Evaluation of Nutritional and Microbial Properties of Bread Developed by Incorporating *Moringa oleifera* Leaves and Fenugreek Leaves

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Authors’ contributions

This work was carried out in collaboration among all authors. Author SMM did the conceptualization, data curation, formal analysis, methodology, writing- original draft, funding acquisition. Author SM managed the project administration, supervision, visualization, methodology, review and editing. Authors SK and DB did the reviewing and editing. All authors read and approved the final manuscript.

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ABSTRACT

Bread supplies a huge part of the supplement needed for development, support of wellbeing and prosperity. It is a brilliant wellspring of protein, minerals, fiber and carbohydrates. Hence, main objective of our study was to evaluate the nutritional and medicinal benefits of bread consisting of *Moringa oleifera* leaf powder (MOLP) and fenugreek leaf powder (FLP), as leaves of *Moringa oleifera* and fenugreek are considered to have high proportion of essential micronutrients and have many nutraceutical properties. Flaxseed was also added to enhance the nutritional benefits of bread as flaxseed contain alpha linolenic acid (ALA) and omega-3-fatty acid. Bread was developed by incorporating different flour blends and analysed to know its nutritional and microbial properties. T₀ include 100% multigrain flour, T₁ include 97.5% multigrain flour, 2.5% moringa leaves powder (T₁ a) and 95% multigrain flour, 5% moringa leaves powder (T₁ b) whereas T₂ include 95% multigrain flour, 2.5% moringa leaves, 2.5% fenugreek leaves (T₂ a) and 90% multigrain flour, 5% moringa leaves and 5% fenugreek leaves (T₂ b). During the experimentation moisture content of bread samples decreased with the increasing content of MOLP and FLP whereas protein, fibre and
ash content of bread samples were increased by increasing the percentage of different blend formulations. Sensory evaluation of the bread samples was done by using the 9-point hedonic scale to know the overall acceptability of the product. Although the nutritional value of developed bread increased but the overall acceptability of product decreased by increasing the supplementation contents.

Keywords: Acceptability; concentration; fenugreek leaf powder; Moringa oleifera leaf powder; nutritional; supplementation.

1. INTRODUCTION

Bread is one of the most conventional man-made foods mainly prepared from wheat flour dough cultured with yeast, allowed to gently rise, and finally baked in a microwave oven. Bread is a primary food of the Middle East, Central Asia, North Africa, Europe, and other European-derived cultures like those in America, Australia, Southern Africa. With the marked increase in customer mindfulness, improved instructive status, and lifestyle changes, the pattern in the consumption of good quality food sources has drastically changed. The consumers are readily accepting various products which give the combined benefits of different nutrients in a single diet plan maintaining a balanced diet [1]. This brings up the idea to provide more nutritional value to the existing products on which our paper is based upon. The Multigrain products consist of general mixture of organic grains like cultivated wheat, oat, barley, maize, polished rice, and other items processed effectively. It also allows leading makers to intentionally create essential items with an inventive appearance, colour and taste. with a helpful dietary profile. It contributes to a sound digestive system and have multiple health benefits. It is also an excellent source of essential micronutrients like calcium, potassium, iron, vitamin B, etc. [1]. With the expanding utilization of bread and other baked items in many nations, the composite flour program promises to save critical requirement of many and give nutritious food to more and more individuals at lower cost [2].

For our study we have considered adding two different components which would add value to the existing multigrain breads and provide other additional benefits. The items considered in our experiment are Moringa leaves and Fenugreek leaves.

Moringa plant known as 'Miracle Tree' is among the best plant source for providing nutrition. Moringa leaves are highly nutritious and are very helpful in the treatment of many afflictions because of their nutritional and medicinal properties [2]. The leaves of Moringa oleifera contain vitamin A, B groups, and C. They are rich in minerals such as iron, calcium, and potassium. Their leaves are also a good source of protein [3]. One tablespoon of moringa leaves provides about 14% of protein, 40% of calcium, 23% of iron, and other essential nutrient required to a child, and around six tablespoons of powdered leaves will fulfill the daily requirement of iron and calcium essential during pregnancy and breastfeeding time [4].

The fenugreek leaves have their specific benefits. The leaves are mainstream for their fragrant and remarkable flavour. Fenugreek has a solid, wonderful, and peculiar smell. Fenugreek (Trigonella foenum graecum) belongs to the Leguminosae family and is admired for its medicinal applications like antimicrobial, antidiabetic, anticancer, antioxidant [5]. Fenugreek leaves contain a high amount of protein, fiber, vitamins and minerals, and beta carotene, etc [6]. While these leaves give a decent measure of different minerals and nutrients, they are particularly rich in Choline [7].

Additionally, we also used Flaxseed which plays an important role in bakery products as it contains a high amount of alpha-linolenic acid (ALA), omega-3 fatty acid [8].

2. MATERIALS AND METHODS

2.1 Procurement of Raw Materials

Multigrain flour, flaxseed and other ingredients used for baking (sugar, fat, dry yeast, salt, skim milk powder) were purchased from a supermarket in Lucknow while mature leaves of Moringa oleifera were obtained from the field of Babasaheb Bhimrao Ambedkar University, Lucknow. Fresh and matured leaves of fenugreek were obtained from the vegetable market of Gomti Nagar, Lucknow.
2.2 Sample Preparation

De-stalking, washing and drying of moringa leaves were done by using the method described by Kokoh et Al. [9]. The fresh fenugreek leaves were washed to remove the dirt particle and dried under the sunlight for 4-5 days till the leaves were completely dried having moisture content 6-8%. The dried fenugreek leaves were reduced to fine powder using a mechanical grinder and then packed into an air-tight container for further use [10].

2.3 Bread Preparation

Five blend formulations (Table 1) were prepared using the straight dough method [11]. All the dry ingredients (flour blends, sugar, skim milk powder, salt, baking powder) were mixed in a large bowl. In a small bowl, dry yeast was mixed with lukewarm water and left for 20 minutes. The yeast mixture was poured into dry ingredients mixture which was then thoroughly blended with melted butter along with some water to make a firm dough. The dough prepared was then kept in a bowl greased with oil and was left for fermentation in the warm temperature. This resulted in formation of bubbles which was removed by knocking off the bubbles out of the dough with the hands. The dough was placed on a lightly floured surface and knead 3-4 times. The dough was patted until round, and the sides were dabbed to form oval shape closing the cracks if any.

The dough was then kept in lightly oiled bread tin mould and was covered with a cloth and left in warm place until it ascends to the highest point of mould. Furthermore, the baking process was carried out by pre-heating the oven and baking the dough at 230 °C for 15 minutes and then turning the mould to the unbaked side to bake it for further 20-25 minutes at 180 °C [12].

2.4 Proximate Analysis of Bread

2.4.1 Moisture content

Moisture content of all bread samples was determined by using AOAC official method [13].

2.4.2 Crude protein

Protein determination of samples was done by using the kjeldahl method described by AOAC [13].

2.4.3 Crude fat

Fat estimation of bread samples was done by using soxhlet apparatus (AOAC method) [13].

2.4.4 Crude fibre and ash

Crude fibre and total ash content of the bread samples were determined by using standard official method of AOAC.

2.4.5 Carbohydrate

Determination of carbohydrate in bread samples was done by using the difference method by AOAC.

\[
\% \text{ carbohydrate} = 100 - \left(\% \text{ protein} + \% \text{ fat} + \% \text{ fibre} + \% \text{ ash} + \% \text{ moisture}\right).
\]
Table 1. Multigrain flour, moringa and fenugreek leaf powder blends for bread making

<table>
<thead>
<tr>
<th>Blends</th>
<th>T₀</th>
<th>T₁(a)</th>
<th>T₁(b)</th>
<th>T₂(a)</th>
<th>T₂(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF</td>
<td>100%</td>
<td>97.5%</td>
<td>95%</td>
<td>95%</td>
<td>90%</td>
</tr>
<tr>
<td>MOLP</td>
<td>-</td>
<td>2.5%</td>
<td>5%</td>
<td>2.5%</td>
<td>5%</td>
</tr>
<tr>
<td>FLP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5%</td>
<td>5%</td>
</tr>
</tbody>
</table>

*MF: Multigrain Flour, MOLP: Moringa oleifera Leaf Powder, FLP: Fenugreek Leaf Powder

2.5 Microbial Evaluation

Total viable bacterial, yeast and mold counts were carried out to determine the microbial load of developed bread samples as described by American Public Health Association [14]. Bread samples were mixed in peptone water followed by serial dilution of the same. Samples were diluted decimally, and 0.1 ml diluent was spread plated on Plate Count Agar (PCA), Mac Conkey Agar (MCA) and Potato Dextrose Agar (PDA) for the determination of total bacterial count, coliforms and fungi counts respectively. Total colonies were counted after incubation of PCA and MCA plates at 37 °C for 24-48 hours and PDA plates at room temperature (28±3 °C) for 3-5 days. The colonies were expressed as cfu/gm.

The mean and standard deviation of the data collected from the sample was then evaluated using the formula for mean and standard deviation.

Mean:

\[ \bar{x} = \frac{\sum x}{N} \]

Standard Deviation:

\[ s = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}} \]

*\(S\) = standard deviation, \(X\) = each value, \(\bar{x}\) = sample mean, \(n\) = number of values in the sample.

2.6 Scanning Electron Microscopy

A freeze-dried sample of 2-4 mm was analysed using Scanning electron microscopy (SEM) method to extract the morphology of the sample. The samples were mounted on to the Aluminium stubs with carbon tape and coating of the samples was done by using the Sputter coater to make the sample conductive. All the samples were observed at 10 KV using a high-resolution field emission of Scanning Electron Microscope (JSM 6490).

2.7 Sensory Evaluation

The sensory evaluation of the prepared samples was performed after 24 hours of baking. Evaluation was done on the basis of some major sensory attributes i.e., texture, colour, taste, aroma, appearance and overall acceptability of product [15]. The evaluation of bread samples was done by 20 untrained people who were habitual consumers of bread.

2.8 Statistical Analysis

The experiment was performed in triplicates and the data obtained for each parameter from their respective evaluation methods was collected successfully in the form of table.

3. RESULTS

3.1 Proximate Analysis of Bread

The proximate analysis of different bread samples is represented in Table 2. The moisture content of developed bread varied from 32.24% to 27.28%. The highest moisture content of 32.24% was obtained in the bread sample consisting of 100% multigrain flour whereas the lowest moisture content of 27.28% was observed in the bread sample made with a blend of multigrain flour, moringa leaf powder, and fenugreek leaf powder in the ratio 90:5:5. The moisture content of developed bread decreased in variation with addition of supplements like moringa leaf powder as the concentration of moringa was increased from 2.5% to 5%. Similar changes were also observed in bread developed by incorporating moringa and fenugreek leaf powder into multigrain flour i.e., on increasing concentration of moringa and fenugreek leaf powder from 2.5% to 5%, moisture content of developed bread decreased from 29.03% to 27.28%.

The highest protein content of 14.11% was obtained from the sample developed with MOLP and FLP supplementation whereas the lowest protein content of 10.13% was observed in the control sample made with 100% multigrain flour.
The concentration of protein depends directly on the concentration of blends.

Bread developed with the blend of MOLP and FLP were found to have higher crude fiber (4.54%) which is somewhat higher than the fiber present in the control sample (2.78%). Total ash content was also increased from 2.91% to 3.42%. Bread developed with 5% moringa leaf powder supplementation obtained 2.06% ash content while bread made with 5% moringa and 5% fenugreek leaf powder contained 2.45% of total ash content.

The fat percentage of developed bread varied from 2.91% to 3.42%. A slight difference was observed in the fat percentage of bread samples as the concentration of blends increased.

The carbohydrate content was higher in the control sample made with only multigrain flour (50.44%) as compared to the other bread samples made with different blend formulations whereas the least carbohydrate content of 48.18% was found in bread made with moringa and fenugreek leaf supplementation.

### 3.2 Microbial Evaluation

The total bacterial count of developed bread varied from 3.5 to 1.1 cfu/gm and fungal count in sample T₀ was found $4.5 \times 10^1$ and the lowest fungal count was present in the sample made from 2.5% MOLP and 2.5% FLP (Table 3). The fungal count of the bread samples was increased during the storage. Bread made from 100% multigrain flour was found to have a higher concentration of total viable bacteria (3.5 cfu/g) as compared to the bread developed with the blend of moringa leaf powder and a mixture of moringa leaf and fenugreek leaf powder. The result showed that the number of bacterial counts decreased as the concentration of moringa leaf powder was increased from 2.5 to 5%. A change was also observed in the bread developed with the supplementation of moringa and fenugreek leaf powder mixture as it was also decreased on increasing the blend concentration from 2.5% to 5%. Whereas on increasing the concentration of multigrain flour, fenugreek and moringa leave blend in the samples the increase in the fungal count was observed. No coliform growth was observed in any of those bread samples.

### Table 2. Proximate analysis of bread samples

<table>
<thead>
<tr>
<th>Blends</th>
<th>Moisture</th>
<th>Protein</th>
<th>Fibre</th>
<th>Ash</th>
<th>Fat</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>32.24 ± 0.24</td>
<td>10.13 ± 0.13</td>
<td>2.78 ± 0.18</td>
<td>1.51 ± 0.08</td>
<td>2.91± 0.06</td>
<td>50.44 ± 0.56</td>
</tr>
<tr>
<td>T₁(a)</td>
<td>29.52 ± 0.14</td>
<td>11.76 ± 0.28</td>
<td>3.64 ± 0.07</td>
<td>1.86 ± 0.11</td>
<td>3.05 ± 0.04</td>
<td>50.16 ± 0.40</td>
</tr>
<tr>
<td>T₁(b)</td>
<td>27.60 ± 0.15</td>
<td>13.73 ± 0.26</td>
<td>4.13 ± 0.12</td>
<td>2.06 ± 0.06</td>
<td>3.11 ± 0.05</td>
<td>49.41 ± 0.13</td>
</tr>
<tr>
<td>T₂(a)</td>
<td>29.03 ± 0.09</td>
<td>12.09 ± 0.07</td>
<td>3.93 ± 0.07</td>
<td>2.02 ± 0.05</td>
<td>3.24 ± 0.06</td>
<td>49.68 ± 0.16</td>
</tr>
<tr>
<td>T₂(b)</td>
<td>27.28 ± 0.03</td>
<td>14.11 ± 0.10</td>
<td>4.54 ± 0.09</td>
<td>2.45 ± 0.09</td>
<td>3.42 ± 0.04</td>
<td>48.18 ± 0.03</td>
</tr>
</tbody>
</table>

*Values are represented in mean± standard error of three replicants. *Refer Table 1 for more info

### Table 3. Microbial load of different bread samples

<table>
<thead>
<tr>
<th>Bread Samples</th>
<th>TBC</th>
<th>Yeast and Mold</th>
<th>Coliform</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>$3.5 \times 10^6$</td>
<td>$4.5 \times 10^1$</td>
<td>NG</td>
</tr>
<tr>
<td>T₁(a)</td>
<td>$2.8 \times 10^6$</td>
<td>$2.8 \times 10^1$</td>
<td>NG</td>
</tr>
<tr>
<td>T₁(b)</td>
<td>$1.3 \times 10^5$</td>
<td>$1.6 \times 10^1$</td>
<td>NG</td>
</tr>
<tr>
<td>T₂(a)</td>
<td>$1.9 \times 10^5$</td>
<td>$2.5 \times 10^1$</td>
<td>NG</td>
</tr>
<tr>
<td>T₂(b)</td>
<td>$1.1 \times 10^5$</td>
<td>$3.1 \times 10^1$</td>
<td>NG</td>
</tr>
</tbody>
</table>

*Cfu/gm: colony forming per unit gram, NG: No Growth, TBC: Total Bacterial Count

### Table 4. Sensory evaluation of developed bread with supplementation of MOLP and FLP

<table>
<thead>
<tr>
<th>Bread Samples</th>
<th>Taste</th>
<th>Colour</th>
<th>Texture</th>
<th>Aroma</th>
<th>Overall Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>8.73 ± 0.44</td>
<td>8.78 ± 0.41</td>
<td>8.63 ± 0.48</td>
<td>8.81 ± 0.39</td>
<td>8.83 ± 0.32</td>
</tr>
<tr>
<td>T₁(a)</td>
<td>8.20 ± 0.51</td>
<td>8.05 ± 0.56</td>
<td>8.58 ± 0.49</td>
<td>8.58 ± 0.59</td>
<td>8.68 ± 0.46</td>
</tr>
<tr>
<td>T₁(b)</td>
<td>7.51 ± 0.53</td>
<td>7.11 ± 0.69</td>
<td>6.91 ± 0.64</td>
<td>6.71 ± 0.64</td>
<td>6.35 ± 0.66</td>
</tr>
<tr>
<td>T₂(a)</td>
<td>7.36 ± 0.55</td>
<td>7.08 ± 0.53</td>
<td>7.10 ± 0.62</td>
<td>6.98 ± 0.56</td>
<td>6.65 ± 0.48</td>
</tr>
<tr>
<td>T₂(b)</td>
<td>4.81 ± 0.98</td>
<td>5.58 ± 0.59</td>
<td>5.45 ± 0.69</td>
<td>4.86 ± 0.94</td>
<td>4.38 ± 0.66</td>
</tr>
</tbody>
</table>

*Values are represented in mean ± standard deviation (n=20)
Fig. 2. Scanning electron microscopy of developed bread samples

Notes: All bread samples were observed at 10KV using a high-resolution field. The experiment was performed in triplicates.
3.3 Scanning Electron Microscopy

Breadcrumbs of different flour blends was prepared to carry out SEM analysis to assess the microscopic structure of bread samples (Fig. 2). Bread made with 100% multigrain flour was found to have a different structural form compared with the other bread samples. The incorporation of moringa leaves, fenugreek leaves affected the structure of the gluten network. The addition of these rough particles like moringa leaves and fenugreek leaves causes the discontinuous structure of the gluten network. The mixing of Moringa powder in the flour resulted in the formation of complex network of protein and gluten structure.

3.4 Sensory Evaluation

Sensory evaluation of developed bread samples was affected by the different ratios and blends of moringa and fenugreek leaf supplementation (Table 3). The result showed that the scores of sensory attributes of bread samples were decreased by increasing supplementation of moringa and fenugreek leaf powder. According to the hedonic ratings, the overall acceptability of the product is inversely proportional to the increase in the concentration of moringa and fenugreek blends. The least value of 4.81%, 5.58%, 5.45%, 4.86%, 4.38% for taste, colour, texture, aroma, overall acceptability respectively was observed on bread developed by incorporating 5% MOLP and 5% FLP blend. Bread developed from 100% multigrain flour was more appreciated as compared to the other bread samples. Bread made from 2.5% of moringa leaf incorporation was also obtained good values for the overall acceptability of the product. It showed 8.20%, 8.05%, 8.58%, 8.58%, and 8.68% for taste, colour, texture, aroma, and overall acceptability respectively.

4. DISCUSSION

Proximate composition of bread samples shows that an increase in supplementation increases the nutritional value of bread. The Moisture content of a food product is an important quality factor for determining its acceptability, physical and microbial properties. The Moisture content of developed bread decreased as the concentration of the preferred supplement increased. This could be due to the low moisture content present in the blend used for the preparation of the product. Sengev et al. [2] also found that moisture content of composite flour (wheat flour and Moringa leaf powder) decreased significantly as the percentage of Moringa leaf was increased. Bahzad Afzal [16] also reported that moisture content of different formulations also decreased with an increase in the supplementation of fenugreek leaves. Olaoye et al. [17] also obtained similar results for bread made with composite flour of wheat, plantain, and soybean.

Crude protein content in bread samples was increased with the addition of supplements like MOLP and FLP. Mishra SP et al. [18] and Moyo B et al. [19] reported that the leaves of Moringa oleifera contain all the amino acids essential for humans. Breads made with moringa leaves are beneficial to the people suffering from malnourishment and also to pregnant women. An increase in fiber ash, crude fiber content was also seen in the developed bread. This result follows the findings found by Sengev et al. [2].

Microbial evaluation of bread samples showed that the supplements of MOLP and FLP, decreased the microbial load of total bacteria, yeast, and mold.

Amabye [20] reported that this might be due to the antimicrobial activity of Moringa oleifera powder. The results observed complies with the findings of Bahzad Afzal [16]. According to their observation addition of fenugreek decreases the microbial load of the product due to its antimicrobial activity. According to the American public health association [14], microbiological standards of developed product can be acceptable if it lies between 10³ to 10⁶ cfu/gm. An increase in the fungal count during storage was also reported by Mishra S and Ajith J [21]. No growth of coliform bacteria represented that the products are safe for consumption and there is no faecal contamination present in developed products. This result is in agreement with the observation seen by Ijah et al. [15].

Scanning electron microscopy results showed that incorporation of moringa and fenugreek causes complex structure between protein and starch granules. Fleming and Sosulski [22] observed that the microstructure of bread when supplanted by protein-rich flours shows irregularity in the very much characterized protein-starch complex of wheat flour bread and the frail gluten structure, bringing about a lessening in bread volume. Agrahar Murugkar and Dixit-Bajpai [23] reported the same result in their studies as the incorporation of finger millet,
moringa leaves, sesame, cumin causes the irregularity in the structure of starch granules and broken gluten matrix. However, the nutritional properties of bread samples increased with the supplementation of leaves powder. Porcel et al [24] also observed that the addition of Okara flour interferes with the structure of the matrix and excess of insoluble dietary fiber affected the gluten network.

Sensory evaluation of the developed product shows that incorporation of moringa and fenugreek leaf powder increases the nutritional property of bread whereas the increase in concentration decreases the acceptability of bread samples. The evaluation of the products represented that the bread made with 100% multigrain flour and 2.5% of MOLP supplementation were more acceptable while the bread made with supplementation of 5% MOLP and 5% FLP was highly unacceptable. The reason behind the decrease in preferences for highly concentrated blends might be due to the odour and colour of dried green leaves. These results comply with the studies done by Sengev et al. [2] and Ebrahem et al. [25].

5. CONCLUSION

The Bread was successfully developed by incorporating moringa and fenugreek leaf powder. The incorporation improved the nutritional value of bread. The Microbial load of developed bread decreased as MOLP and FLP were added into the products. Thus, incorporating moringa and fenugreek leaves in products could be beneficial from the nutritional and microbial perspective. Morphological study of the bread samples showed that after the addition of the moringa and fenugreek there was irregular structure of gluten and starch granules. The sensory evaluation concludes that the acceptability of the product is inversely related to the concentration of blends. Thus, an acceptable and nutritious product can be developed by maintaining the concentration of blends.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


