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Research Article

Preparation and Quality Evaluation of Yogurt by Incorporation with *Moringa oleifera* Leaves Powder

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Abstract

Moringa oleifera leaves is fill with a pack of nutrients therefore, this study was carried out in order to enrich the nutritional value of traditional yoghurt. The powdered leaves of *Moringa oleifera* were analyzed for moisture content, vitamin C, protein, ash and fat content and the result was found to be 7.34 ± 0.04%, 17.24 ± 0.10 mg/100g, 25.89 ± 1.42%, 9.67 ± 0.40% and 2.1 ± 0.2% respectively and mesh size was 140 µm. From the sensory analysis the product having 0.5% *Moringa oleifera* leaves powder incorporated yoghurt was chosen for further analysis as it showed comparable result with control yoghurt. The chemical analysis of moringa incorporated yoghurt showed that fat, lactose, protein, total solid, acidity, calcium and pH were 2.9 ± 0.11%, 3.71 ± 0.02, 4.51 ± 0.09%, 22.18 ± 0.19%, 0.82 ± 0.02, 356.83 ± 10.19 (mg/100g) and 4.3 ± 0.02 whereas that of control yoghurt were found to be 2.76 ± 0.07%, 3.86 ± 0.03, 3.7 ± 0.4%, 19.90 ± 0.28%, 0.69 ± 0.01, 244.67 ± 10.19 (mg/100g) and 4.5 ± 0.01 respectively.

The fresh yoghurt was found to contain no any coliform, yeast and mold count. Setting time and syneresis for moringa leaves incorporated yoghurt (0.5%) i.e. sample A was found 270 minutes and 34.05 ± 1.30%, respectively whereas setting time for control sample i.e. Sample E was 300 minutes and syneresis was 22.43 ± 2.12%. Acidity was continuously observed in the interval of 30 minutes till the yoghurt sets which showed rapid increase in acidity in *Moringa oleifera* incorporated yoghurt than that of control yoghurt. Acidity rose from 0.69 to 1.14 in control yoghurt and for *Moringa oleifera* incorporated yoghurt it rose from 0.82 to 1.40 during 12 days storage period. The total plate count of the yoghurt decreased during the storage period of 12 days from 1.20×10^9 cfu/ml to 1.1×10^3 cfu/ml for Sample A and from 1.76×10^9 cfu/ml to 4.4×10^3 cfu/ml for Sample E i.e. control yoghurt.

Keywords: *Moringa oleifera;* Syneresis; Incorporated Yoghurt; Chemical Analysis; Sensory Analysis

Introduction

Yogurt is defined as a coagulated milk product that results from the fermentation of lactic acid in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophiles* (CAC, 1992) [4]. According to the Code of Federal Regulations of the USA Food and Drug Administration (FDA) [5], yogurt are often defined as food produced by culturing one or more of the optional dairy ingredients namely, cream, milk, partially skim milk, and skimmed milk, used alone or together with a characteristic bacterial culture that contains carboxylic acid i.e. lactic acid producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophiles*.

Moringa oleifera "The Queen of Green", tree belonging to Moringaceae family is medium sized tree about 10m. They are available in the mid-hills and Terai of Nepal [12]. Because of several nutritional, pharma logical value [3] and industrial application [6,14]. *Moringa oleifera* is mentioned to as a "Miracle tree" or "Wonder tree" by Kasolo., *et al.* [10]. Plant leaves is rich in vitamin B complex, calcium, potassium, iron and protein containing all essential amino acids in good proportion [16]. Moringa leaves are great source of antioxidants due to their high levels of ascorbic acid, flavonoids, and carotenoids [21].

In Nepal, *Moringa oleifera* can easily be grown in Terai and mid hill region. It is commonly known as Sohijan, Saijun, Sajiun and Sitalchini. According to the Ministry of agriculture, about 54 metric ton Moringa is produced in 67 hectors of land in 13 districts of Terai. In Nepal it has successfully grown in home garden across the tropical region, foot hills, and some parts of mid-hill. Despite its multi-purpose uses and potentiality for cultivation, this plant has not received much attention for cultivation and conservation in Nepal [19].

The general objective is to utilize *Moringa oleifera* leaves powder for the production of yoghurt. The specific objectives of the research are to study the acceptability of the product by sensory evaluation, to carry out chemical analysis of prepared yoghurts and to study the effect on yoghurts acidity and microbial load during storage.

Moringa oleifera leaves powder contain higher amounts of several important nutrients and have high potential in food application but very limited research is available. In Nepal, the cost of *Moringa oleifera* leaves powder alone is high, and addition of powder in less amount in delicious yoghurt can enrich the nutritional value. Production of such yogurt has not been practices yet in Nepal and using *Moringa oleifera* leaves powder as an additional ingredient of yoghurt will help to diversify the product, also increases the utilization of *Moringa oleifera* leaves revealing its nutritional significance. There is a very limited study about the technological, physico-chemical, organoleptic, and microbiological properties of such yoghurts. As people consume yoghurt with breakfast, as a dessert in parties; the incorporation of *Moringa oleifera* leaves powder in yoghurt could act as supplement for various amino acids, minerals, antioxidants and vitamins.

Materials and Methods

Moringa oleifera leaves powder was bought from supplier, Moringa Nepal located at Nag pokhari marg, Kathmandu and grated to mesh size 140 µm. Starter Culture i.e. blend of mesophilic and thermophilic bacteria (DVS culture) of Chr. Hanson was brought from a market located in Kathmandu. The standardize milk produced by Dairy Development Committee (DDC) was used for the preparation of yoghurt and other ingredients like sugar, skim milk powder (SMP) were bought from a market in Kathmandu. The utensils, glassware and other required equipment were used from laboratory of College of Applied Food and Dairy Technology (CAFODAT). The research was intended to prepare *Moringa oleifera* leaves powder incorporated yoghurt. So, different proportions of *Moringa oleifera* leaves powder (0.5%, 1.0%, 1.5% and 2.0%) were taken for the preparation of yoghurt.

Method of yoghurt preparation

The method explained by Hassan., *et al.* [8] was followed with modification. The milk was divided into five portions and coded as A, B, C, D and E. Sample E was control sample while other samples A, B, C and D were 0.5%, 1.0%, 1.5% and 2.0% *Moringa oleifera* incorporated yoghurt respectively. For the preparation of yoghurt, the milk was preheated to 45°C followed by the addition of 2% SMP, 4% sugar and the different proportion (0.5%, 1.0%, 1.5% and 2.0%) of *Moringa oleifera* leaves powder was added separately and heated to 80°C for 15 minutes. It was cooled to 45°C and starter culture was added and left for the incubation of 4 - 6 hours at 42 ± 1°C. The product was refrigerated at 5 ± 1°C and kept for further analysis. *Moringa oleifera* incorporated sample which was chosen on the basis of sensory analysis was compared with the control sample.

Sensory evaluation

Sensory analysis of product was performed by 9-point hedonic ranking test (9=like extremely and 1=dislike extremely) for color, aroma, taste, consistency, and overall acceptability. The evaluation was carried out by 10 semi-trained panelists. Each panelist was provided with coded samples and a sheet of sensory evaluation card was provided to score for the quality attributes of color, aroma, taste, consistency, and overall acceptability as per their preference.

Analysis for raw materials and products

Protein content of *Moringa oleifera* leaves powder was determined by Kjeldahl method as per as AOAC [2], total ash was determined as incineration method as per AOAC [2]. Vitamin C was determined as suggested by K.C and Rai [11] and crude fat was determined using soxhlet apparatus described in AOAC [2].

For yoghurt, crude protein was determined by formol titration method which relies on the measurement of carboxylic group present in the amino acids and protein by titrating with 0.1N NaOH; fat was determined by the Gerber method as described in AOAC [2]; pH was analyzed using calibrated pH meter. Calcium content was determined by the titration method using the indicator Solo chrome Blue; Lactose by using procedure from AOAC [2]; whereas total solids and acidity were determined following Quality hand book of DDC as described in the laboratory hand book for dairy

industry, NDDB [17]. Microbial analysis was done as suggested in NDDB [17] by IDF [9] and syneresis was determined by a method used by Lee and Lucey [13].

Statistical analysis

The experimental data were analyzed using one-way ANOVA using the Statistical Analysis System using the SPSS 16.0 program. Significant levels were defined as probabilities of 0.05 or 5% level of significant. All processing treatments were triplicates. The means were treated with ANOVA and seen if there was significantly different among products using Duncan.

Result and Discussion

Sensory analysis

The result of sensory evaluation is shown in table 1.

Sample	Color	Aroma	Taste	Consistency	Overall acceptability
A	7.6 ± 0.52^{a}	7.6 ± 0.52^{a}	7.5 ± 0.53^{a}	7.4 ± 1.26^{a}	7.6 ± 0.52^{a}
B	6.7 ± 0.67^{b}	6.0 ± 1.25^{b}	6.2 ± 1.23^{b}	6.0 ± 1.05^{b}	6.0 ± 0.94^{b}
C	6.3 ± 1.06^{cbd}	4.8 ± 1.32^{c}	5.2 ± 1.23^{c}	6.2 ± 1.14^{b}	5.3 ± 0.82^{c}
D	5.8 ± 1.14^{dc}	4.4 ± 1.35^{dc}	4.8 ± 1.14^{dc}	5.8 ± 1.23^{b}	4.9 ± 0.74^{dc}
E	7.7 ± 0.67^{ea}	7.4 ± 0.70^{ea}	7.6 ± 0.70^{ea}	7.1 ± 0.57^{a}	7.8 ± 0.63^{ea}

Table 1: Mean sensory score of different yoghurts prepared.

* Values are the means ± standard deviations.

Key:

A= 0.5% *Moringa oleifera* leaves powder added yoghurt

B= 1.0% *Moringa oleifera* leaves powder added yoghurt

C= 1.5% *Moringa oleifera* leaves powder added yoghurt

D= 2.0% *Moringa oleifera* leaves powder added yoghurt

E= Control yoghurt.

The mean scores of color, taste and overall acceptability were highest for the control sample E followed by sample A (0.5%). Sample A had received the highest mean score for aroma and consistency.

Color

The mean score of color of different samples ranged from 5.8 - 7.7. Statistically, there was no significant difference ($p > 0.05$) between Sample A and Sample E. However, highest mean score for sample E may be ascribed to familiarity of white color in yoghurt by people.

Aroma

In case of aroma, the mean sensory scores ranged from 4.4-7.6, sample A receiving the highest mean score on basis of aroma whereas D has lowest. There was no significant difference ($p > 0.05$) in the aroma of Sample A and E but they were significantly different with other samples at 5% significant level.

Taste

The mean sensory scores for taste ranged from 4.8-7.6, Sample E was found to be highest whereas Sample D has lowest. Statistically, there was no significant difference in the taste of sample A and E at 5% significance level. However, sample A and E were significantly different with other samples.

Consistency

In case of consistency, the mean sensory scores for Sample A, Sample B, Sample C, Sample D and Sample E were 7.4 ± 1.26, 6.0 ± 1.05, 6.2 ± 1.14, 5.8 ± 1.23 and 7.1 ± 0.57 respectively. The mean score of Sample A was found to be highest whereas Sample D has lowest. There was no significant difference between sample A and E whereas they significantly differ with sample B, C and D.

Overall acceptability

The mean sensory score for overall acceptability of Sample A, B, C, D and E with standard deviation (±) were 7.6 ± 0.52, 6.0 ± 0.94, 5.3 ± 0.82, 4.9 ± 0.74 and 7.8 ± 0.63 respectively. Among the different formulation of *Moringa oleifera* leaves powder incorporated yoghurt, sample A; 0.5% *Moringa oleifera* leaves powder was found to be best with higher acceptance according to the sensory score. There was no significant difference in the sample A and E in overall acceptance while they differ significantly from others.

Chemical composition of *Moringa oleifera* leaves powder

The chemical composition of *Moringa oleifera* leaves powder used for the preparation of yoghurt has been presented in table 2.

The moisture content of *Moringa oleifera* leaves powder was found to be 7.34 ± 0.04%. Protein, fat, total ash and vitamin C content of the Moringa oleifera leaves powder was found to be 25.89 ± 1.42%, 2.1 ± 0.20%, 9.67 ± 0.4% and 17.24 ± 0.1 mg/100g respectively. The values presented in the table 1 for ash and fat are in line with the studies carried out by Putaya, H.A.N, (2015) [20] and Mbaiguinam Mbailao, (2014) [15]. Protein plays vital role in body building and this dried leaves contain appreciable amount of protein. However, variation in protein content was observed, this variations may be attributed to the different areas of study which might have been impacted by factors like climatic and geographical conditions. The value of Vitamin C was in close agreement with Fuglie Lowell [7], who reported value as 17.3 mg/100g.

Parameters	Obtained Values	Putaya, H.A.N 2015 [20]	Mbaiguinam Mbailao [15]
Protein(g/100g)	25.89 ± 1.42	19.95	31.78 ± 0.17
Ash %	9.67 ± 0.40	9.26	7.22 ± 0.54
Vitamin C(mg/100g)	17.24 0.10	ND	ND
Fat%	2.1 ± 0.20	1.29	2.78 ± 0.53

Table 2: Chemical composition of *Moringa oleifera* leaves powder per 100g

Note: The results in the above table are means of triplicate value ± standard deviations.

Chemical composition of selected and controlled product

On the basis of sensory analysis, yoghurt with 0.5% *Moringa oleifera* leaves powder incorporated yoghurt (Sample A) was selected as it was found most organoleptic among incorporated yoghurts. The results of chemical properties of selected product and controlled product is given in the table 3.

- **Total solids**: The total solid content of Sample A was 22.18 ± 0.19% and that of sample E was 19.90 ± 0.28%. Higher solid content in moringa incorporated yoghurt will lead to stability in yoghurt. Statistically, addition of *Moringa oleifera* leaves powder while preparing yoghurt significantly affected ($p < 0.05$) the total solid content.
- **Fat content:** Fat content of control sample was 2.76 ± 0.07% and low in comparison to *Moringa oleifera* leaves powder incorporated yoghurt with the value 2.9 ± 0.11%. The samples were not significantly different ($p > 0.05$) from one another.
- **Protein content:** From the result obtained, the protein content of Sample A was 4.51 ± 0.09% whereas that of Sample E was 3.7 ± 0.4%. Statistical analysis showed significant influence ($p < 0.05$) of addition of *Moringa oleifera* leaves powder in yoghurt. The increase in protein content can be attributed to addition of moringa leaves and the result corresponds to Sodamode., *et al.* (2013) [26] who insinuated that addition of leaves protein concentrate increases the protein and could be used as nutritionally valuable healthy ingredients to improve protein deficiency of person.
- **Lactose and pH**: The lactose content were found to be 3.71 ± 0.02 and 3.86 ± 0.03 for sample A and E respectively. The lactose content for Sample A was lower than sample E. This might be due to increase in acidity of *Moringa oleifera* leaves powder incorporated yoghurt faster than that of control yoghurt as lactose gets converted to lactic acid. The value for pH for Sample A was 4.3 ± 0.02 and 4.5 ± 0.01 for sample E. The processing significantly affected ($p < 0.05$) both lactose and pH content of yoghurt.
- **Acidity:** The acidity pattern of both yoghurts are presented in figure 1 and were found to be increasing. The increase in acidity in Moringa incorporated yoghurt was rapid than control yoghurt. Due to inclusion of acidity obtained from Vitamin C present in Moringa leaves [25], the rapid increase in acidity of Moringa incorporated yoghurt might have occurred. The values of acidity in coagulum state of control sample E was 0.69 ± 0.01 at 5 hours and that of sample A was 0.82 ± 0.02 at 4.5 hours. The values of the sample were significantly different ($P < 0.05$) from one another.
- **Ash content:** From the result obtained, ash content of sample A and sample E were 0.88 ± 0.53% and 0.74 ± 0.01%. *Moringa oleifera* leaf is rich in minerals like iron, magnesium, zinc, calcium, phosphorus, sodium etc. which might have contributed for the higher ash content of sample A than sample E. Statistical analysis at 5% significance level showed that addition of moringa leaves significantly affected ($p < 0.05$) ash content of the prepared yoghurts.

- **Calcium content:** Calcium content of *Moringa oleifera* leaves powder incorporated yoghurt i.e. sample A was 356.83 ± 10.19 mg/100g and that of control yoghurt i.e. sample E was 244.67 ± 10.19 mg/100g. The difference in calcium content was observed and might be due to inclusion of calcium present in the moringa leaves as research suggested that it contains four times calcium than milk (Tree for life, 2005) [27]. Statistically, addition of moringa leaves significantly influenced ($p < 0.05$) calcium content of the prepared yoghurts at 5% significance level.

Parameters	Sample A	Sample E
Total solid %	22.18 ± 0.19 [a]	19.90 ± 0.28 [b]
Fat %	2.9 ± 0.11 [a]	2.76 ± 0.07 [a]
Protein %	4.51 ± 0.09 [a]	3.7 ± 0.4 [b]
Lactose	3.71 ± 0.02 [a]	3.86 ± 0.03 [b]
pH	4.3 ± 0.02 [a]	4.5 ± 0.01 [b]
Acidity as lactic acid	0.82 ± 0.02 [a]	0.69 ± 0.01 [b]
Ash %	0.88 ± 0.53 [a]	0.74 ± 0.01 [b]
Calcium (mg)	356.83 ± 10.19 [a]	244.67 ± 10.19 [b]

Table 3: Result of chemical analysis of yoghurt.

*Values are the mean of triplicates ± standard deviation. Values in the row bearing different superscript are significantly different ($p < 0.05$).

Figure 1: Acidity pattern of yoghurts until coagulation.

Microbial count of the final product

The result of microbial analysis of the product is shown in table 4. The fresh yoghurt was free from any yeast and mold count, coliform count under storage at 5 ± 1°C. Nil coliform count indicates that the quality of yoghurt was maintained hygienically and the sanitation condition was good. This finding was similar with the findings of Ahmed [1] who found that there were no coliform bacteria in yoghurt drink samples.

Parameters	Control yoghurt	Sample A (0.5%)
Coliform count	Nil	Nil
Yeast	Nil	Nil
TPC	1.76×10^9cfu/ml	1.2×10^9cfu/ml

Table 4: Microbial analysis of the product.

Physical analysis

The result of setting time and syneresis is shown in table 5.

Sample	Setting time(minutes)	Syneresis (%)
A	270	34.05 (1.30)
E	300	22.43 (2.12)

Table 5: Setting time and syneresis of the product

Physical analysis showed reduction in setting time of 0.5% *Moringa oleifera* leaves powder incorporated yoghurt compared to control yoghurt whereas syneresis was found to be higher in 0.5% *Moringa oleifera* leaves powder incorporated yoghurt. The reduction in setting time of *Moringa oleifera* leaves powder incorporated yoghurt might have occurred due to the addition of vitamin C present in *Moringa oleifera* leaves which coagulated the constituents of milk. The lactic acid produced by starter culture and acidity added through *Moringa oleifera* leaves powder destabilizes the casein micelles and coagulation occurred.

Syneresis of the *Moringa oleifera* leaves incorporated yoghurt was found to be increased. As syneresis is dependent of various factor, one of the reason could be increase in the acidity of the moringa incorporated yoghurt which might have cause the whey separation and destabilize the gel network [22].

Effect of storage period on acidity of yoghurt

Yoghurt samples A and E were prepared and subjected for acidity determination from first to twelve days with four days interval and their relation is presented in figure 2.

Figure 2: Change in acidity of yoghurt at 5 ± 1°C for twelve days.

The mean value of acidity of yoghurt E for first, fourth, eighth and twelfth days were found to be 0.69, 0.90, 0.98, and 1.15 whereas for yoghurt A the mean values were 0.82, 1.05, 1.22 and 1.40 respectively. Statistical analysis at 5% level of significance showed that there was significant difference ($p < 0.05$) in acidity of yoghurt A and yoghurt E. The initial acidity (lactic acid) of *Moringa oleifera* leaves powder incorporated yoghurt A was 0.82 which rose to 1.4% at 12 days, when stored at 5 ± 1°C. The changes in the titrable acidity of yoghurt could be fermentation process by microorganism and degradation of lactose. The acidity further increased gradually in all types of yoghurt i.e. significant increase in acidity during storage could be due to conversion of lactose to lactic acid by lactic acid bacteria [23].

Effect of storage period on microbial quality of yoghurt

The freshly prepared plain/control yoghurt and the *Moringa oleifera* leaves powder incorporated yoghurt had 1.76×10^9 and 1.2×10^9 cfu/ml of total plate count, which decreased on twelve days to 4.4×10^3 and 1.1×10^3 cfu/ml respectively when stored at 5 ± 1˚C respectively.

Days	Number of microorganisms (cfu/ml)	
	Sample A	Sample E
0	1.20×10^9	1.76×10^9
4	1.1×10^7	1.3×10^7
8	9.5×10^4	1.25×10^5
12	1.1×10^3	4.4×10^3

Table 6: Study of TPC of yoghurt at various storage days.

The moringa incorporated yoghurt had low total plate count as compared to control samples. This may be due to higher acidity in the yoghurt and also due to anti-microbial action of moringa leaf powder [18]. These findings are in close agreement with the results of Sangheetha., *et al.* [24] who observed that the bacterial colony in yoghurt decreased during the storage period.

Conclusion

Among different formulation of incorporated yoghurt, 0.5% *Moringa oleifera* leaves powder incorporated was rated most organoleptic than other and it can be ascribed to increasing bitter after-taste of *Moringa oleifera* incorporated yoghurt to sensory panelist. Addition of *Moringa oleifera* leaves powder significantly affected the total solid, protein, lactose, pH, acidity, ash and calcium content of yoghurts while no significant difference was observed in fat content of prepared yoghurts. The nutritional quality of yoghurt increases with incorporation of *Moringa oleifera* leaves powder whereas setting time is reduced in 0.5% *Moringa oleifera* leaves powder incorporated yoghurt. It is expected to soar up the utilization of *Moringa oleifera* leaves.

Recommendations

As higher percentage incorporated yoghurts were not rated as good to 0.5% moringa incorporated and control yoghurt, it is recommended to identify lingering bitter after taste compound and workout on it. Also, anti-oxidant properties of the prepared yoghurt can be studied and anti-microbial properties of *Moringa oleifera* leaves powder on the lactic culture can be studied.

Conflict of Interest

The authors declare no conflict of interest.

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