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Tanuj Kumar Tanwar
Ph.D. Scholar, Department of Livestock Products Technology, Collage of Veterinary Sciences, GADVASU, Ludhiana, Punjab, India

Corresponding Author:
Tanuj Kumar Tanwar
Ph.D. Scholar, Department of Livestock Products Technology, Collage of Veterinary Sciences, GADVASU, Ludhiana, Punjab, India

Effect of *Moringa* leaf powder on storage quality of paneer at refrigeration temperature (4±1°C)

Tanuj Kumar Tanwar

Abstract
The present study was undertaken to explore the antimicrobial and antioxidant properties of *Moringa* leaf powder in enhancing the shelf-life of paneer. Paneer is an important heat and acid coagulated milk product. It is a rich source of high quality animal protein, fat, minerals and vitamins. It is a non-fermentative, non-renneted, non-melting and unripened type of cheese. Therefore, paneer fortified with 2, 4, and 6% of *Moringa* leaf powder extracts of along with control was studied to explore the potency of these herbs on oxidative stability and storage quality of paneer on 0,7, 14, 21 and 28 days in refrigerated (4 ± 1^0C) condition. Paneer prepared with 4% of *Moringa* leaf powder were adjudged to the best among all based on sensory attributes. Paneer incorporated with *Moringa* leaf powder were safe for consumption till 28 days at refrigerated storage (4 ± 1^0C) on the basis of pH, moisture, FFA, TBARS, microbiological profile and sensory evaluation of paneer. The developed value added paneer could be conveniently packed in LDPE for a period of 28 days in refrigerated (4 ± 1^0C) condition without any marked loss of physico-chemical, microbial and sensory quality.

Keywords: *Moringa* leaf powder, Paneer, oxidative stability, microbiological evaluation, sensory

Introduction
Paneer is an important indigenous milk product which is obtained by heating the milk followed by acid coagulation by using suitable coagulant such as citric acid, lactic acid, tartaric acid, sour whey. The whey is removed to some extent through filtration and pressing. Paneer is a non-fermentative, non-renneted and non-melting type of cheese, obtained by acid and heat coagulation of milk. It is considered one of the most extensively consumed dairy product in India. The paneer market in India reached a value of INR 365.5 Billion in 2020. Looking forward, IMARC Group expects the market to grow at a CAGR of 15% during 2021-2026.
According to the PFA (2010), paneer means product obtained from cow or buffalo milk or combination thereof, by the precipitation with sour milk, lactic acid or citric acid. It shall contain not more than 70 percent moisture and the fat content should not be less than 50 percent expressed on dry matter. Milk solids may also be used in preparation of paneer. Bureau of Indian standards (BIS 1983) imposed maximum of 60 percent moisture and minimum of 50 percent fat in dry matter for paneer. Good quality paneer is characterized by a marble white colour, mildly acidic taste, sweetish, nutty flavor, and spongy body, closely knit and smooth texture. (Aneja, 2007) [3].
Paneer is of great value in diet, especially in the Indian vegetarian context, because it contains a high level of fat and proteins as well as some minerals, especially calcium and phosphorous. It is also fairly high level of fat soluble vitamins A and D. Over and above its high protein content and digestibility, the biological value of protein in paneer is in the range of 80 to 86 (Shrivastava and Goyal, 2007) [25]. Paneer is mainly used as base material in preparation of various dishes like potatoes, peas and spinach etc.
Milk Products are subject to spoilage having negative effects on the quality in terms of sensory and nutritional quality by either of the two major causes i.e. microbial and chemical (Rababah *et al.*, 2004) [21] and this can be controlled or minimized by the addition of synthetic or natural antioxidants. However, the consumer has concerns about the safety of synthetic antioxidants, which had led to the utilization of natural antioxidants. In this context, plant derivatives which are rich sources of antioxidant and nutraceutical substances are used to enhance quality and shelf life of products. Natural antioxidants extracted from plants such as cinnamom, curry leaves extract, citrus peel and sesame seed can be used as alternatives to the synthetic antioxidants.
In addition, plants are the source of several bioactive ingredients including phenolic compounds, dietary fibres and other phytochemicals.

Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardio protective and vasodilatory effects. Major groups of phyto-chemicals obtained from plant extracts include polyphenols, quinones, flavanols/ flavanoids, alkaloids, and lectins.

Moringa (*Moringa oleifera*) belong to family olieferaceae is fast growing deciduous tree. *Moringa oleifera* is a multipurpose and exceptionally nutrious vegetable tree. It is a subtropical species that is known different names as drumstick tree, saijhan, mooringal and sajna. It has very high nutritional properties that would used as a food supplement and medical application (Pande, 2013) [20]. Almost all part of drumstick is a rich sources of proteins, vitamins and minerals including potassium, calcium, phosphorus, iron, folic acid as well as carotene (Anjorin *et al*., 2010) [4]. Moringa leaves posses high protein and all essential amino acids. The leaves are rich in phenolic compounds including phenolic acids and flavonoids (Makkar and Becker, 1996) [10]. Leaves possess remarkable nutritional and medicinal quality. They contain high amount of vitamin C which act as a antioxidant and prevent many disease. Leaves are also good source of calcium, potassium and zinc which are essential for bone, brain and nerve.

2. Materials and Methods

2.1 Procurement of ingredients

Milk was purchase from local dairy. *Moringa* leaf powder was purchase from local market of Ludhiana.

2.2 Standardization of milk

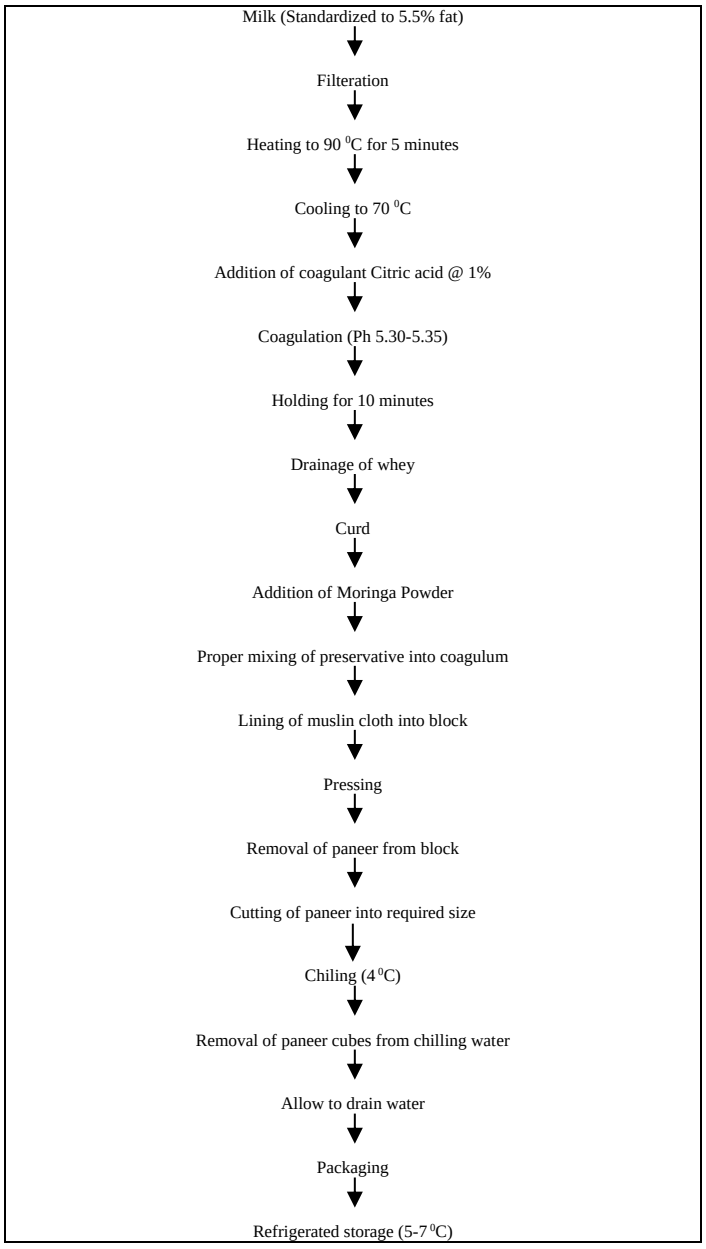
Milk was standardized to 5.5% Fat and 9% solid not fat.

2.3 Packaging and storage of paneer

Control, 2%, 4% and 6% *Moringa* leaf powder incorporated paneer sample of 20 grams each were prepared and packed in LDPE bag and stored at 4±1°C.

2.4 Preperation of Paneer

Paneer was prepared from cow milk with slight modification using the method given by Singh and Kanawjia (1991) [26].



Flow diagram for preparation of Paneer incorporated with Moringa powder

2.5 Physico-chemical analysis

The pH of paneer was measured as described by Awad *et al.* (2005) [7]. Approximately 20 g paneer was mixed with 20 ml warm distilled water (35-40 °C) and slurry was prepared. pH of paneer was measured directly by inserting the electrode into the slurry.

2.5.1 Moisture Content

10 gm of sample was transferred in pre-weight flat bottom aluminum moisture cup, which was transferred to hot air oven at 110±2ºC and kept for 16±1 hrs. Moisture content was calculated by applying the following formula: (AOAC, 1995) [5].

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where, W1 = Weight of empty cup,
W2= weight of cup + sample,
W3= Weight of cup+ dried sample.

2.5.2 Thio Barbituric Acid (TBA)

It was determined using the method of Witte *et al.* (1970) [28]. 10 g of paneer sample was blended finely with 50 ml of 20% TCA in a waring blender/ homogenizer for 2 minutes. The extract was filtered through Whatman filter paper No.42 and in a test tube. 3 ml of this extract was mixed with equal volume of 0.1% (w/v) TBA reagent and blank sample was prepared by mixing 3 ml 20% TCA with equal volume of 0.1% TBA reagent. The content of each test tube was thoroughly mixed and boiled for 35 minutes and further were allowed to cool down. The spectrophotometer absorbance was measured at 532 nm. TBA value was calculated by comparing the absorbance of test sample with a standard graph prepared by using known concentrations of malondialdehyde.

2.5.3 Free Fatty Acid (FFA)

For the determination of free fatty acids, the method described by Koniecko (1979) [15] was followed. 10 gram sample blended for two minute with 50 ml of chloroform in presence of 10g anhydrous sodium sulphate. The mix was filtered through Whatman No.1 filter paper into a 300ml conical flask. About 1 to 2 drop of 0.2% phenolphthalein indicator was added to the chloroform extract, which was further titrated against 0.1N alcoholic potassium hydroxide. Percent free fatty acid was calculated as

$$\text{FFA (\% Oleic acid)} = \frac{0.1 \text{ x ml 0.1N alcoholic KOH x } 0.282}{\text{Wt. of sample}} \text{ x } 100$$

2.6 Microbiological Profile

Total plate count and Yeast and Mould count in the sample were determined by method described by APHA (1984) [6]. Readymade media (Hi-Media) were used for the analysis.

2.7 Sensory Evaluation

Nine-point Hedonic scale method (Lawless and Heymann, 2010) [17] was used for sensory evaluation of all the samples by a panel of 7 semi trained judges.

2.8 Statistical Analysis

Data were analyzed statistically on IBM SPSS Statistics-20.0 software, USA packages as per standard methods. Duplicate samples were drawn for each parameter and replicated thrice (n=6). Sensory evaluation was performed by a panel of seven judges, total observations were 21 (n=21). Means between the periods of storage, between treatments, and within treatments were compared by two-way analysis of variance (ANOVA) and critical difference test as per Snedecor and Cochran (1980) [27]. The statistical significance was estimated at 5% level ($P<0.05$) and evaluated with Duncan's multiple range test. The results were presented in the form of Mean±S.E.

2.9 Experimental Design

The *Moringa* leaf powder was added in standardized formulation of paneer replacing coagulum proportionately (wt./wt.) at the level of 2%, 4% and 6%. The products were evaluated based on physico-chemical, sensory and microbiological profile on 0, 7th, 14th, 21th and 28th day during refrigeration storage at (4±1°C).

3. Results and Discussion

3.1 Physico-chemical parameters

3.1.1 pH

The pH of paneer incorporated with *Moringa* leaf powder was recorded to be significantly lower with increased in level of *Moringa* leaf powder. It may be due to the fact that it contains ascorbic acid and other phenol and flavonoids which are acidic in nature. This was supported by the finding of Mishra *et al.*, (2021) [19].The pH was decreased significantly on successive storage till 28th days irrespective of levels of incorporation of *Moringa* leaf powder in paneer including control may be attributed to bacterial spoilage by lactose fermentating organism. Sachdeva and Singh (1990) [23] also observed decrease in pH of paneer samples during storage. (Table 1)

3.1.2 Moisture

The moisture level recorded in paneer incorporated with *Moringa* leaf powder including control decreased significantly ($P<0.05$). This may be due to the fact that the powder prepared contains less moisture levels as present in coagulum of paneer. The moisture level observed in paneer incorporated with *Moringa* leaf powder and control decreased significantly ($P<0.05$) on successive refrigeration storage days. (Table 1). This result is also in accordance with Rai *et al.*, (2008) [22] who reported decreasing moisture content of paneer during storage.

3.1.3 TBARS

The TBARS value is an indicative of lipolysis due to the external environment and microbes. The TBARS values showed significantly ($P<0.05$) increases throughout the storage study period irrespective of the incorporation or non-incorporation of *Moringa* leaf powder into the paneer. The treatment product showed lower levels of increase as compared to that of the control. The TBARS content of the control product reached greater than 1 mg malonaldehyde /kg on the 28th day of storage whereas the TBARS values of any of the treatment products could not reach to level of 1 malonaldehyde/kg even on the 28th day of storage. This is a clear cut indication of the fact that the *Moringa* leaf powder exhibited anti-lipolytic properties. This finding was in congruence with the finding of Chauhan *et al.*, (2015) [9]. For paneer Fasseas *et al.* (2007) [12] reported extract of various

herbal plants and found out reduction in TBA value and lipid oxidation significantly. (Table 1)

3.1.4 Free Fatty Acid (FFA) Value

The FFA value of paneer incorporated with *Moringa* leaf powder recorded significantly low ($P<0.05$) as depicted in Table-1. This also suggests that paneer incorporated with *Moringa* leaf powder have better shelf life than control. It was due to the fact that flavonoids present in *Moringa* leaf has antilipolytic activity due to which lipolysis didn't occur and free fatty acid production was less. The FFA level in the paneer samples noticed in this study agree with the reports of Kumar and Bector (1991) [16] and Boghra et al. (1997) [8]. (Table 1)

Table 1: Effect of refrigerated storage on physico-chemical characteristics of paneer treated with different levels of *Moringa* leaf powder. (Mean±SE)*

Treatments	0-day	7th day	14th day	21st day	28th day
pH					
Control (0%)	5.58±0.019^{Aa}	5.29±0.007^{Ab}	5.04±0.029^{Ac}	4.83±0.030^{Ad}	4.61±0.030^{Ae}
Moringa (2%)	5.51±0.016^{Aa}	5.23±0.004^{Ab}	4.99±0.028^{Ac}	4.78±0.05A^{Ad}	4.58±0.057^{Ae}
Moringa (4%)	5.45±0.033^{Aa}	5.15±0.027^{Ab}	4.93±0.027^{Ac}	4.72±0.019^{Ad}	4.53±0.019^{Ae}
Moringa (6%)	5.39±0.039^{Aa}	5.08±0.018^{Ab}	4.85±0.094^{Ac}	4.67±0.029^{Ad}	4.49±0.029^{Ae}
Moisture					
Control (0%)	54.60±0.36^{aA}	53.45±0.41^{bA}	52.37±0.44^{cA}	51.25±0.18^{dA}	50.20±0.38^{eA}
Moringa (2%)	54.10±0.86^{aB}	52.95±0.33^{bB}	51.65±0.34^{cB}	50.65±0.39^{dB}	49.62±0.29^{eB}
Moringa (4%)	53.68±0.34^{aC}	52.36±0.35^{bC}	51.05±0.72^{cC}	50.08±0.39^{dC}	49.05±0.59^{eC}
Moringa (6%)	53.10±0.48^{aD}	51.85±0.19^{bD}	50.53±0.16^{cD}	49.56±0.55^{dD}	48.52±0.05^{eD}
TBA (mg malonaldehyde/kg)					
Control (0%)	0.314±0.007^{Ae}	0.440±0.006^{Ad}	0.682±0.013^{Ac}	0.931±0.013^{Ab}	1.335±0.248^{Aa}
Moringa (2%)	0.251±0.010^{Ae}	0.341±0.005^{Bd}	0.573±0.009^{Bc}	0.820±0.011^{Bb}	1.230.±0.011^{Ba}
Moringa (4%)	0.186±0.014^{Be}	0.242±0.005^{Cd}	0.491±0.011^{Cc}	0.710±0.005^{Cb}	0.982±0.005^{Ca}
Moringa (6%)	0.169±0.009^{Be}	0.233±0.009^{Cd}	0.472±0.015^{Cc}	0.690±0.005^{Cb}	0.882±0.005^{Da}
FFA (% oleic acid)					
Control (0%)	0.114±0.0026^{Ae}	0.133±0.0007^{Ad}	0.214±0.0012^{Ac}	0.307±0.0008^{Ab}	0.512±0.0006^{Aa}
Moringa (2%)	0.109±0.0014^{Ae}	0.118±0.0014^{Bd}	0.184±0.0015^{Bc}	0.235±0.0012^{Bb}	0.344±0.0014^{Ba}
Moringa (4%)	0.079±0.0013^{Be}	0.084±0.0013^{Cd}	0.123±0.0008^{Cc}	0.153±0.0013^{Cb}	0.270±0.0012^{Ca}
Moringa (6%)	0.073±0.0006^{Be}	0.078±0.0009^{Cd}	0.118±0.0012^{Cc}	0.148±0.0011^{Cb}	0.260±0.0016^{Da}

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly ($P<0.05$).n=6 for each treatment.

4. Microbiological Characters

Mean microbiological scores of paneer incorporated with different level of *Moringa* leaf powder as well as control during storage at 4±1^{0}C are presented in Table 2.

4.1 Total Plate Count (TPC)

The mean values of TPC was significantly ($P<0.05$) low in *Moringa* leaf powder incorporated paneer than control (Fahey, 2005) [11]. The total plate count has increased significantly ($P<0.05$) on successive refrigeration storage days in *Moringa* leaf powder incorporated paneer including control. The TPC of control paneer was indicative of fact that the product was not suitable for consumption on 28th day of storage. The TPC value of *Moringa* leaf powder incorporated paneer were found to be in the range of 5 $\log_{10}$ cfu/gm which is indicative of the fact that *Moringa* leaf powder extract incorporated paneer were suitable for consumption even on 28th day of refrigeration storage. Similarly, incorporation of *Moringa* leaf extracts was reported to reduce the total plate count during the storage of various meat products (Shah *et al*., 2015) [24]. In addition, Jayawardana *et al*. (2015) [13] reported that the total plate count of chicken sausages formulated with 0.5%, 0.75%, and 1% moringa leaves was significantly lower than that of the control during the storage period.

4.2 Yeast and Molds

The yeast and molds were not detected till 14th day of storage but it appeared in all product from 21th day onward. The yeast and molds count of *Moringa* leaf powder incorporated paneer was significantly ($P<0.05$) lower than control on 21th and 28st day of storage (Table-2). It was also indicative of the fact that control paneer was not suitable but *Moringa* leaf powder incorporated paneer was found to be suitable for human consumption even on 28th day of refrigeration storage. Moreover these extract possess natural fungicidal effect against food borne fungi as supported by Amabye and Tedesse (2016) [2], who worked on anti-fungal and antimicrobial properties of *Moringa* leaf powder.

Table 2: Effect of refrigerated storage on microbiological characteristics of paneer treated with different levels of *Moringa* leaf powder. (Mean±SE)*

Treatments	0-day	7th day	14th day	21st day	28th day
Total Plate Count (logcfu/g) Refrigeration temperature (4±1°C)					
Control (0%)	2.50±0.013^{Ae}	3.40±0.009^{Ad}	3.68±0.022^{Ac}	4.30±0.022^{Ab}	5.30±0.032^{Aa}
Moringa (2%)	2.35±0.013^{Be}	3.15±0.015^{Bd}	3.35±0.012^{Bc}	3.84±0.007^{Bb}	4.32±0.017^{Ba}
Moringa (4%)	1.80±0.015^{Ce}	2.58±0.010^{Cd}	2.85±0.011^{Cc}	3.18±0.171^{Cb}	3.94±0.022^{Ca}
Moringa (6%)	1.60±0.005^{De}	2.30±0.011^{Dd}	2.55±0.009^{Dc}	2.90±0.007^{Db}	3.30±0.022^{Da}
Yeast and Molds Count (logcfu/g) Refrigeration temperature (4±1°C)					
Control (0%)	ND	ND	1.72±0.011^{Ac}	2.83±0.011^{Ab}	3.65±0.011^{Aa}
Moringa (2%)	ND	ND	1.55±0.011^{Bc}	2.34±0.011^{Bb}	3.21±0.013^{Ba}
Moringa (4%)	ND	ND	1.40±0.010^{Cc}	2.08±0.010^{Cb}	2.78±0.011^{Ca}
Moringa (6%)	ND	ND	1.25±0.008^{Dc}	1.50±0.008^{Db}	2.15±0.009^{Da}

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05).n=6 for each treatment.

5. Sensory Parameters

All the sensory parameters viz. colour and appearance, flavour, texture and overall acceptability were found to be significantly (P<0.05) lower on successive refrigeration storage in paneer incorporated with *Moringa* leaf powder including control.

The colour & appearance,flavour and texture of 4% incorporated *Moringa* leaf powder paneer was found to be significantly higher than 2 and 6% *Moringa* leaf powder incorporated and control paneer (Table-3). There was a gradual and significant (P<0.05) decrease in flavour and texture of control, treated paneer. The Overall acceptability of 4% incorporated *Moringa* leaf powder in paneer was significantly higher than 2 and 6% *Moringa* leaf powder incorporated and control paneer.

The herbal extract can act as a very good flavoring agent. It can also act as a binding agent. The sensory character can also be enhanced with herbal extract incorporation in various milk and meat products. It has positive effect by inhibiting discolouration and off odour formation in different milk product during refrigeration storage (Deshmukh *et al*., 2009 [10] and Ahmed & Bajwa (2019) [1].

Table 3: Effect of refrigerated storage on sensory attributes of paneer treated with different levels of *Moringa* leaf powder. (Mean±SE)*

Treatments	0-day	7th day	14th day	21st day	28th day
Colour and appearance					
Control (0%)	7.59±0.10^{Da}	6.92±0.076^{Db}	6.10±0.099^{Cc}	5.29±0.107^{Dd}	S
Moringa (2%)	8.25±0.11^{Ba}	7.68±0.116^{Bb}	6.94±0.106^{Bc}	6.22±0.080^{Bd}	5.45±0.131^{Be}
Moringa (4%)	8.66±0.12^{Aa}	7.78±0.108^{Ab}	7.12±0.083^{Ac}	6.48±0.086^{Ad}	5.88±0.080^{Ae}
Moringa (6%)	7.87±0.15^{Ca}	7.05±0.076^{Cb}	6.38±0.106^{Dc}	5.75±0.112^{Cd}	5.10±0.137^{Ce}
Flavour					
Control (0%)	7.42±0.15^{Da}	6.97±0.116^{Da}	6.28±0.106^{Dc}	5.40±0.122^{Dd}	S
Moringa (2%)	8.15±0.15^{Ba}	7.19±0.073^{Bb}	6.50±0.084^{Bc}	5.97±0.094^{Bd}	5.47±0.080^{Be}
Moringa (4%)	8.50±0.15^{Aa}	7.35±0.092^{Ab}	6.66±0.099^{Ac}	6.14±0.109^{Ad}	5.69±0.106^{Ae}
Moringa (6%)	7.75±0.15^{Ca}	7.14±0.092^{Cb}	6.35±0.106^{Cc}	5.65±0.135^{Cd}	5.04±0.086^{Ce}
Texture					
Control (0%)	7.49±0.10^{Da}	6.93±0.058^{Db}	6.18±0.123^{Dc}	5.34±0.095^{Dd}	S
Moringa (2%)	8.20±0.11^{Ba}	7.45±0.079^{Bb}	6.97±0.080^{Bc}	6.15±0.074^{Bd}	5.64±0.120$^{A e}$
Moringa (4%)	8.58±0.12^{Aa}	7.68±0.079^{Ab}	6.91±0.081^{Ac}	6.25±0.127^{Ad}	5.73±0.131^{Be}
Moringa (6%)	7.82±0.15^{Ca}	7.16±0.073^{Cb}	6.37±0.094^{Cc}	5.70±0.097^{Cd}	5.07±0.121^{Ce}
Overall acceptability					
Control (0%)	7.66±0.10^{Da}	6.98±0.076^{Db}	6.16±0.099^{Cc}	5.35±0.107^{Dd}	S
Moringa (2%)	8.31±0.11^{Ba}	7.75±0.116^{Bb}	6.99±0.106^{Bc}	6.29±0.080^{Bd}	5.51±0.131^{Be}
Moringa (4%)	8.73±0.12^{Aa}	7.85±0.108^{Ab}	7.19±0.083^{Ac}	6.55±0.086^{Ad}	5.95±0.080^{Ae}
Moringa (6%)	7.91±0.15^{Ca}	7.17±0.076^{Cb}	6.45±0.106^{Dc}	5.42±0.112^{Cd}	5.11±0.137^{De}

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05).n=6 for each treatment.

6. Conclusion

The *Moringa* leaf powder was used in preparation of value added paneer. The developed product exhibited significant (p>0.05) anti- oxidant, anti lipolytic and anti microbial activity. The incorporation of *Moringa* leaf powder (4%) in value added paneer has enhanced its sensory scores as well as shelf life. The result revealed the possible application of *Moringa* leaf powder (4%) as a natural source of anti microbial and anti oxidant in development of value added milk product with potential health benefits.

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