Sensorial and chemical analysis of biscuits prepared by incorporating *Moringa* flower powder and leaf powder

Yadav KC, Sharad Bhattarai, Lila Devi Shiwakoti, Samrat Paudel, Milan Subedi, Bhoj Raj Pant, Mahendra Paudel, Shristi Dhugana, Saraswati Bhattarai, Tribhuwan Tiwari, Niranjan Koirala, Nada H. Aljaarba, Saad Alkahtani, Gaber El-Saber Batiha, Ramesh Shiwakoti, and Jitendra Upadhyaya

**Central Campus of Technology, Department of Food Technology, Tribhuvan University, Dharan, Nepal;**

**National Tea and Coffee Development Board, Hile, Dhankuta, Nepal;**

**Department of Agriculture, Institute of Agriculture and Animal Science (IAAS), Tribhuvan University, Rampur Campus, Rampur, Nepal;**

**Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal;**

**Department of Agriculture, University Institute of Agricultural Sciences, Chandigarh University, Gharuan, India;**

**Department of Agriculture, Institute of Agriculture and Animal Science, Prithu Technical College, Tribhuvan University;**

**Department of Science, College of Science, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia;**

**Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia;**

**Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt;**

**Forest Directorate, Ministry of Forest, Environment and Soil Conservation, Government of Nepal, Dharan, Nepal**

**ABSTRACT**

*Moringa oleifera* is a nutrient-rich plant, also referred to as a miracle tree, and is commonly used in the preparation of functional foods including herbal biscuits. Despite having a wide range of biomolecules, *M. oleifera* has not been studied for its nutritional benefits in Nepal. To fill this gap, five different formulations of flower and leaf powder ratios of 11:4, 11.75:3.25, 12.5:2.5, 13.25:1.75, 14:1 named as A, B, C, D, E, and control formulations were tested for their sensory and chemical characteristics. The results showed that calcium content (115.73 mg/100 g) was higher in biscuits with a higher percentage of the leaf (11:4) while TPC was minimum. Further, biscuits containing a higher percentage of flower powder contained fewer tannins. The sensory analysis concluded that D was deemed the best in overall attributes by panelists upon statistical analysis, however formulations A and B were superior to other samples regarding the chemical properties. These findings confirm that there is a huge potential for improving herbal biscuits.

**ARTICLE HISTORY**

Received 7 March 2022
Revised 17 April 2022
Accepted 19 April 2022

**KEYWORDS**

Flower; Hedonic test; Herbal biscuits; Leaves powder; *Moringa oleifera*; Proximate analysis

**CONTACT**

Jitendra Upadhyaya
jitu.upadhyaya@gmail.com
Institute of Agriculture and Animal Science, Tribhuvan University, Rampur Campus, Chitwan, Nepal

© 2022 Yadav KC, Sharad Bhattarai, Lila Devi Shiwakoti, Samrat Paudel, Milan Subedi, Bhoj Raj Pant, Mahendra Paudel, Shristi Dhugana, Saraswati Bhattarai, Tribhuwan Tiwari, Niranjan Koirala, Nada H. Aljaarba, Saad Alkahtani, Gaber El-Saber Batiha, Ramesh Shiwakoti and Jitendra Upadhyaya. Published with license by Taylor & Francis Group, LLC. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Introduction**

Biscuits are among the most popular cereal snack foods across the globe, particularly among children. These are generally small (usually flat) baked goods made from wheat flour. Today, people are more health and nutrition-conscious, making biscuits exclusively made from wheat may not be a healthy choice, promoting functional/herbal food in the mainstream. Thus, in recent times, manufacturers have adjusted their focus on the use of herbs in conventional foods like tea, juices, and snack chips by giving names like “special diet foods,” “medical foods,” “dietary supplements” due to the growing trend of consumption of herbal products for wellness. According to the Food and Drug Administration, functional foods contain ingredients (either naturally occurring or added) that
provide health benefits beyond the traditional nutritional value of the food.[3] Unlike conventional foods, there is no such regulatory category of functional food as per FDA and other regulatory bodies; however, herbal ingredients need to be approved as GRAS to be used in functional food.[4]

Moringa oleifera is referred to as a miracle plant or tree of life-based, particularly on its uses regarding medicine and extremely high nutritional value.[5] This plant has been studied extensively for its multipurpose role,[6] including herbal biscuit products.[7] Flowers of Moringa oleifera are fragrant, yellowish-white bisexuals with dimensions of 1 cm long by 2 cm wide, set in the basal cup of the thalamus,[8] and serve as a good source of a wide variety of nutrients, including proteins, potassium, phosphorous, iron, calcium antioxidants (α and γ tocopherol) and polyunsaturated fatty acids, leading them to be ready food or tea and dietary supplement after processing.[9] Further, there is enormous bioactive potential in the Moringa flower, with its crude extract showing antibacterial, antifungal, anti-larval, antioxidant, anti-inflammatory and anti-cancer properties. Additionally, flowers also possess diuretic, hepato-protective, and anti-tussive properties, and are used as a preventative for a wide variety of illnesses, like pertussis, asthma, combating infertility, and muscle and spleen problems.[9] The leaves of the Moringa plant contain all amino acids, uncommon among plant sources, and a very high amount of unsaturated fatty acids including linoleic acid. Additionally, Moringa leave is abundant in almost all vitamins, including vitamin A, vitamin B1 including folic acid, pyridoxine, and nicotinic acid, vitamin C, vitamin D, and vitamin E.[5,10] A study by Hasaballa et al.[11] also reported that Moringa leaf contained the highest amount of calcium and iron compared to other parts. In addition, polyphenols present in Moringa also contribute to sensory qualities of natural foods derived from it.[12] The research regarding the bread incorporated with moringa escalated the nutritional standards[13,14] while moringa seed flour can replace some proportion of wheat flour to increase the quality of bread.[15] Moringa leaves also offer the potential to be used in biscuits.[16,17] The studies are being conducted to utilize the different parts of moringa in different food products due to their potential benefits and different processes are being carried out for the optimum consumer acceptance. However, limited research is carried out for incorporating flour from both leaves and flowers in the biscuits. This research is expected to establish the nutritional importance and acquaint the fortified with biscuits to the consumers.

Materials and methods

Collection of raw materials

Moringa flowers and leaves were collected and sun-dried in Arkhaule village (Mahalakshmi municipality) in Dhankuta district, Nepal (27° 05′ 51″ N/87° 19′ 48″ E). The remaining ingredients (sugar, shortening, salt, and baking powder) were procured from the local market in Dharan, and biscuits were prepared at the Central Campus of Technology in Dharan, Nepal. Further, the leaves and flowers of Moringa were sun-dried for 3 days, ground into powder, and packaged in a polyethylene bag.

Preparation of biscuits

Five experimental biscuits (A, B, C, D, and E) were prepared by using flower and leaf ration of 11:4, 11.75:3.25, 12.5:2.5, 13.25:1.75, and 14:1, respectively, that substituted 15 parts of wheat for the control sample. A preliminary pilot-scale study was conducted and the taste was harsh when the moringa content was more than 15%. A similar experiment was performed where the effects of replacing wheat flour with 0–15% debittered moringa seed flour on wheat flour and the chemical properties of bread was studied.[15] Other ingredients (sugar, shortening, salt, and baking powder) were added to the formulated ratio to make dough for biscuits, which were baked at a temperature of 200°C, and later were packed airtight for analytical works based on AACC with slight modification to the original procedure with reference to Srivastava et al.[18]
Proximate and nutrient analysis

Raw materials and biscuit samples were analyzed in triplicate for moisture content, crude fats, crude protein, crude fiber, total ash, calcium, and iron. Standard AOAC method AOAC (935.29) was used to determine moisture content. Crude proteins were analyzed based on AOAC (923.03). Further, crude fats, total ash, and crude fiber were analyzed corresponding to AOAC (992.23), AOAC (923.03), and AOAC (962.09) respectively. Calcium content was determined using the method described by Ranganna titrimetrically from the prepared ash solution. Iron was determined colorimetrically at the absorption of 480 nm from ash solution as described by Ranganna.

Total polyphenol content

Determination of total phenol was carried out with the Folin-Ciocalteu reagent (FCR) method. In the FCR, phenols react with phosphomolybdic acid in an alkaline medium to produce a blue-colored complex (molybdenum blue). For this, 0.5 ml of extract and 1 ml of FCR was mixed and incubated for 15 minutes at room temperature, followed by the addition of 2.5 ml saturated sodium carbonate followed by 30 minutes incubation at room temperature, then the absorbance was read at 760 nm.

Total flavonoids content

Total flavonoid content was determined using the aluminum chloride assay method with slight modification. Ten g powdered sample in 100 ml 80% methanol was left overnight for extraction. The extract was filtered and heated to 45°C on a hot plate. 0.2 ml of 5% NaNO₂ was added to 2 ml of extracted sample and left for 6 minutes. 0.2 ml of 10% AlCl₃ was added and stood for another 6 min. Finally, 2 ml of 1 N NaOH was added, and volume was made up to 5 ml with 80% methanol, stand for 15 min and absorbance was measured at 510 nm. Approximately 80% methanol was used instead of sample for blank. For the standard curve, 2 ml of different concentrations (20%, 40%, 60%, 80%, and 100%) of quercetin in 80% methanol was prepared and absorbance was measured at 510 nm.

Antioxidant activity (DPPH inhibition)

Antioxidant activity of the extracts of samples and products was determined using the method described by Hawa et al. with slight variation. Using 80% methanol, samples of different concentrations were made. 0.1 mM DPPH solution was mixed with 1 ml of the prepared extract and left in dark for 30 minutes. Then, the absorbance was measured at 517 nm. Blank samples were prepared with methanol and DPPH. The % inhibition was expressed in percentage using the equation:

\[
\% \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%
\]

The 50% inhibitory concentration (IC₅₀) was expressed as the quantity of extracts to react with half of DPPH.

Tannin content

The tannin content in the samples was determined by the Folin–Ciocalteu method. In brief, 0.1 mL of methanolic extract was added to the volumetric flask that contained 7.5 mL distilled water, 0.5 mL Folin-Ciocalteu reagent, and 1 mL 35% Na₂CO₃ solution. Then, the volume of the mixture solution was made up to 10 mL with distilled water and shaken well. After holding for 30 min at room temperature, the absorbance was measured at 725 nm on UV/visible. The test results were correlated with the standard gallic acid curve, and tannin content was expressed as mg Gallic acid equivalent (mg GAE/g) of dry extract.
Sensory analysis

An assessment of sensory parameters including appearance, color, smell, taste, hardness, crunchiness, and overall acceptability was performed by panelists using a 9-point Hedonic rating scale. Thirty healthy panelists were trained for 7 days (2 hours daily) to familiarize panel members with sensory attributes. The panelists were then provided with uniform quantity (1 piece) of biscuit in stainless steel plate and evaluation of appearance, color, aroma, taste, hardness, crunchiness and overall acceptability was done usually at 1:00–2:30 pm.

Statistical analysis

The triplicate data of proximate composition and nutritional analysis were analyzed by one-way analysis of variance (ANOVA) and this was carried out by using software GenStat Release 12.1 (Copyright 2009, VSN International Ltd.). In case of significant difference, Tukey’s HSD post hoc test was used to separate the means at a 5% level of significance. R-studio was used for the figures.

Results and discussion

Chemical analysis of raw materials

The chemical analysis of raw materials is presented in (Table 1). The moisture, crude protein, crude fat, crude fiber, total ash, and carbohydrate content of the wheat flour used in biscuit making was found to be 11.64 ± 0.76%, 10.15 ± 0.36%, 1.32 ± 0.32%, 0.54 ± 0.06%, 0.73 ± 0.04% and 87.26 ± 0.25%, respectively, which were similar to findings of Ocheme et al. The results also indicated that the protein content of flowers (17.1 ± 0.74%), the fiber content of leaves (10.50 ± 0.43%), and ash content of leaves (11.50 ± 0.43%) were like those published by Hasaballa et al. while the fiber content of flower (10.55 ± 1.13%) and protein contents of leaves (31.82 ± 0.56%) were higher than his results. Furthermore, the iron (56.37 ± 4.32%) and calcium (1016.15 ± 7.63 mg/100 g) contents of the flower were like those reported by Hasaballa et al. However, they were found to be higher than those found by Dhakar et al. Additionally, the Calcium content of the leaf (1283.49 ± 12.45 mg/100 g) was similar but iron (15.56 ± 3.67 mg/100 g) content was lower, which corresponds to the result obtained by Valdez-Solana et al. The TPC, TFC, and tannin content of the flower were relatively high, but the antioxidant activity was lower.

Values are means of triplicate ± standard deviation.

| Particulars                   | Moringa flower powder | Moringa leaf powder |
|-------------------------------|-----------------------|---------------------|
| Moisture (%)                  | 4.37 ± 0.09           | 6.1 ± 0.23          |
| Crude protein (%)             | 17.1 ± 0.74           | 31.82 ± 0.56        |
| Crude fat (%)                 | 11.00 ± 0.03          | 2.57 ± 0.6          |
| Crude fiber (%)               | 10.55 ± 1.13          | 10.30 ± 0.96        |
| Ash content (%)               | 6.79 ± 0.67           | 11.50 ± 0.43        |
| Carbohydrate (%)              | 57.72 ± 0.97          | 38 ± 1              |
| Iron (mg/100 gdb)             | 56.37 ± 4.32          | 16 ± 4              |
| Calcium (mg/100 gdb)          | 1016 ± 8              | 1283 ± 12           |
| TPC (mg GAE/100 g dry extract)| 10425 ± 88            | 7513 ± 71           |
| TFC (mg QE/100 g dry extract) | 3320 ± 22             | 2908 ± 25           |
| AA (DPPH)                     | 51 ± 3                | 26 ± 2              |
| Tannin (mg/100 g dry extract) | 2379 ± 25             | 934 ± 20            |
| Energy value (kcal/100 g)     | 380.8                 | 282.8               |
Chemical analysis of biscuit

The analysis of chemical characteristics of supplemented biscuits is presented in (Table 2). The ANOVA indicated that herbal biscuits were found to have a low moisture content when compared to control biscuits, ranging from 2.84% to 3.34%, similar to the result obtained by Sengev et al.\cite{26} Low moisture content of supplemented biscuit was due to the low water absorptivity\cite{27} and low moisture content of Moringa leaf and flower powder.

The crude protein content is more in samples A and B and lesser in samples C, D, and E, although this behavior is nonlinear, since the leaf has higher protein content than flower, and from samples A to E, the proportion of the leaf decreases. These results correlate with the findings of Alam et al.\cite{28}

The fat content in the supplemented biscuit was found to be higher than that of the control biscuit, possibly due to the higher fat content of flowers. Similar type of data was obtained by Devi et al.\cite{29,30} Since both flowers and leaves are great sources of fiber,\cite{11,26} the dietary fiber content increased compared to control. Even a slight change in composition brought varied results in terms of total ash content on a dry basis. The results indicate that MFP and MLP supplemented biscuits are richer in mineral profile than control with a significant increase in iron (from 0.47% in control up to 2.62–3.35%) and calcium (from 26.25% in control up to 98.7–115%) in herbal biscuits. Danchana et al.\cite{23} have also reported improvement of mineral fiber protein in biscuits supplemented by green leafy vegetables.

TPC content was the highest in sample E (72.60 mg GAE/100 g dry extract) due to the highest flower content, while flavonoid content ranged from 308.08 to 347.04 mg/100 g quercetin equivalent in herbal biscuits. Patel and Pandey\cite{31} reported that increased levels of wheat flour decreased the antioxidative activity of biscuits, but increased levels of antioxidant-rich components increased antioxidative activity significantly. This study also found that wheat biscuits have the least antioxidant activity, having the highest IC\textsubscript{50} values of 1435.19, compared to other herbal biscuits, which ranged from 116.7 to 99.68. Further, Thorat et al.\cite{27} indicated that baking does not affect the level of TPCs or antioxidants. Therefore, herbal biscuits were found to be higher in antioxidant activity than control biscuits because of the flower and leaf powders that contributed to their antioxidant properties. An increase in tannin content was observed in all herbal biscuits; however biscuits with a higher proportion of Moringa flower had a comparatively lower tannin content. Samuel et al.\cite{32} also found an increase in tannin content in cookies as the amount of okra, a tannin-rich component increased in cookies. The randomized pattern for the energy yield is due to variations in the fat, carbohydrate, and protein content in these biscuits of varying compositions of raw materials used. Data obtained for energy value calculated for his herbal biscuits of varying compositions of Moringa and Tulshi was also irregular\cite{28}.

Sensory analysis of biscuits

Statistical analysis was done on the sensory score obtained from 25 panelists using a 9-point hedonic rating test (9 = like extremely, 1 = dislike extremely). Biscuits were evaluated based on appearance, color, aroma, taste, hardness, crunchiness, and overall acceptability, and the mean sensory score for each biscuit samples by the panelist is presented in (Figure 1).

The statistical analysis shows that there is almost no significant difference (p < .05) in terms of appearance among the prepared samples that can be seen clearly from (Figure 1). Control biscuits got the highest score probably because panelists were habituated to plain biscuits. The score gradually increased from A to E because the darker greener appearance of biscuits might have played an important role in appearance because of the percentage of leaves incorporated in them. However, sample E got a lesser score because the biscuits were less puffed and looked more like crisps with uneven surfaces. In addition, the baking condition always has a significant role in appearance along with the ingredients. The replacement of whole-wheat flour with increasing levels of MLP and MFP
Table 2. Chemical characteristics of supplemented biscuits.

| Parameters                  | Control | A           | B           | C           | D           | E           |
|-----------------------------|---------|-------------|-------------|-------------|-------------|-------------|
| Moisture (%)                | 3.86b ± 0.54 | 2.84b ± 0.65 | 2.96b ± 0.97 | 2.99b ± 0.72 | 2.90b ± 0.64 | 3.34b ± 0.47 |
| Crude protein (%)           | 3.491a ± 0.81 | 3.908a ± 0.38 | 4.103a ± 0.92 | 3.851a ± 0.69 | 3.722a ± 0.46 | 3.928a ± 0.94 |
| Crude fat (%)               | 19.999a ± 0.34 | 22.10c ± 0.64 | 22.22c ± 0.28 | 20.78b ± 0.23 | 20.60b ± 0.09 | 21.61a ± 0.24 |
| Crude fiber (%)             | 0.744a ± 0.38 | 1.757a ± 0.08 | 1.66c ± 0.34  | 0.99a ± 0.17  | 1.44c ± 0.40  | 1.26bc ± 0.34 |
| Ash (%)                     | 2.395a ± 0.13 | 3.60a ± 0.05  | 3.42a ± 0.14  | 3.23a ± 0.07  | 3.24c ± 0.08  | 2.56a ± 0.04  |
| Carbohydrate (%)            | 73.38c ± 0.43 | 68.64a ± 0.76 | 68.61a ± 0.64 | 71.14a ± 0.84 | 70.98b ± 0.37 | 70.64b ± 0.83 |
| Iron (mg/100 g, db)         | 0.476a ± 0.01 | 3.35b ± 0.01  | 3.06b ± 0.02  | 2.97d ± 0.003 | 3.21d ± 0.005 | 2.62a ± 0.02  |
| Calcium (mg/100 g, db)      | 26.25b ± 0.06 | 115.73 ± 0.69 | 108.75a ± 0.16 | 104.06c ± 0.35 | 98.7e ± 0.17  | 99.37b ± 0.08  |
| TPC (mg GAE/100 g dry extract) | 72.60b ± 4.33 | 489.75b ± 6.42 | 478.53b ± 5.53 | 520.29b ± 8.47 | 544.93b ± 15.29 | 579.58b ± 13.65 |
| TFC (mg QE/100 g dry extract) | 45.53b ± 12.56 | 308.08b ± 21.82 | 314.28b ± 6.53 | 327.46bc ± 7.43 | 347.04bc ± 10.12 | 334.05bc ± 12.34 |
| AA (IC50)                   | 1435.19b ± 55.12 | 100.97b ± 4.23 | 99.68b ± 3.64  | 114.92b ± 3.45 | 116.01b ± 4.01 | 116.70b ± 5.29 |
| Tannin (mg/100 g, dry extract) | 0.68b ± 0.25 | 96.71b ± 2.18  | 90.78b ± 3.5   | 86.63b ± 3.87  | 47.27b ± 1.12  | 50.67b ± 2.31  |
| Energy (Kcal/100 g)         | 474.92 | 475.15 | 475.28 | 472.44 | 470.21 | 476.33 |

Note: Values are means of triplicate ± standard deviation. Values in the row bearing different superscripts are significantly different (P < 0.05). The flower and leaf powder ratio 11.4, 11.75:3.25, 12.5:2.5, 13.25:1.75, and 14:1, represented A, B, C, D, and E respectively.
might have decreased the spread ratio, preventing light, airy texture making biscuits more like crisps which must be attributed to a lower score for appearance to all formulated biscuits that caused dilution of gluten and less water available for gluten hydration.\textsuperscript{[12,33,34]}  

According to Yang et al.\textsuperscript{[9]} flower powder is more suitable than leaves for food additives as it does not affect food color and appearance. This might be the reason for the higher score for color for sample E gradually reducing toward sample A as these biscuits had a higher percentage of flower and lesser percentage of the leaf. Because the chlorophyll content of leaves that masked the yellowness of biscuit colors was unusual to the panelist, it was attributed to the highest score for the color of control biscuit. The peculiarity of color for panelists played a significant role in lower scores to formulated biscuits. All \textit{Moringa}-supplemented samples were noticeably green, even at 1% incorporation in bread.\textsuperscript{[26]}  

Sample A got the lowest score in aroma, which might be due to the grassy or earthy smell due to the higher percentage of leaf powder. Even though the aroma was improved to an extent by increasing the percentage of flower content the grassy smell was not completely unnoticeable in other biscuits as well thus sample D and E were liked mostly with no significant difference between these two samples as per statistical result. The flower contains several components that impart flavor and aroma as reported by Yang et al.\textsuperscript{[9]} that might have masked undesirable aroma making panelists prefer biscuits with a higher percentage of flowers.

A slight sensation of grassy taste was observed in all the treatments, similar to the findings of Sengev et al.\textsuperscript{[26]} who found grassy taste by incorporating leaf powder as low as 1%. The increasing pattern of the score on taste might be due to a reduction in leaf percentage that had contributed to a grassy/bitter taste. (Figure 1) shows that sample D and sample E were liked by panelists and did not have much significant difference between them in terms of taste. The presence of saponins in \textit{Moringa} leaf might have contributed to the bitter taste in biscuits with a higher leaves percentage in them.\textsuperscript{[35]}  

Sample E was found hardest and undesirable by the panelists. Statistical analysis shows no significant difference between samples A, B, C, and D. Sample E had the highest percentage of flowers in it thus higher fiber content. Breannan and Samyue\textsuperscript{[36]} found the reduction of firmness upon evaluating textural characteristics of biscuits enriched with dietary fibers. Belitz et al.\textsuperscript{[33]} state that gluten can form a thick, cohesive, elastic mass that when placed in the oven puffs up too many times its original volume and sets a light, airy texture. Substitution of flour by \textit{Moringa} flower and leaf powder that prevented gluten development and thus puffing which might have caused undesirable hardness for newly formulated biscuits. Therefore, the control biscuit had a higher score for hardness as it was firmer and more desirable to panelists.

The crunchiness was least affected by the incorporation of herbal powder. Control biscuit with a mean score of 8.4 was the best-liked biscuit in terms of crunchiness while biscuit B was most unlikely in terms of crunchiness. A and C are different from D and E had scores of 7.2, 7.2, and 7.9 and 7.8 respectively. A similar reduction in crunchiness was seen in biscuits made with unripe banana flour that might be due to baking conditions, the quantity of an ingredient, and the protein content of flour.\textsuperscript{[37]}  

Sample D was considered the best by the score given by the panelist (7.7). Control biscuit got a higher score than sample D (8). Sample E had some similarities with sample D (7.2) in terms of overall acceptance. Sample A was disliked by most as it got the lowest score of 6.6. Samples B and C got the intermediate score. These samples had similarities in acceptance when compared to sample E but sample D has some sort of different features than other samples in terms of acceptance by panelists. Acceptability of biscuits seems to increase with the increase in flower powder and with the reduction in leaf powder; however, sample D is highly accepted than sample E because sample E had undesired hardness and poor appearance. Although every formulated biscuit got lower ranks in terms of overall acceptability, the acceptance of biscuits can be further increased if consumers are made aware of the beneficial health attributes and nutritional information of the herbal biscuits.\textsuperscript{[38,39]}
Figure 1. The graph represents the score for the Moringa biscuit against the different parameters tested. The top graph shows the overall acceptability for the samples tested. A, B, C, D, and E represent sample A, sample B, sample C, sample D, and sample E respectively.
Physical parameters of biscuits

Physical parameters of biscuits such as weight, thickness, diameter, and spread ratio were substantially affected using composite flour with flower and leaf powder than wheat flour. The different values obtained from the analysis are presented in (Table 3). Weight of biscuits increased in prepared herbal biscuits which might be due to an increase in protein content in the formulated biscuit. Similar results were obtained by Adeola and Ohizua,\cite{37} incorporating pigeon pea flour. Spread ratio or diameter is used to determine the quality of flour used in preparing biscuits and the ability of the biscuit to rise.\cite{40} A higher spread ratio of biscuits is more desirable, so flour blends of biscuits A, D, and E are more desirable in terms of spread ratio. The replacement of whole-wheat flour with an increasing level of MLP might have decreased the spread ratio in samples B, C which must be attributed to dilution of gluten and less water available for gluten hydration according to Sharma et al.\cite{34} and Ajibola et al.\cite{12}

Correlation plot for different chemical parameters

The strong positive correlations between TPC, TFC calcium and iron content was revealed while antioxidant activity (IC50) was negatively correlated with TPC, TFC tannin, calcium content and iron content (Figure 2) signifying positive correlation with higher value of DPPH .\cite{23} The IC50 value is inversely proportional to the free radical scavenging activity/antioxidant property of the sample. Although calcium alone does not act as an antioxidant, but it takes part in forming antioxidants.\cite{41}

Table 3. Physical parameters of biscuits.

| Samples | Weight (g) | Thickness (mm) | Diameter (mm) | Spread ratio |
|---------|------------|----------------|---------------|--------------|
| Control | 10.4 ± 0.45| 9.26 ± 0.20    | 61.67 ± 0.57  | 6.65 ± 0.20  |
| A       | 12.7 ± 1.24| 8.06 ± 0.31    | 60.63 ± 2.08  | 7.52 ± 0.25  |
| B       | 13.3 ± 1.38| 9.50 ± 0.10    | 61.54 ± 0.57  | 6.45 ± 0.03  |
| C       | 13.2 ± 1.78| 9.27 ± 0.15    | 60.42 ± 1.52  | 6.54 ± 0.26  |
| D       | 12.6 ± 1.55| 8.76 ± 0.35    | 61.67 ± 1.53  | 7.04 ± 0.29  |
| E       | 13.4 ± 1.27| 8.66 ± 0.15    | 61.33 ± 0.58  | 7.07 ± 0.08  |

Note: Values are means of triplicate ± standard deviation. Values in the row bearing different superscripts are significantly different (P < 0.05)

Figure 2. Correlation plot between TFC, TPC, IC50, Tannin and calcium and iron content.
On the paradoxical side, fruits and leafy green vegetables contain non-heme iron that is a good source of antioxidants that will help in slowing the adverse effect of iron on human health.\textsuperscript{[42]} Phenolic and flavonoid molecules are important antioxidant components that are responsible for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals. They also have ideal structural characteristics for free radical scavenging. Different literature reports indicate a linear correlation of total phenolic and flavonoid content with antioxidant capacity. By comparing the correlation coefficients (Figure 2), it is possible to suggest that phenolic and flavonoid groups are highly responsible for the antioxidant activity of the selected samples.\textsuperscript{[43]}

**Principal component analysis (PCA)**

PCA was done to select the optimum sample after treatment of the different proportion of the moringa leaf and flower powder where the first principal component (Dim.1) was responsible for 92.8% variation with eigen value of 5.56, while second principal component (Dim.2) was reported to 6.2% variation with eigen value of 0.37 these two components together account for 98.99% variations.

The distribution of different biscuit samples was influenced by TFC, TPC, IC50, Tannin, and calcium and iron content. Only the control sample was in the left-hand side (Figure 3) while other samples of biscuit A, B, C, D and E were on the right side. Among these samples, the samples coded as A B and C were positioned on the first quadrant (Figure 3) which illustrates they were superior to other samples regarding the chemical properties.

**Conclusion**

*Moringa oleifera* flower and leaf can be used to prepare herbal biscuits utilizing the plant’s beneficial properties. In conclusion, this study demonstrated that the preparation of herbal biscuits using local plants could be a noble and innovative step toward a healthy and nutritious lifestyle with an enhanced chemical composition that is easy to manufacture and distribute. Based on sensory results, biscuit composition can be altered to improve the acceptance of each comparable parameter. As of now, panelists prefer control biscuits to all other formulated biscuits. Acceptance of biscuits can be further...
increased if consumers are made aware of the beneficial health attributes and are habituated to these sorts of biscuits. Rooms for further improvement are further possible though.

Acknowledgments

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2022R62), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2022R62), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia

ORCID

Yadav KC http://orcid.org/0000-0003-0203-6667
Lila Devi Shiwakoti http://orcid.org/0000-0002-7655-2173

References

[1] Bello, F. A.; Akpan, M. E.; Sodipo, M. A. Physicochemical and Sensory Properties of Cookies Produced from Wheat, Unripe Plantain, and Germinated Fluted Pumpkin Seed Composite Flour. *Food Sci. Qual. Manag.* 2020, 96, 36–43.

[2] Kwak, N. S.; Jukes, D. J. Functional Foods. Part 2: The Impact on Current Regulatory Terminology. *Food Control*. 2001, 12(2), 109–117. DOI: 10.1016/S0956-7135(00)00029-3.

[3] Zhou, W., and Hui, Y. H. *Bakery Products Science and Technology*, Second ed.; UK: John Wiley & Sons, Ltd, 2014.

[4] Robert, E. C., and Robert, W. *Handbook of Nutraceuticals and Functional Foods*, Second Ed ed.; New York: Taylor and Francis, 2006.

[5] Dhakar, R. C.; Maurya, S. D.; Pooniya, B. K.; Bairwa, G.; Sanwarmal, N. M. *Moringa*: The Herbal Gold to Combat Malnutrition. *Chron. Of Y Sci*. 2020, 2(3), 119–125. DOI: 10.4103/2229-5186.90887.

[6] Anwar, F.; Latif, S.; Ashraf, M.; Gilani, A. H. *Moringa Oleifera*: A Food Plant with Multiple Medicinal Uses. *Phytotherapy Research*. 2007, 21(1), 17–25. DOI: 10.1002/ptr.2023.

[7] Claufton, S. M.; Pearce, R. J. Protein Enrichment of Sugar-snap Cookies with Sunflower Protein Isolate. *Journal of Food Science*. 1989, 54(2), 354–356. DOI: 10.1111/j.1365-2621.1989.tb03079.x.

[8] Kalappurayll, T. M.; Joseph, B. P. A Review of Pharmacognostical Studies on *Moringa Oleifera* Lam. Flowers. *Pharmacognosy Journal*. 2017, 9(1), 1–7. DOI: 10.5530/pj.2017.1.1.

[9] Yang, L.; Wang, X.; Wei, X.; Gao, Z.; Han, J. Values, Properties and Utility of Different Parts of *Moringa Oleifera*: An Overview. *Chin. Herb. Med.* 2018, 10(4), 371–378. DOI: 10.1016/j.chemed.2018.09.002.

[10] Qi, N.; Feng, X.; Wang, X.; Xu, Y.; Lin, L.; Lin, L. Simultaneous Analysis of Eight Vitamin E Isomers in *Moringa Oleifera* Lam. Leaves by Ultra Performance Convergence Chromatography. *F Chem*. 2016, 207, 157–161. DOI: 10.1016/j.foodchem.2016.03.089.

[11] Hasaballa, M. A.; Elsohaimy, S. A.; Shaltout, O. E.; Zeitoun, M. A. M. Chemical Composition and Bioactive Compounds of Leaves, Flowers and Seeds of Moringa Plant. *J. Adv. Agri. Res.* 2017, 22, 662–674.

[12] Ajibola, C. F.; Oyirinde, V. O.; Adeniyi, O. S. Physicochemical and Antioxidant Properties of Whole-wheat Biscuits Incorporated with *Moringa Oleifera* Leaves and Cocoa Powder. *J. Sci. Res. Rep.* 2015, 7, 195–206.

[13] Firdausy, H. M.; Widodo, R.; Panjaitan, T. W. S. D. D. Texture and Organoleptic of Steamed Bread Addition with Moringa Leaf Flour. *FOOD SCI TECH*. 2020, 3(1), 11. DOI: 10.25139/ft.v0i0.2640.

[14] Bourkeoua, H.; Różyło, R.; Gawlik-Dziki, U.; Benatallah, L.; Zidoune, M. N.; Dziki, D. Evaluation of Physical, Sensorial, and Antioxidant Properties of Gluten-free Bread Enriched with Moringa Oleifera Leaf Powder. *Eur. Food Res. Technol.* 2018, 244(2), 189–195. DOI: 10.1007/s00217-017-2942-y.

[15] Ogunsina, B. S.; Radha, C.; Indrani, D. Quality Characteristics of Bread and Cookies Enriched with Debittered *Moringa Oleifera* Seed Flour. *Int. J. Food Sci. Nutr.* 2011, 62(2), 185–194. DOI: 10.3109/09637486.2010.526928.
[41] Ben Amor, N.; Megdiche, W.; Jiménez, A.; Sevilla, F.; Abdelly, C. The Effect of Calcium on the Antioxidant Systems in the Halophyte Cakile Maritima under Salt Stress. *Acta Physiol. Plant.* 2010, 32(3), 453–461. DOI: 10.1007/s11738-009-0420-2.

[42] Romeu, M.; Aranda, N.; Giralt, M.; Ribot, B.; Nogues, M. R.; Arija, V. Avocado Consumption Is Associated with Better Diet Quality and Nutrient Intake, and Lower Metabolic Syndrome Risk in US Adults: Results from the National Health and Nutrition Examination Survey (NHANES) 2001–2008. *Nutrition Journal.* 2013, 12(1), 1–9. DOI: 10.1186/1475-2891-12-1.

[43] Afonso, P.; Correa, P.; Pinto, F.; Sampaio, C. Shrinkage Evaluation of Five Different Varieties of Coffee Berries during the Drying Process. *Biosyst. Eng.* 2003, 86(4), 481–485. DOI: 10.1016/j.biosystemseng.2003.08.012.