obtained in all the experiments were analysed statistically by applying two-way ANOVA by approved statistical methods of SPSS (version 28.0.1.1).

3. Results and Discussion
3.1 Physicochemical analysis of yoghurt
The Mean ± SE values of physico-chemical properties of control and treatment yoghurt samples are presented in Table 2. Statistical analysis revealed no significant differences in the fat and total solids content of the control and treatment yoghurt samples. However, the pH and fermentation time was significantly reduced in the ME yoghurt when compared with the control yoghurt. The results are in accordance with the findings of Kim *et al*. (1992) [9], who observed a gradual decrease in pH on increasing the level of incorporation of phenolic components in the yoghurt. These authors also opined that the decline in pH was presumably due to increased fermentation by the starter, especially *Lactobacillus delbrueckii* ssp. *bulgaricus*. Therefore, inclusion of ME did had a significant bearing on the physicochemical properties of yoghurt.

Table 2: Physicochemical properties of yoghurt samples (Mean ± SE)

Parameters	Control	T_1	T_2	T_3	T_4	T_5
Setting time (hours)	4.25 ± 0.19	4.12 ± 0.18	4.01 ± 0.21	3.82 ± 0.19	3.67 ± 0.23	3.50 ± 0.22
pH	4.67 ± 0.03	4.64 ± 0.04	4.60 ± 0.03	4.58 ± 0.05	4.55 ± 0.04	4.51 ± 0.05
Fat (%)	3.00 ± 0.14	3.02 ± 0.16	3.08 ± 0.11	3.01 ± 0.13	3.05 ± 0.12	3.07 ± 0.12
Total solids (%)	23.88 ± 0.02	23.87 ± 0.15	23.86 ± 0.22	23.89 ± 0.24	23.87 ± 0.22	23.87 ± 0.22

Different mean values in a row do not differ significantly (P ≥ 0.05)

3.2 Textural profile analysis of yoghurt
The results pertaining to the textural properties of control and treatment yoghurt samples are presented in Table 3. Statistical analysis revealed a significant (P < 0.05) difference in the firmness and consistency values of control and treatment yoghurt samples. As the ME inclusion level increased, the firmness and consistency scores of the treatments were also increased. This supported by the findings of El-Gammal *et al*. (2017) [10], who analysed the functional yoghurt made with aqueous extract of *Moringa oleifera* and reported that the different concentrations of aqueous extract of *Moringa oleifera* significantly affected the textural properties of the yoghurt samples. They also reported that the hardness, cohesiveness and gumminess values of the experimental yoghurt samples were increased significantly with the increase in concentration of *Moringa oleifera* leaf extract.

Table 3: Textural profile analysis of yoghurt (Mean ± SE)

Parameters	Firmness (g)	Consistency (g-sec)
Control	105.56^{b} ± 20.64	1971.60^{c} ± 127.57
T_1	121.79^{b} ± 23.51	2096.88^{c} ± 129.32
T_2	139.47^{b} ± 24.61	2158.84^{c} ± 136.14
T_3	153.06^{b} ± 23.92	2297.14^{c} ± 121.24
T_4	162.06^{a} ± 24.04	2030.93^{b} ± 112.78
T_5	175.05^{a} ± 21.93	2298.61^{a} ± 107.19

Means bearing differ superscripts in a column differ significantly (*p*<0.05)

3.3 Microbial analysis of yoghurt
Table 4 shows the Mean ± SE values of probiotic viability and microbial qualities of control and treatment yoghurt samples. Statistical analysis revealed that increasing the level of inclusion of ME in yoghurt significantly (P < 0.05) increased the probiotic bacterial count. Consequently, the maximum probiotic viability was shown by the yoghurt with highest ME concentration. Zhang *et al*. (2019) [11] analysed the *Moringa oleifera* leaf extract (ME) incorporated yoghurt and reported that the viable cell counts of *S. thermophilus* were dynamically higher in ME-supplemented yoghurt when compared to the control. These authors also reported that the increased growth of these lactic acid bacteria could be attributed to the components of ME such as polyphenols. In fact, ME contains dietary polyphenolic substances (Siddhuraju and Becker, 2003) [12], which could have promoted the rate of fermentation of lactic acid bacteria. The higher rate of metabolism and survival of lactic acid bacteria should be associated with this accelerated fermentation.

The yeast and mould count of control and treatments exhibited no significant difference among them and were within the FSSAI (2012) [13] prescribed limits of 100 per gram (maximum) respectively. In addition, the yeast and mould count showed a decreasing trend with increase in the level of inclusion of ME in the yoghurt. This is supported by the findings of Georgakouli *et al*. (2016) [14], who analysed the olive fruit polyphenol-enriched yoghurt and reported that the polyphenols prevented the potential spoilage of yoghurt by inhibiting the growth of spoilage microorganisms such as yeasts and moulds during fermentation and refrigerated storage. The coliforms were found to be absent in both control and treatment yoghurt samples, which confirms that the product has been produced under the hygienic standards.

Table 4: Microbial analysis (log_{10} cfu/ml) of yoghurt (Mean ± SE)

Parameters	*S. thermophilus*	*L. bulgaricus*	Yeast and mould	Coliforms
Control	8.66 ± 0.02	8.55 ± 0.02	1.33 ± 0.01	Nil
T_1	8.67 ± 0.02	8.56 ± 0.02	1.32 ± 0.01	Nil
T_2	8.67 ± 0.03	8.57 ± 0.02	1.31 ± 0.01	Nil
T_3	8.68 ± 0.02	8.57 ± 0.03	1.31 ± 0.02	Nil
T_4	8.69 ± 0.01	8.59 ± 0.02	1.30 ± 0.01	Nil
T_5	8.70 ± 0.01	8.62 ± 0.01	1.29 ± 0.02	Nil

Different mean values in a column do not differ significantly (*p*≥0.05)

3.4 Sensory evaluation of yoghurt
Table 5 shows the average sensory scores of different treatment of *Moringa oleifera* leaf extract incorporated functional yoghurt. Statistical analysis revealed that no significant (P ≥ 0.05) differences was observed between different treatments and control except T_4 and T_5, which scored lower in all sensory parameters and highly differs significantly (P≤0.01) with other three treatments and control. All the sensory scores were good up to 1.5% level of incorporation of *Moringa oleifera* leaf extract in the

functional yoghurt. Beyond that level, sensory scores were reduced drastically.

Table 5: Sensory properties of yoghurt samples (Mean ± SE)

Parameters	Colour and Appearance	Flavour	Body and Texture	Overall acceptability
Control	8.83 ^{a} ± 0.11	8.75 ^{a} ± 0.11	8.83 ^{a} ± 0.11	8.67 ^{a} ± 0.11
T_1	8.58 ^{a} ± 0.15	8.58 ^{a} ± 0.15	8.75 ^{a} ± 0.11	8.42 ^{a} ± 0.15
T_2	8.42 ^{a} ± 0.08	8.33 ^{a} ± 0.11	8.50 ^{a} ± 0.13	8.33 ^{a} ± 0.11
T_3	8.33 ^{a} ± 0.11	8.25 ^{a} ± 0.11	8.42 ^{a} ± 0.08	8.25 ^{a} ± 0.11
T_4	7.67 ^{ab} ± 0.21	7.50 ^{b} ± 0.13	7.75 ^{b} ± 0.17	7.83 ^{ab} ± 0.11
T_5	7.17 ^{b} ± 0.25	6.92 ^{c} ± 0.15	7.67 ^{b} ± 0.17	7.58 ^{b} ± 0.15

Means bearing different superscripts in a row differ significantly ($P < 0.05$)

Increase in the level of addition of *Moringa oleifera* leaf extract reduced the attractive bright colour and glossy smooth appearance, and the flavour of the product, which may be attributed to the greenish colour and bitter taste of the *Moringa oleifera* respectively. In addition, incorporation of *Moringa oleifera* leaf extract reduced the body and texture scores of the product (Vijay *et al*., 2022) [15]. Hence, *Moringa oleifera* leaf extract level of 1.5 percent was selected as the maximum acceptable level for inclusion in the functional yoghurt.

4. Conclusion

From the results, it can be concluded that the functional yoghurt samples can be prepared by incorporating *Moringa oleifera* leaf extract up to 1.5 per cent without affecting the sensory and textural properties. In addition, no marked variation has been noticed in the physicochemical and microbiological properties of the developed functional yoghurt. Therefore, the consumers can harvest the benefits of consuming *Moringa oleifera* and catch up on a new yoghurt variety with good probiotic viability and numerous therapeutic properties.

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