RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 43 Issue 1: 142-147 (March 2024)

Development of *Chhana* Spread by Incorporating Moringa (*Moringa oleifera* L.) Leaves Extract as a Source of Antioxidants and Phenolics

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10.18805/ajdfr.DR-2082

ABSTRACT

Background: Herbs are abundantly grown throughout India in all the states, which encourages the development of herbs incorporated products that provide health advantages in addition to flavour and taste to functional dairy products. Hence, the present study was undertaken to develop functional *chhana* spread by incorporating moringa leaves extract as a source of antioxidants and phenolics.

Methods: The *chhana* spread was formulated with incorporation of moringa leaves extract at different incorporation levels of 4%, 6% and 8% levels. The developed moringa *chhana* spread was investigated for its sensory characteristics, physico-chemical, antioxidant and phenolic properties against control.

Result: The moringa leaves extract with 6% incorporation level in *chhana* showed higher overall acceptability than others. Among the physico-chemical properties, there were no significant differences in moisture and lactose percentage between control and treated groups. As extract levels in the *chhana* increased, protein, fat and ash percentages were significantly increased. Antioxidants and phenolic contents were found significantly higher in 8% of moringa leaves extract group and lowest was found in control group. Consequently, moringa leaves extract could be used as a natural antioxidant and phenolic ingredient to develop a novel *chhana* spread.

Key words: Antioxidant activity, Chhana spread, Moringa leaves extract, Phenolic content.

INTRODUCTION

India produces traditional milk products with about 50-55% of its milk production. Traditional milk products play a prominent role in the economy of our country. Since milk is now regarded as a nearly complete food and is known as a “Bank of Nutrients”. Milk and milk-based products are becoming more and more popular. Indian traditional dairy products like khoa, *chhana*, *etc*. are prepared using the majority of the country’s milk production (Gupta *et al*., 2022). Cow milk is preferred for making *chhana* because the finished product has a soft, velvety body and a smooth texture, which are highly desired qualities for making *chhana*-based sweetmeats, particularly rasogolla (Kumar *et al*., 2015). However, buffalo milk presents a number of technological challenges in the production of high-quality *chhana* and sweets based on *chhana*, particularly rasogolla, due to its inherent physico-chemical differences from cow milk.

Chhana is considered to be the Indian substitute for soft cottage cheese. The color and texture of *chhana* are marble white, with a spongy texture and a mild acidic taste (Ammu *et al*., 2020). It serves as the basis for the creation of a wide range of sweets, including rasogolla, sandesh, rasomalai, chum chum and *chhana* murki. It must not contain more than 70% moisture and must have a minimum of 50% fat expressed on the basis of dry matter. Additionally, milk solids can be used to make *chhana*. *Chhana*’s quality is determined by the strength and type of coagulant used. *Chhana*’s yield is mostly determined by percentage of milk solids in the milk, the amount of milk solids recovered and the amount of moisture retained by the *chhana* (Begum *et al*., 2019).

Moringa oliefera, commonly known by the locals as the ‘tree of miracles’ since ages, owing to its immense nutritional and bioactive properties has been studied time and again to exploit its potential into the food applications. This plant leaf is abundant in macronutrients (Protein rich, high carbohydrate content, dietary fibers), micronutrients (vitamin B complex, A, C and D) and phytochemicals (Ansari *et al*., 2020). Furthermore, it has been reported that the leaves of this plant have a number of biological properties, including diuretic, immune-stimulating, hypotensive, anti-inflammatory,

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How to cite this article: Singh, B., Yadav, M.P.S., Yadav, P., Prakash, V., Rai, D.C. and Rathaur, A. (2024). Development of *Chhana* Spread by Incorporating Moringa (*Moringa oleifera* L.) Leaves Extract as a Source of Antioxidants and Phenolics. Asian Journal of Dairy and Food Research. 43(1): 142-147. doi: 10.18805/ajdfr.DR-2082.

Submitted: 23-02-2023 **Accepted:** 30-03-2023 **Online:** 03-05-2023

anti-ulcer, anti-hepatotoxic, anti-tumor, thyroid hormone status regulation, hypocholesterolemic, radio protective, hypolipidaemic, anti-atherosclerotic, antidiabetic and antioxidant properties (Yadav and Ghimire, 2019; Boulal *et al*., 2021; Das *et al*., 2022). Addition of *Moringa oleifera* leaves powder (1.0-1.5% w/v) to yoghurt enhanced the nutritional profile of the yoghurt indicated by increased total solids, protein and fat content of the herbal yoghurt over the plain yoghurt (Akajiaku *et al*., 2018). The leaf extract, which mostly consists of phenolics and flavonoids, has been shown to have antioxidant action both *in vitro* and *in vivo* (Vongsak *et al*., 2013).

As a result of the Covid-19 pandemic, health-conscious consumers have been focusing on preserving and promoting their health, nutrition and immunity by leveraging the functional and medicinal health attributes of herbs (Paswan *et al*., 2021). Hence considering all the facts the present study was aimed to prepare *chhana* spread with different level of moringa leaves extract (MLE) using calcium lactate and determine their sensorial characteristics, physico-chemical properties, antioxidant and phenolic activity.

MATERIALS AND METHODS

This research was performed in the Laboratory of Department of Animal Husbandry and Dairying, C.S. Azad University of Agriculture and Technology, Kanpur. Cow milk was procured from Dairy Farm at C.S. Azad University of Agriculture and Technology Kanpur, India and was standardized to 3.5% fat and 8.5% SNF. Fresh moringa leaves were procured from the local market of Rawatpur, Kanpur. Hi Media Laboratories Pvt. Ltd., Mumbai, India provided all chemicals and reagents used in this study.

Moringa leaves extract was prepared as per (Okorie *et al*., 2021) with slight modification. Fresh green leaves (250 g) of Moringa were taken and ground by a conventional electric mixer (10000 rpm). The mixture was poured into 1000 ml of distilled water then mixture was boiled for 15 min at a temperature of 70°C. At last extractions was decanted into a sterilized container after filtration with the muslin cloth.

$$\text{Yield \%} = \frac{\text{(Weight of the beaker + extract)-Weight of empty beaker}}{\text{Initial weight of sample beaker}} \times 100$$

The moringa *chhana* spread was prepared in laboratory of the Department of Animal Husbandry and Dairying, C.S. Azad University of Agriculture and Technology, Kanpur. The methods for the formation of *chhana* and preparation of sample were according to De (2005) after slight modification. Fig 1 illustrates the making process of moringa *chhana* spread. The cow milk was heated in Karahi, over an open fire to 80-85°C. Using a ladle while heating, the milk was stirred to avoid burning. When temperature of milk reached at given temperature 70°C then different levels of moringa leaves extract was poured simultaneously. T1 (control *chhana*); T2 (*chhana* consisting 4% (w/v) MLE); T3 (*chhana* consisting 6% (w/v) MLE); T4 (*chhana* consisting 8% (w/v) MLE). After that coagulation

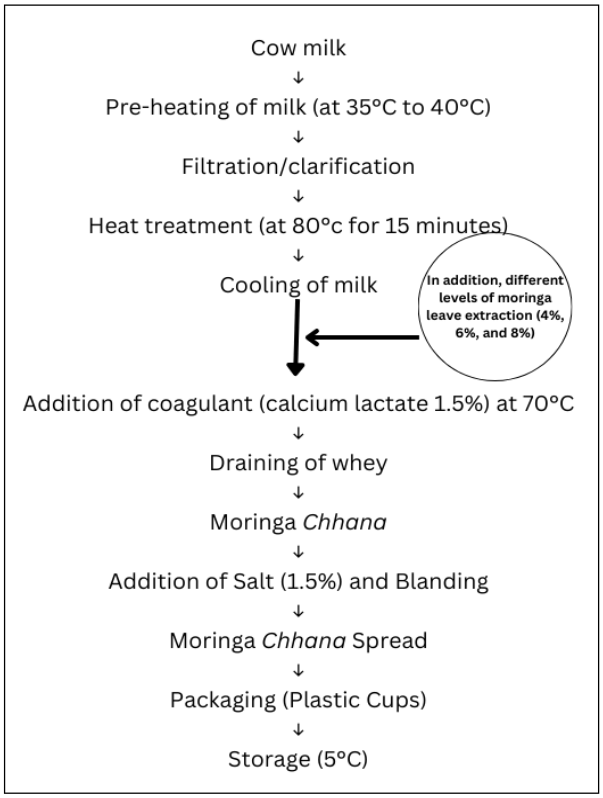


Fig 1: Flow diagram of manufacturing of moringa *chhana* spread.

with 1.5% solution of calcium lactate was added slowly to the milk with constant stirring until complete coagulation took place. Once coagulation occurred the stirring was stopped and contents were poured over a clean piece of muslin cloth for straining of whey. After whey draining channa was collected and weighed. The common salt at 1.5% was mixed with the help of mixer after weighed. Required amount of whey was added. The obtained mix was stirred well to have channa spread. Finally, Moringa *Channa* spread was collected and packed in plastic cups.

The sensory parameters of the moringa *chhana* spread was find out in the Department of Animal Husbandry and Dairying, CSAUA and T Kanpur following with method represented by Singh *et al*. (2022) with slight modifications. 5 males and 5 females judges were chosen at random manner among faculty, research scholar and post graduate at Department of Animal Husbandry and Dairying. Before the sensory test, the judges were given instructions to familiarize themselves with the process. Using a 9-point hedonic scale, individuals evaluated the sensory characteristics of moringa *chhana* spread when it was kept at room temperature (25°C±2°C). Based on a scale of 1-9, the degree of liking was assigned to each sample (1: dislike extremely and 9: like extremely).

Analysis of physico-chemical characteristics (*e.g*. Moisture, Fat, Protein, Lactose and Ash) of *chhana* spread and *chhana* spread with different levels of MLE were done by as per (Rathaur *et al*., 2020; Gupta *et al*., 2020).

The method developed by Patel *et al.* (2022) was used to calculate DPPH scavenging activity. A UV-VIS spectrophotometer was used to measure the absorbance of 200 µl of sample at a wavelength of 517 nm (Shimadzu, Japan). The percentage DPPH inhibition of the formulated samples was calculated using the following formula:

$$\text{Per cent (\%) inhibition} = 100 \times \frac{(A_1 - A_0)}{A_1}$$

Where,

A_1 = Absorbance of the control.

A_0 = Observed final absorbance of sample extract at the wave length of 517 nm. Methanol (95%) was used as blank.

The total phenolic content of the samples was determined as per Krawitzky *et al*. (2014). For this study, 100 µl of sample was collected and the absorbance was measured using a UV-VIS spectrophotometer (Shimadzu, Japan) at 760 nm. Total phenolic content was calculated and expressed as gallic acid equivalent (µg GAE/ml).

The entire experiment was conducted in triplicate in order to quantify the sensory, physicochemical antioxidant and phenolic characteristics of moringa *chhana* spread. With SPSS version 25, used one-way analysis of variance to determine the significance of differences between samples. Statistically significant parameter effects were considered when the difference exceeded ($p<0.05$).

RESULTS AND DISCUSSION

The result obtained from the research was analyzed and discussed below:

Sensory evaluation:

Flavour

The flavor score presented in Table 1 shown that the highest score was recorded in *chhana* spread with 6 per cent MLE (8.6±0.05) amongst all the treatments, the lowest score was found in *chhana* spread with 8 per cent MLE (8.1±0.21). The flavour score was observed quite similar in *chhana* spread with 4 and 6 per cent MLE. The reasons for the low score may be due to the deep aroma of extract found at a higher level whereas mild dull flavour found in the lower level which was not liked by judges. The differences in scores obtained by all the treatments were found to be significant.

Body and texture

The body and texture of the *chhana* spread was increased from 8.0±0.21 to 8.6±0.10 by increasing the substitution of MLE is shown in Fig 2. The composite moringa *chhana* spread sample T3 had a high mean score for body and texture (8.6±0.10) followed by sample T2 with a mean score of (8.4±0.20). The lowest score was obtained in *chhana* spread prepared with 8 per cent MLE (8.0±0.21). The control sample T1 had the third mean value (8.2±0.19) and this brought a significant difference ($p<0.05$) between the body and texture of the control and moringa *chhana* spread.

Color and appearance

Having a pleasing colour and appearance is the most important factor in a consumer's acceptance of the product. The score values obtained revealed that the *chhana* spread prepared by using 6 per cent MLE was found superior amongst all the treatments in colour and appearance which recorded the maximum score (8.9±0.10), followed by *chhana* spread with 4 per cent MLE (8.5±0.13) represented in Table 1. The lowest score was obtained by the product with 8 per cent MLE (8.1±0.12). It was observed that the *chhana* spread with 6 per cent MLE showed slight greenish moringa leaves colour with a clear and clean appearance which was liked very much by the judges. The variation in score may be due to the intensity of colour observed that light dull green to dark greenish 8 per cent level of MLE which may not be liked by the judges.

Table 1: Effect of different levels of moringa leaves extract on sensory quality of *chhana* spread.

Treatment	Flavour	Body and texture	Color and appearance	Spreadability	Overall acceptability
T1	8.2±0.20^{bc}	8.2±0.19^{bc}	8.3±0.11^{b}	8.3±0.22^{b}	8.3±0.15^{b}
T2	8.4±0.22^{ab}	8.4±0.20^{ab}	8.5±0.13^{b}	8.4±0.18^{b}	8.4±0.14^{b}
T3	8.6±0.05^{a}	8.6±0.10^{a}	8.9±0.10^{a}	8.7±0.05^{a}	8.7±0.10^{a}
T4	8.1±0.21^{c}	8.0±0.21^{c}	8.1±0.12^{c}	8.1±0.26^{c}	8.0±0.16^{c}
SEM	0.08	0.11	0.05	0.12	0.18
P-value	0.005	0.008	<0.01	0.004	0.002

Table 2: Effect of different levels of moringa leaves extract on phyisco-chemical properties of *chhana* spread.

Treatment	Moisture	Fat	Protein	Lactose	Ash
T1	63.22±0.02	15.50±0.18	17.20±0.12	2.10±0.05	1.98±0.02
T2	62.21±0.02	15.70±0.21	17.35±0.14	2.12±0.08	2.10±0.12
T3	62.20±0.22	15.76±0.16	17.42±0.17	2.13±0.10	2.17±0.06
T4	62.22±0.18	15.81±0.19	17.47±0.10	2.11±0.12	2.22±0.10
SEM	0.18	0.11	0.05	0.12	0.18
P-value	0.568	0.008	<0.01	0.354	0.036

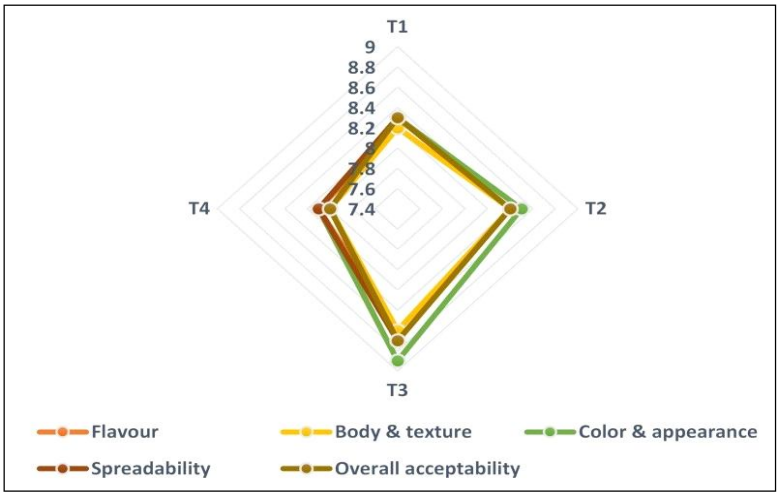


Fig 2: Sensory evaluation of moringa leaves extract incorporated *chhana* spread.

Table 3: Effect of different levels of moringa leaves extract on Antioxidants and phenolic content of *chhana* spread.

Treatment	Phenolic content (µg GAE/ml)	Antioxidant (%)
T1	115.37±2.20^{d}	70.35±2.11^{d}
T2	122.41±1.97^{c}	74.35±1.73^{c}
T3	132.30±2.07^{b}	82.45±2.07^{b}
T4	144.50±2.12^{a}	88.53±2.16^{a}
SEM	2.73	2.62
P-value	<0.01	<0.01

Spreadability

The spreadability of the *chhana* spread was increased from 8.1±0.21 to 8.7±0.05 by increasing the substitution of MLE. The composite moringa *chhana* spread sample T3 had a high mean score for spreadability (8.7±0.05) followed by sample T2 with a mean score of (8.4±0.18). The lowest score was found inA *chhana* spread prepared with 8 per cent MLE (8.1±0.26). The control sample T1 had the third mean value (8.3±0.22) and this brought a significant difference ($p<0.05$) between the spreadability of the control and moringa *chhana* spread.

Overall acceptability

Overall acceptability is the indicative parameter of the sensory quality of products in totality. The overall acceptability of *chhana* spread prepared by using 6 per cent MLE scored highest points (8.7±0.10). The overall score of *chhana* spread with MLE T4 and control group was observed a quite similar score 8.4±0.14 and 8.3±0.15, respectively which indicates that overall acceptability of the *chhana* spread was found to be good for all the treatment groups. The lowest score was found in *chhana* spread prepared with 8 per cent MLE (8.0±0.16). The differences in scores possessed by all the treatments were highly significant. Similar result reported by Gupta *et al.* (2022) that sensory quality of *chhana* spread with moringa leaves extract more acceptable compared to control. Afaf *et al.* (2015) carried out Sensory evaluation of whey guava beverage and the results depicted that the beverage with 2.5 or 5 per cent moringa extract was found to be more acceptable. Based on the results, we can affirmatively state that amongst the different levels of MLE, T3 treatment (6% MLE) was found to be most acceptable by the judges *i.e.*, good quality *chhana* spread was obtained with the addition of 6 per cent moringa leaves extract.

Characteristics of Moringa *chhana* spread

The effect of different levels of MLE on physico-chemical characteristics of *chhana* spread is shown in Table 2. There were no significant differences in moisture and lactose contents among the groups. Fat and protein contents were ($p<0.05$) increased with increases levels of extract. Similar result was found by Badola *et al.* (2018) that fat and protein content of paneer was also increased after the incorporation of cardamom and black pepper powder. Reddy *et al.* (2020) reported that different levels of moringa leaves powder enhance the physico-chemical composition of moringa idly mix compared to control sample. *Moringa oleifera* leaf extract is rich in polyphenols, flavonoids, tannins, vitamins, minerals (iron) and proteins. Its inclusion to milk before coagulation might represent an excellent way to enrich the coagulated milk products. Ash content levels was significantly ($p<0.05$) increased with increases levels of extract. Highest ash content was found in 8% MLE group and lowest was found in control group. The content of minerals within a food sample can be determined by how much ash the food contains. In general, low ash content indicates that a food product does not contain a lot of minerals.

Quantification of antioxidant and phenolic properties of *chhana* spread

Total phenolic content (TPC) and antioxidant property of *chhana* and moringa *chhana* spread is shown in Table 3. The TPC of moringa *chhana* spread were (T2 122.41±1.97, T3 132.30±2.07 and T4 144.50±2.12 µg GAE/ml) respectively and *chhana* spread was (T1 115.37±2.20 µg GAE/ml). The percentage DPPH inhibition of moringa

chhana spread were (T2 74.35±1.73, T3 82.45±2.07 and T4 88.53±2.16%) respectively and *chhana* spread was (T1 70.35±2.11%). In a study by Mohammed *et al.* (2018), *Moringa oleifera* extract was added to cream cheese at different ratios of 2.50, 3.50 and 5.00 grams/100 grams to increase the shelf-life total phenol content and antioxidant activity of the final product. Badola *et al.* (2018) also reported that 0.25 per cent black pepper and 0.50 per cent cardamon powder resulted in enhancement of phenolic content (0.472 mg GAE/gram) which was slightly higher than the control paneer (0.459 mg GAE/gram). Moringa, contains bioactive components such as polyphenols in its composition and also has an antioxidant capacity. Generally, by increasing the MLE amount from 4 to 8%, an increase in the TPC and antioxidants was noticed in moringa *chhana*.

CONCLUSION

In this study, functional *chhana* spread consisting moringa leaves extract (MLE) was evaluated for sensorial and functional properties. Furthermore, these findings indicate that the functional dairy product will be benefited from MLE, since MLE can be incorporated into a various dairy-based products because of its high stability of sensorial attributes and rich bioactive profile. Moreover, sensory properties of *chhana* spread showed that MLE inclusion did significantly changed the flavour, color, body and texture and appearance and overall acceptability of *chhana* spread. Additionally, it is concluded that the bioactive compounds from variety of herbal extract can be used in food products as a natural bioactive ingredients in order to improve of their sensorial as well as nutritional properties.

ACKNOWLEDGEMENT

The author would like to express their gratitude to the Department of Animal Husbandry and Dairying, C.S. Azad University of Agriculture and Technology, Kanpur, for giving facilities for this work.

Conflict of interest

All authors declared that there is no conflict of interest.

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