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Studies on quality enhancement of Shrikhand using *Moringa oleifera* leaf extract

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Abstract
The study aimed to enhance Shrikhand, a dairy dessert, with *Moringa oleifera* leaf extract. Shrikhand samples were developed with different aqueous Moringa extract concentrations @ 2%, 3%, 4% as T_1, T_2, T_3 respectively. T_0 was the control sample with no added Moringa extract. Evaluation of T_0, T_1, T_2 and T_3 encompassed physicochemical traits (Total solids, moisture, ash, protein, carbohydrates, sucrose, fat, titratable acidity, pH, colour index), sensory aspects (colour and appearance, body and taste, flavour and taste and overall acceptability), and microbiological factors (SPC, YMC, Coliform). T_3 obtained the highest scores for ash content, protein, titratable acidity, lightness value of colour index measurement and Standard Plate Count whereas it obtained reduced scores for carbohydrate content. T_3 also secured top scores for organoleptic evaluation signifying positive consumer perception. Successful 4% aqueous Moringa extract incorporation into Shrikhand augmented health benefits.

Keywords: Shrikhand, *Moringa oleifera*, Moringa leaf extract, Chemical analysis, Microbiological analysis

1. Introduction
India's traditional dairy products, including fermented milk products like Shrikhand, hold significant cultural significance and enjoy unparalleled market prospects and growth rates. Approximately 50 to 55% of India's milk output is transformed into a variety of confections and treats that are deeply ingrained in age-old customs (Aneja *et al.*, 2002) [1].

Shrikhand is one such popular dairy delicacy from India prepared from whole milk. It is a sweet and sour semi-solid dessert that is widely consumed in certain regions of the country. Recognized for its taste and potential health advantages, Shrikhand is a renowned cultured milk product that contains a notable amount of milk proteins and phospholipids originating from lactic acid fermentation facilitated by various bacterias. (Jaybhay *et al.*, 2019, Gupta *et al.*, 2018) [2, 3]. Shrikhand not only stands out as a delectable dessert but also offers potential health advantages for specific cardiovascular and gastrointestinal issues such as lactose intolerance, constipation, and colon cancer. It enhances digestive function and fortifies the immune system, making it a wholesome dietary choice. Shrikhand contains moisture content 39% and total solids 61%. It has 10.0% fat, 78.0% carbohydrates, 11.5% proteins, and 0.5% ash calculated on dry matter. The pH level falls within the range of 4.2–4.4. (Boghra and Mathur, 2000) [4].

Furthermore, there is potential for fortifying Shrikhand with various additional ingredients to enhance its nutritional value. Researchers have incorporated ingredients like fruit pulp (Sahu *et al.*, 2021) [7], powders (Ojha *et al.*, 2018) [6], and herbal extracts (David, J. *et al*., 2015) [5] into Shrikhand. One such ingredient very suitable for fortification is *Moringa oleifera*, a tree native to India known for its nutritional and medicinal properties. The incorporation of *Moringa oleifera* leaf powder into Shrikhand offeres additional health benefits and contributed to the growing market for Moringa-based products (Dubey *et al.*, 2018) [8].

Moringa oleifera, also called as the "Miracle tree," is rapidly growing tree that thrives in arid conditions. It is revered for its safety profile, nutritional and medicinal attributes, making it an increasingly popular choice for food fortification. The world-wide market for Moringa-based products has been expected to grow significantly, with India itself satisfying approximately 80% of the global demand for Moringa. (Busani *et al*., 2011, Stohs and Hartman, 2015) [9, 10] (https://agriexchange.apeda.gov.in/Weekly_eReport/Moringa_Report.pdf)

Moringa oleifera leaves have a well-balanced composition of carbohydrates, dietary fibre,

protein, iron, calcium, vitamin C, potassium, magnesium, and vitamin A. They are rich in vitamins, minerals, polyphenols, phenolic acids, flavonoids, tannins, and proteins, which contribute to their potent antioxidant and antimicrobial properties. (Foidl *et al.*, 2001; Ogbe and Afikku, 2011) [11, 12]. Moringa leaves have a high nutritional value and remain largely unaffected by cooking or preservation methods, whether fresh, cooked, or dried as a powder (Razis *et al*., 2014) [16]. For ages, Moringa has found a place in traditional medicine across diverse global cultures. Its applications encompass addressing skin infections, anemia, anxiety, asthma, blackheads, chest congestion, blood impurities, cholera, conjunctivitis, bronchitis, cough, diarrhea, eye and ear infections, glandular swelling, fever, headpains, high or low blood pressure, hysteria, joint discomfort, face acnes, and respiratory issues (Mahmood *et al.*, 2010) [14]. The leaves of *Moringa oleifera* encompass crucial amino acids, leaf-based carotenoids, and compounds boasting nutraceutical traits. It serves as an anti-inflammatory, antioxidant, antibiotic, antifungal, antiviral, and antidepressant agent. Additionally, it exhibits analgesic, diuretic, antihypertensive, and antitumor properties. Numerous other pharmacological attributes are evident, including but not limited to antiarthritic, antispasmodic, antiurolithic, hepatoprotective, anaphylactic, and antihyperglycemic characteristics (Pandey *et al.*, 2012) [15].

The rising interest in natural alternatives and the pursuit of a healthier lifestyle have contributed to the growing popularity of Moringa and other plant-based products. It has been successfully integrated as a food fortifier in various products like biscuits, bread, yogurt, cheese, soups, etc. and has enhanced the overall quality of the product (Islam *et al.*, 2021, Oyeyinka & Oyeyinka, 2018) [13, 17].

2. Materials and Methods

The materials and methods adopted along the span of this research are reported below. The order of operations designed to carry on the work is mentioned below.

Experimental site: Research and Technology lab, College of Dairy and Food Technology, Maharana Pratap University of Agriculture and Technology, Udaipur-313001, Raj. (India).

2.1 Preparation of Moringa Leaf Extract

100 ml by adding distilled water was added to 10 g of leaf powder. It was boiled at 100 °C/30 min. The obtained extract was then cooled and filtered using Whatman filter paper no. 1.

2.2 Preparation steps for Experimental and Control Shrikhand treatments

2.2.1 Preparation of Curd

Standardized milk (4.5% fat and 8.5% SNF) was heated to 90 ºC and then cooled to 30-32 ºC. It was then inoculated @ 1% with the Curd culture (CURD 3370 LYO 250 DCU), mixed well, and incubated at 30 ºC until the curd was set firmly (acidity 0.7-0.8% lactic acid).

2.2.2 Preparation of Chakka

The formed curd was broken and transferred to nylon bag and was then hung on a peg for drainage of whey. Time taken was around 15 hours at approximately 5 ºC. The whey from the curd drained off and obtained Chakka was used further.

2.2.3 Preparation of Shrikhand

The Chakka was then admixed with sugar @ 30% and kneaded for uniform mixing. The product so obtained was the Control Shrikhand sample which had no moringa leaf extract.

2.2.4 Preparation of Experimental Shrikhand

For the Experimental Shrikhand, the Chakka was mixed with the already moringa extract in varying percentages as mentioned in the table. The obtained product was then filled in cups and sealed, cooled and stored at 5 ºC.

Table 1: Details of different chakka treatments

Materials	Treatments given to Chakka (4.5% fat and 8.5% SNF)			
	T_0	T_1	T_2	T_3
Moringa Leaf Extract concentration (%)	-	2	3	4

2.3 Technical Programme

2.3.1 Organoleptic Evaluation of Shrikhand Treatments

Freshly prepared Shrikhand treatments was served to 3 panelists. The sample was then evaluated using a 9-point hedonic scale. This was referenced through Amerine *et al*., (1965) [21].

2.3.2 Chemical Analysis of Shrikhand Treatments

2.3.2.1 Total solid content

The total solid in Shrikhand treatments was evaluated according to FSSAI Manual (2016) [20]. In cultured dairy products, total solids are determined after neutralization of developed acidity with alkali using hot air oven method. Total

$$\text{solids \% } \left(\frac{w}{w}\right) = \frac{100\ (\text{Weight of residue left after drying} - a)}{\text{Weight of prepared sample taken}}$$

$$a = \frac{\text{Normality of NaOH} \times \text{Titre value} \times 40}{1000 \times 2}$$

2.3.2.2 Moisture

Moisture content in Shrikhand treatments was found as per method suggested in FSSAI Manual (2016) [20].

2.3.2.3 Ash

Ash content was assessed in accordance to FSSAI Manual (2016) [20]. The sample was heated in muffle furnace (550 ± 20 °C).

$$\text{Total Ash \% Dry matter basis} = \frac{\text{Mass of the crucible with ash (g)} - \text{mass of the empty crucible (g)} \times 100}{(100 - \textit{moisture \%}) \times (\textit{mass of crucible with material taken for test in g} - \textit{mass of empty crucible in g})}$$

2.3.2.4 Protein

Shrikhand samples protein analysis was determined by Kjeldahl method as per FSSAI Manual (2016) [20].

$$\text{Nitrogen \% (N)} = \frac{14.01 \times 0.1\ \text{N} \times (\text{Titre value (ml)} - \text{blank value (ml)}) \times 100}{\textit{weight of sample} \times 1000}$$

0.014= M eq. of N_2

Protein % dry matter basis = N% × 6.38

2.3.2.5 Carbohydrate
The carbohydrate content was estimated as per Jain and Mogra, (2006) [22].

Carbohydrates % dry matter basis ($\frac{\mathbf{g}}{\mathbf{100\ g}}$)= 100- (moisture + ash + protein + fat)

2.3.2.6 Sucrose
Sucrose estimation was done as per Lane-Eynon's method described in IS:1479, Part II, 1961.

2.3.2.7 Fat
Estimation of fat for the samples was done using the Mojonnier fat extraction method mentioned in FSSAI Manual (2016) [20]. The fat extraction involved addition of ammonia, ethyl alcohol, diethyl ether, and petroleum ether, with subsequent separation of layers and repeated extractions followed by weighing.

$$\text{Fat \% dry matter basis } \left(\frac{w}{w}\right) = \frac{\text{Weight of extracted fat} \times 100}{\text{Weight of sample taken}}$$

2.3.2.8 Titratable acidity
Method mentioned in FSSAI Manual (2016) [20] was adopted to estimate the titratable acidity of Shrikhand variations. 30 ml warm water was added to 10 g sample followed by adding 1 ml phenolphthalein and then titrating against standard solution of NaOH.

$$\text{Titratable acidity as \% LA} = \frac{9 \times \text{Volume of Standard NaOH required} \times \text{normality of Std NaOH solution}}{\text{weight of sample}}$$

2.3.2.9 pH estimation
It was done using standard method (AOAC, 2005) [19]. A pH-meter (HM Digital PH-80 Hydrotester) was used to determine the pH values of the samples.

2.3.2.10 Colour Index
The colour reflectance measurement was determined using a colorimeter (Hunter Lab Colour Flex, Hunter Associates Laboratory Inc., Reston, VA, USA) in accordance to the standard method (AOAC, 2005) [19].

2.3.3 Microbiological Analysis of Shrikhand Treatments
2.3.3.1 Standard plate count (SPC)
Standard plate count of Shrikhand sample was determined as per BIS: 1981, SP:18 Part X1. 1 ml of ($x10^3$) dilution was added to sterile petri plates, followed by 10-15 ml of pre-melted Nutrient agar, plates were tilted for even distribution and solidified in a laminar airflow cabinet followed by incubating at 37 °C for 48 hours. The average colony count per gram was then calculated.

2.3.3.2 Yeast and Mould count (YMC)
Yeast and Mould count of Shrikhand sample was analysed as per the method BIS: 1981, SP:18 Part X1. Same plating procedure as of SPC analysis was employed, except for the aseptic acidification of Potato Dextrose Agar (PDA) to pH 3.5 using pre-sterile 10% tartaric acid followed by incubation at 22±1 °C for a duration of 3 to 5 days.

2.3.3.3 Coliform Count
The Coliform Count of Shrikhand sample was analysed as per method BIS: 1981, SP:18 Part X1.
In the Shrikhand plating process, Violet Red Bile Agar (VRBA) was utilized, with an additional overlay of VRBA agar. The inverted plates were then incubated at a precise temperature of 37 °C for 24 hours.

2.3.4 Statistical analysis
Data obtained was analyzed statistically by one-way and two-way ANOVA accompanied by Duncan's post hoc test using IBM SPSS Version 21 Statistics software.

3. Results and Discussion
3.1 Organoleptic Parameters of Shrikhand Treatments
Colour and Appearance
The highest score for colour and appearance (9.00) was obtained from the treatment T_0 and T_3 followed by T_2 (8.66). The least score (8.33) was obtained in T_1. There were no significant differences found among the treatments with respect to colour and appearance of the samples.

Flavour and Taste
The highest score for flavour and taste (9.00) was obtained from the treatment T_3 followed by T_2 (8.33). The lowest score (8.00) was obtained in T_1 and T_0. There were no noteworthy differences found among the treatments with respect to flavour and taste of Shrikhand treatments.

Body and Texture
All the Shrikhand samples (T_0, T_1, T_2, T_3) scored 8.66 for the body and texture attribute showing no notable differences found among the treatments with respect to body and texture.

Overall Acceptability
The highest score for overall acceptability (9.00) was obtained for the treatment T_3 followed by T_2 (8.66). The least score (8.33) was obtained in T_0 and T_1. There were no significant differences found on overall acceptability among the treatments.

Table 3.1: Average data of Organoleptic Scores of Shrikhand treatments

Parameters	Shrikhand treatments				CD
	T_0	T_1	T_2	T_3	
Colour and appearance	9	8.33	8.66	9	0.561
Flavour and taste	8	8	8.33	9	0.193
Body and texture	8.66	8.66	8.66	8.66	1
Overall acceptability	8.33	8.33	8.66	9	0.363

* Significant at 5 % level

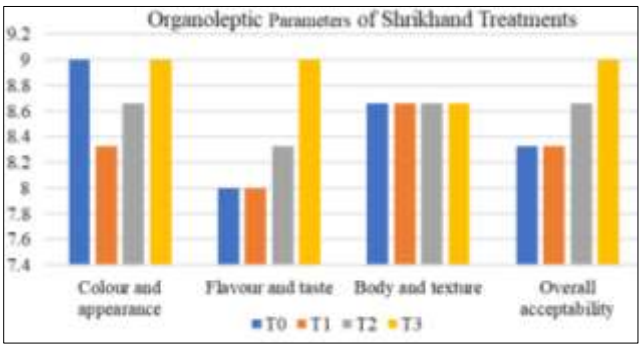

Fig 1: Organoleptic evaluation of Shrikhand treatments

3.2 Physical and Chemical parameter analysis of Shrikhand Treatments

3.2.1 Total solid content

The highest mean value for total solid percentage in Shrikhand treatments (59.93) was obtained from the treatment T_0 i.e. The control sample followed by treatment T_1 (58.33) and T_2 (58.11). The least score (57.85) was obtained in T_3. Significant differences were found among the treatments. F Value obtained was 1615.883 which depicted that there was crucial effect of treatment on total solids percentage.

3.2.2 Moisture

The highest mean moisture percentage value in Shrikhand treatments (42.24) was obtained from the treatment T_3 followed by T_2 (41.88) and T_1 (41.66). The lowest score (40.06) was obtained in T_0 (control). Significant differences were observed among the treatments. F Value was 1615.883, showing significant effects of treatment on moisture percentage.

3.2.3 Ash

The highest value for the ash percent dry matter in Shrikhand treatments (0.9) was obtained from the treatment T_3 followed by T_2 (0.89) and T_1 (0.88). The minimum score (0.68) was obtained in T_0 (control). There were significant differences obtained among the various treatments. F Value was 639.278, designating notable effect of treatment on ash percentage.

3.2.4 Protein

The highest value for protein percent dry matter in Shrikhand treatments (9.42) was obtained from the treatment T_3. The second highest score was by T_2 (9.37) followed by T_1 (9.27). The minimum score (8.5) was obtained in T_0 (control). There were significant differences found among the treatments. F Value was 177.299, indicating remarkable effect of treatment on protein value.

3.2.5 Carbohydrates

The highest value for carbohydrates percent dry matter in herbal Shrikhand (42.09) was obtained from the treatment T_0 (control) followed by T_1 (39.48) and T_2 (39.12). The minimum score (38.8) was obtained in T_3. There were significant differences found among the treatments. F Value was 1224.646, indicating significant effect of treatment on carbohydrate.

3.2.6 Sucrose

The highest value for sucrose percentage on dry matter in Shrikhand treatments (30.13) was obtained from the treatment T_1. Second highest scorer was T_2 (30.07) followed by T_0 (30.1). The minimum score (30.01) was scored by T_3. There were significant differences observed among the samples. F Value obtained was 5.308.

3.2.7 Fat

The highest mean value for fat percentage on dry matter in Shrikhand treatments (8.72) was obtained from the treatment T_2 and T_3 both followed by T_1 (8.70). The minimum score (8.62) was obtained in T_0 (control). There were no significant differences found among the treatments. F Value was 1.994, indicating no noteworthy effect of treatment on fat percentage.

3.2.8 Titratable acidity

The highest value for titratable acidity in Shrikhand treatments (1.11) was obtained from the treatment T_3. Second highest scorer was T_2 (1.03) followed by T_1 (0.97). The least score (0.88) was obtained in T_0 (control). There were significant differences obtained in the treatments. F Value was 471.429 showing notable effect of extract on treatments.

3.2.9 pH

The highest mean value for pH in Shrikhand treatments (4.41) was obtained from the treatment T_0 (control) followed by T_1 (4.33) and T_1 (4.29). The minimum score (4.24) was obtained in T_3. There were significant differences found among the treatments. F Value was 1868.000, indicating notable effect of treatment on pH.

3.2.10 Colour index

Results stipulated a significant difference in the colour among the four variations. The lightness ranged between 75.86-78.85, T_0 and T_3 acquiring the minimum and maximum values. T_1 and T_2 got values as 76.41 and 76.77 respectively. a value ranged between -1.80- -2.22, T_1 having the lowest and T_0 having the highest value towards greenish colour. T_2 and T_3 got -2.26 and -1.94 respectively. The yellowness of Shrikhand samples varied from 14.58-16.16. T_1 being at lower end and T_0 at higher. T_2 showed a value of 15.25 and T_3-15.33. F Value for L, a and b was found to be 47274.769, 6810.667, 15087.300 respectively indicating significant effect of treatment on colour index of Shrikhand samples.

Table 2: Average data of different physico-chemical parameters of Shrikhand treatments

Parameters	Shrikhand Treatments				F Value	CD
	T_0	T_1	T_2	T_3		
Total solids (%)	59.93	58.33	58.11	57.85	1615.883*	0.000
Moisture (%)	40.06	41.66	41.88	42.24	1615.883*	0.000
Ash (% dry matter basis)	0.68	0.88	0.89	0.9	639.278*	0.000
Protein (% dry matter basis)	8.5	9.27	9.37	9.42	177.299*	0.000
Carbohydrates (% dry matter basis)	42.09	39.48	39.12	38.8	1224.646*	0.000
Sucrose (% dry matter basis)	30.1	30.13	30.07	30.01	5.308*	0.026
Fat (% dry matter basis)	8.62	8.7	8.72	8.72	1.994**	0.194
Titratable acidity (% LA)	0.88	0.97	1.03	1.11	471.429*	0.000
pH	4.41	4.33	4.29	4.24	1868.000*	0.000

* Significant at 5 % level

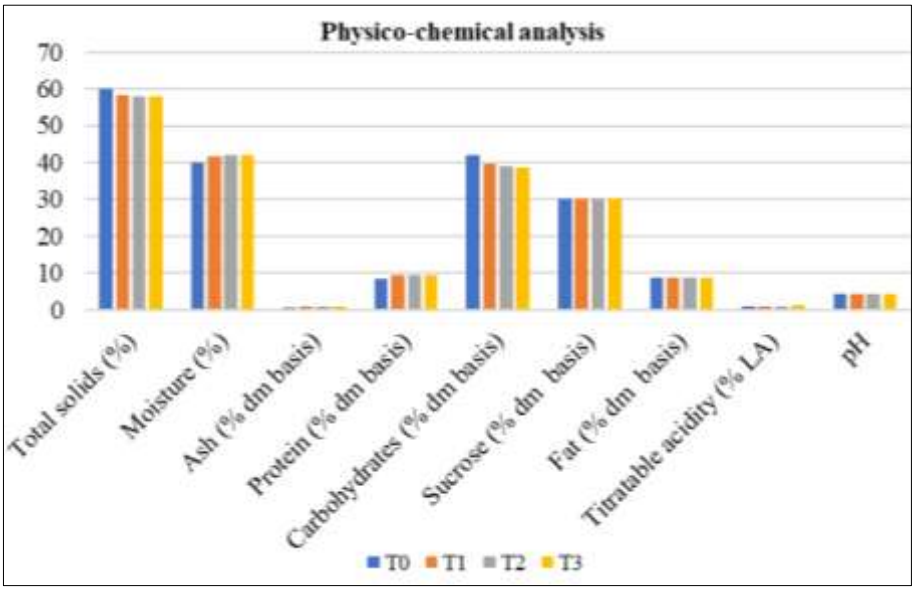

Fig 2: Chemical analysis of Shrikhand treatments

Table 3: Average of L*, a*, b* values (Colour Index) of Shrikhand treatments

Colour Index	Shrikhand treatments				F Value	CD
	T_0	T_1	T_2	T_3		
L*	75.86	76.41	76.77	78.85	47274.769*	0.000
a*	-2.22	-1.8	-2.26	-1.94	6810.667*	0.000
b*	16.16	14.58	15.25	15.33	15087.300*	0.000

* Significant at 5 % level

3.3 Microbiological Analysis

The obtained highest mean score for SPC was 5.80 for the treatment T_3 followed by 5.06 for T_2 and 4.76 for T_1. The minimum score (3.20) was obtained in T_0 (control). No yeasts, moguls or coliforms were detected/ obtained in Shrikhand samples. This indicates that the Shrikhand samples were prepared and kept/stored at proper hygienic place.

Table 4: Average data for microbiological parameters

Parameters	Shrikhand Treatment				F Value	CD
	T_0	T_1	T_2	T_3		
SPC (x 10^3 cfu/ g)	3.2	4.76	5.06	5.8	98.144*	0.000
YMC (cfu/ g)	Nil obtained	Nil obtained	Nil obtained	Nil obtained	-	-
Coliform (cfu/ g)	Nil obtained	Nil obtained	Nil obtained	Nil obtained	-	-

* Significant at 5 % level

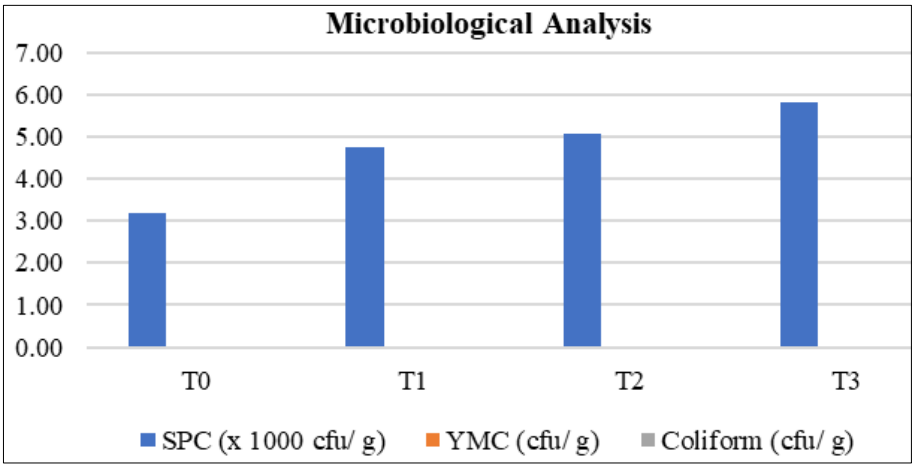

Fig 3: Microbiological analysis of Shrikhand treatments

4. Conclusion

Moringa oleifera stands as a potent natural resource, celebrated for its nutritional prowess and medicinal attributes. Taking the results of the current research into consideration, it is concluded that Shrikhand developed by inducing 4% *Moringa oleifera* leaf extract i.e., sample T_3 scored high for organoleptic characteristics viz, Colour, Appearance, Texture, Body, Flavour, Taste and Overall Acceptability. It also obtained the highest value for lightness in colour index. The chemical analysis results show that T_3 with 4% Moringa leaf extract possesses maximum moisture, protein, ash content and acidity value along with containing lowest amount of carbohydrates and comparable fat content. The non-experimental sample (T_0) has maximum total Solids and pH. Nutrient composition and microbial count of all treatments was found to be within the limits as specified by FSSAI.

There was significant differences obtained between and within the samples. This substantiates the inference that the Shrikhand fortified with 4% *Moringa oleifera* extract indeed outperforms the control sample, spotlighting the extract's capacity to fortify and elevate traditional food preparations.

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