Indian Journal of Chemical Technology
Vol. 32, July 2025, pp. 433-440
DOI: 10.56042/ijct.v32i4.17816

Spiced moringa mix: Physio-chemical characterization and quantitative analysis

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Received 11 March 2025; accepted 11 July 2025

Novel Moringa Spice mix, comprising Moringa leaves and spices, with a focus on its biochemical, physical, and sensory attributes with substantial levels of key bioactive compounds, including phenolics (7.05 mg/mL), flavonoids (11.78 mg/mL), carotenoids (15.92 mg/100g), chlorophylls (6.65 µg/g), ascorbic acid (31.7 mg/100g), accompanied by 59.68% inhibition of antioxidant activity indicated potential health-promoting effects. Analyses provided insights into the proximate composition percentage (moisture: 1.43, ash: 14.28, fiber: 18.59, protein: 26.88, fat: 17.36, carbohydrate: 21.46), colour attributes (L*= 38.95, a*= -0.99, b*= 27.44), and aroma profile (E-Nose) of the Moringa spice mix. HPLC analysis identified specific polyphenolic compounds contributing to its bioactivity. Sensory evaluation affirmed the overall acceptability of the Moringa spice mix, with commendable ratings for taste, aroma, texture, and appearance, underscoring its potential as a palatable and nutritious dietary addition. This underpins the multipurpose potential of the Moringa spice mix as a functional food/beverage product.

Keywords: *Moringa,* Bioactivity, Health Benefits, Sensory, Spice mix

Introduction

Moringa oleifera, known as the "miracle plant" or "tree of life," is native to regions like India, Pakistan, western Asia, sub-Himalayan areas, Africa, and Arabia, and has spread to the Philippines, Cambodia, Central America, and the Caribbean. This plant is renowned for its medicinal, nutritional, and industrial benefits[1]. Moringa is rich in macronutrients, micronutrients, and phytosterols necessary for hormone synthesis, with leaves that are nutrient-dense, containing proteins, carbohydrates, fibers, vitamins (A, B, C, D, and E), minerals (iron, calcium, zinc, potassium, magnesium, and copper), and phytosterols[2]. It surpasses many foods in vitamin C, vitamin A, calcium, potassium, and iron content[3]. Moringa leaf powder (MLP) effectively addresses micronutrient deficiencies, improves food intake, weight gain, and nutrient concentrations, particularly benefiting iron deficiency[2]. Moringa leaf extracts also provide antioxidant, anti-cancer, anti-inflammatory, antidiabetic, and antimicrobial benefits. The potential benefits of various Moringa preparations for disease treatment or prevention are not widely recognized, despite substantial oral history supporting these uses[4].

Cumin, prized for its aromatic flavour, contains bioactive compounds like cuminaldehyde and cuminic alcohol with potential health benefits. Coriander adds a citrusy sweetness and harbors constituents like linalool, geranyl acetate and geraniol[5], known for their antioxidant and antimicrobial properties[6,7]. Turmeric, renowned for its colour and flavour, is rich in curcuminoids, notably curcumin, celebrated for its antioxidant and anti-inflammatory effects[8]. Chili peppers bring heat due to capsaicinoids like capsaicin, which may boost metabolism and possess analgesic properties[9]. Black pepper contains piperine, enhancing nutrient bioavailability and displaying antioxidant effects[10]. Asafoetida, prized for its pungent aroma, contains ferulic acid and umbelliferone, potentially offering antimicrobial and digestive benefits[11]. Ginger, known for its pungent flavour, contains bioactive compounds like gingerol and shogaol, with various pharmacological properties including anti-inflammatory and antioxidative effects[12].

Food fortification enhances the nutritional value of staple foods, improving population health at a reasonable cost, with methods categorized by Allen et al.[13] as mass, targeted, and market-driven fortification. Incorporating *Moringa oleifera* into staple foods, particularly in Africa, aligns with the goal of improving overall nutrition by enhancing these foods' nutritional content.

The development of a Moringa spice mix combines the nutritional benefits of Moringa leaves with the convenience of a spice blend, enhancing dishes with essential nutrients and antioxidants. This study compares the nutraceutical properties and potential health benefits of the Moringa spice mix with raw Moringa leaves, examining how processing and formulation affect nutrient retention. The Moringa spice mix offers an easy way to incorporate Moringa's nutritional benefits into daily diets. The study explores the nutritional composition, antioxidant activity, and sensory attributes of the Moringa spice mix, aiming to highlight its contributions to nutrition, health, and culinary arts. The following sections outline the methodology for analyzing the Moringa spice mix, present the results, discuss their implications, and provide recommendations for future research and practical applications.

Experimental Section

Preparation of Moringa Spice Mix

Moringa leaves were washed, excess moisture removed, and then dried in a hot air oven at 70°C for 3.5 h. The dried leaves (60 g) are ground into granules in an electric mixer. Cumin powder (10 g), coriander powder (7 g), chili powder (7 g), black pepper powder (5 g), turmeric powder (4 g), ginger powder (4 g), salt (2.5 g), and hing powder (0.5 g) are added and mixed briefly for uniformity. The resulting mixture is stored in an airtight container or zip-lock bag.

Biochemical Tests

Total Phenolic Content Estimation (TPC)

Sample and control extracts were prepared by grinding Moringa Spice mix and Dry Moringa Leaves with methanol (1:10 g/mL) and filtering. A 10% (w/v) extract was mixed with 5% Folin-Ciocalteu Reagent and incubated for 5 min. Next, 15% (v/v) of a 20% (v/v) Na_2CO_3 solution was added, with distilled water used to reach the final volume[14]. Absorbance at 765 nm was measured using a Shimadzu UV-1800 spectrophotometer, determining polyphenol content using Gallic acid as a standard.

Total Flavonoid Content Estimation (TFC)

Sample and control extracts were prepared by grinding Moringa Spice mix and Dry Moringa Leaves with methanol (1:10 g/mL) and filtering. A 10% extract was mixed with $NaNO_2$ and $AlCl_3$, incubated for 5 min, then NaOH was added and diluted with water[14]. This was repeated for the control extract. Absorbance at 510 nm was measured using a Shimadzu UV-1800 Spectrophotometer. Total flavonoids were calculated using catechin as a standard.

Antioxidant assay or total free radical scavenging

Sample and control extracts were prepared by grinding Moringa Spice mix and Dry Moringa Leaves with methanol (1:10 g/mL) and filtering. 1 mL extract was added to 4 mL of DPPH solution and incubated for 20 min at room temperature in the dark[14]. Absorbance at 517 nm was measured using a Shimadzu UV-1800 Spectrophotometer, and scavenging activity was calculated.

$$\% \text{ Inhibition} = (1 - A_{sample}/A_{standard}) \times 100$$

Where, $A_{standard}$ = Absorbance of the standard and A_{sample} = Absorbance of the sample.

Estimation of carotenoids

Two grams of Moringa spice mix, 3 g of Na_2SO_4, and 0.5 g of α-Tocopherol (2 mM) were ground with ice-cold acetone (250-500 mL) until colourless. After desolvation at 60 ± 5°C, the residue was dissolved in methanol. A 10% extract was prepared by diluting with methanol[14]. This process was repeated for dry Moringa leaves, with absorbance measured at 450 nm for carotenoid calculation using a Shimadzu UV-1800 spectrophotometer.

$$\text{Carotenoids (µg per g)} = A \times V\,(mL) \times 10/E \times P$$

Where, A = Absorbance (450 nm), V = Total extract volume, p = Sample weight, E = Ex-coefficient of Methanol

Estimation of Ascorbic Acid

A dye solution was prepared by dissolving 50 mg of Dichlorophenol Indophenol in 150 mL of warm distilled water with 42 mg of $NaHCO_3$. After cooling and diluting to 200 mL, it was used to fill the burette. Then, 2 g of Moringa spice mix was ground with 25 mL of 3% HPO_3 solution. From this extract, 10 mL was titrated with the dye solution until the pink end-point appeared[14]. The same process was repeated for the dry Moringa leaves.

$$Ascorbic\ acid\ (mg\ per\ 100g\ or\ mL) = \left(\frac{Titre\ value\ \times vol.made\ up\ to}{Aliquot\ of\ extract\ taken\ for\ estimation \times wt.of\ sample\ for\ estmation} \right) \times 100$$

Dye factor was found by titration of 5 mL Ascorbic acid solution (1:1 mg/mL with 3% HPO_3 solution) with 5 mL of 3% HPO_3 solution.

$$\frac{Dye\ factor}{Titre\ value} = 0.5$$

Chlorophyll estimation

3 g of Moringa spice mix, with 2 g of $CaCO_3$ and 2 g of quartz sand, were ground in 85% acetone solution (250 mL) until colourless. Then, 25 mL of this extract was mixed with 25 mL of ethyl ether in a separating funnel, washed with 10 mL of distilled water (5-6 times) until the acetone smell evaporated. Absorbance was measured at 660 nm and 642.5 nm using a Shimadzu UV-1800 Spectrophotometer for Chlorophyll calculation, following a similar process for dry Moringa leaves.

Total Chlorophyll = [17.12 x Abs (660nm)] + [16.8 x Abs(642.5nm)]

Chlorophyll A = [9.93 x Abs (660nm)] - [0.777 x Abs(642.5nm)]

Chlorophyll B = [17.6 x Abs (642.5nm)] - [2.81 x Abs(660nm)]

Determination of polyphenols by high-performance liquid chromatography (HPLC)

1 g of dry Moringa leaves was placed in a 10 mL brown measuring flask and mixed with methanol. After ultrasonication for 40 min at 20°C, the solutions were filtered and analyzed by HPLC using a C8 column. Mobile phases A (acetonitrile) and B (acetic acid and Milli-Q water) were used for elution, with 20µl of each sample injected and a run time of 22 min per sample. Peaks were compared with standards, prepared in methanol, for the analysis of 9 polyphenols[15].

Physical Tests

Proximate analysis

The AOAC (1990) methods[16] were used for duplicate analysis of moisture, protein, crude fiber, fat, carbohydrate, and ash content in percentages. Moisture content was determined by oven-drying samples at 105°C, ash content by heating at 550°C, protein content by nitrogen values using Protein analyzer with a conversion factor of 6.25, crude fiber content by digestion method, and fat content by soxhlet extraction method.

Colour measurement

The Moringa spice mix and dry Moringa leaves were placed in cuvettes and enclosed in HunterLab colorimeters. These devices emit light onto the samples, measure the reflected light, and convert it into colour information using mathematical models, providing objective color measurements. The readings were obtained using a Konica Minolta CM-5 Spectrophotometer.

Electronic Nose (E-Nose)

For the electronic nose experiment, 3 g of Moringa spice mix and dry Moringa leaves were sealed in 20 mL vials with PTFE/silicone caps. Gas from the headspace was analyzed using an autosampler (Odor Scanner HS 100, Gerstel, Germany). After shaking at 500 rpm and 60°C for 20 min, the gas sample was injected into the GC injector. Analysis was performed using a GC electronic nose, Alpha MOS Hercules Neo, with two FIDs and Alpha Soft software, employing C6-C16 alkanes for Kovat's indices and compound identification, supported by the AroChemBase library within Alpha Soft.

Sensory evaluation

Sensory evaluation involves systematically evaluating taste, colour, texture and flavour with a panel with their individual consent. The ethical permission was not required by the institution. Samples were evaluated sequentially, starting with appearance, followed by aroma, and then taste. The feedback was collected to know and have a detailed characterization of the product.

Results and Discussion

Biochemical Tests

Total phenolic content (TPC)

The total phenolic content was determined using the Gallic acid standard curve[14], with the equation y = 0.001x + 0.0342 and R2 = 0.9952 guiding calculations and plotting. The results are shown in Table 1. Dry Moringa Leaves exhibited a total phenolic content of 1.344 mg/mL, while Moringa spice mix showed 7.048 (±0.43) mg/mL. Boonyadist et al. (2013)[17], reported a slightly higher total phenolic content of 2.61 (±0.35) mg/ml for dry Moringa leaves, indicating potential varietal

Table 1 — Polyphenols profile in extracted dry moringa leaves

Peaks No.	Retention time (min)	Polyphenol detected
1	2.059	Quercetin
3	2.987	Gallic Acid
5	3.360	Ferulic Acid
6	3.531	Ellagic Acid
14	7.648	Epigallactocatechin
15	7.979	Catechin
16	8.139	Caffeic Acid
22	9.824	Vanillin
25	10.784	Gallocatechin gallate

differences or the necessity for more precise extraction techniques. The substantial increase in total phenolic content in Moringa spice mix is attributed to the inclusion of various spices, known for their elevated polyphenol levels, signifying promising antioxidant activity in the extract.

Total flavonoid content estimation (TFC)

The total flavonoid content was determined using the catechin standard curve[14], yielding the equation y = 0.0011x + 0.0158 and R2 = 0.9954. Dry Moringa Leaves displayed a total flavonoid content of 5.930 (±0.098) mg/mL, while Moringa spice mix exhibited 11.776 (±0.122) mg/mL. Djemoui et al. (2019) reported a lower total flavonoid content of 5.192 mg/mL for dry Moringa leaves, suggesting our study's efficient extraction methods and possibly superior leaf quality[18]. The incorporation of various spices in Moringa spice mix significantly boosted flavonoid content, indicating potential health benefits such as improved heart health and diabetes prevention through antioxidant effects and glucose metabolism regulation.

Antioxidant assay or total free radical scavenging activity (DPPH)

The absorbance of control at 517 nm was 0.833, used to calculate each sample's inhibition percentage. Dry Moringa leaves showed 66.78% inhibition, while Moringa spice mix exhibited 59.68%. Differences may stem from spice inclusion, comprising 40% of the mix's weight[18]. Mmabatho et al. reported 75.56% inhibition in dry leaves, hinting at preparation or environmental variations[19]. The extract's DPPH scavenging likely relates to its hydrogen donating ability, notably in mature leaf extracts.

Estimation of carotenoids

The carotenoid content was determined at 450 nm using a reported procedure[14]. Dry Moringa exhibited 8.92(±0.78) mg/100 g, while Moringa spice mix showed 15.92 (±0.39) mg/100g, attributed to the spices comprising 40% of its weight. Saini et al. (2014) reported 5.5 mg/100g for dry Moringa leaves, suggesting varietal or extraction method differences. Our study's higher yield may stem from superior leaf quality or proper extraction techniques. Environmental factors and genetic variability could also influence carotenoid levels across studies.

Estimation of ascorbic acid

The average titer value for the standard solution of ascorbic acid[14] (Rangana, 1997) was 4.1 mL, based on three titrations. The ascorbic acid content was 51.3 (±0.32) mg/100g for dry Moringa and 31.7 (±0.43) mg/100g for Moringa spice mix. Dry Moringa leaves typically contain 51.7 mg/100g of ascorbic acid (USDA database), indicating high-quality leaves used in our study. However, ascorbic acid is sensitive to factors like temperature and light, which may influence its content during processing and storage. Understanding these factors is essential for accurately assessing the nutritional value of Moringa products.

Chlorophyll Estimation

Chlorophyll estimation was conducted[14] at 660 nm and 642.5 nm absorbance wavelengths. For dry Moringa leaves, the chlorophyll content was determined as follows: Chlorophyll A: 8.42 (± 0.62) µg/g, Chlorophyll B: 2.67 (± 0.29) µg/g, and Total Chlorophyll: 11.9 (± 0.37) µg/g. In comparison, Moringa spice mix exhibited the following chlorophyll content: Chlorophyll A: 5.05 (± 0.68) µg/g, Chlorophyll B: 1.60 (± 0.54) µg/g, and Total Chlorophyll: 6.65 (± 0.38) µg/g. The higher chlorophyll content in dry Moringa leaves compared to Moringa spice mix can be attributed to the absence of chlorophyll in the spices constituting 40% of the mix's total weight. Nguyen (2021) reported slightly higher chlorophyll content for dry Moringa leaves (Chlorophyll A: 9.33 µg/g, Chlorophyll B: 3.28 µg/g, Total Chlorophyll: 12.57 µg/g) compared to our test.

Determination of polyphenols by high-performance liquid chromatography (HPLC)

The research employed methods recommended by Zhu et al. to analyze nine polyphenols in Moringa oleifera leaf samples[15]. Fig. 1 shows chromatograms displaying these compounds and the corresponding concentrations determined through HPLC analysis.

The study identified nine polyphenols in dry moringa leaves, confirmed through Liquid Chromatography – Mass Spectroscopy (LC-MS), a technique that separates, identifies, and quantifies compounds by creating and detecting charged ions. Zhu et al. conducted dry moringa leaf extraction using methanol and detected eleven polyphenols, including Gallic acid, Chlorogenic acid, Vanillin, Ferulic acid, Gallocatechin gallate, Rutin, Isoquercetin, Quercitrin, Baicalin, Quercetin, and Kaempferol[15].

However, polyphenols were not determined in the moringa spice mix using HPLC due to the complex composition, which includes 40% spices making it challenging to identify individual polyphenols in the chromatogram.

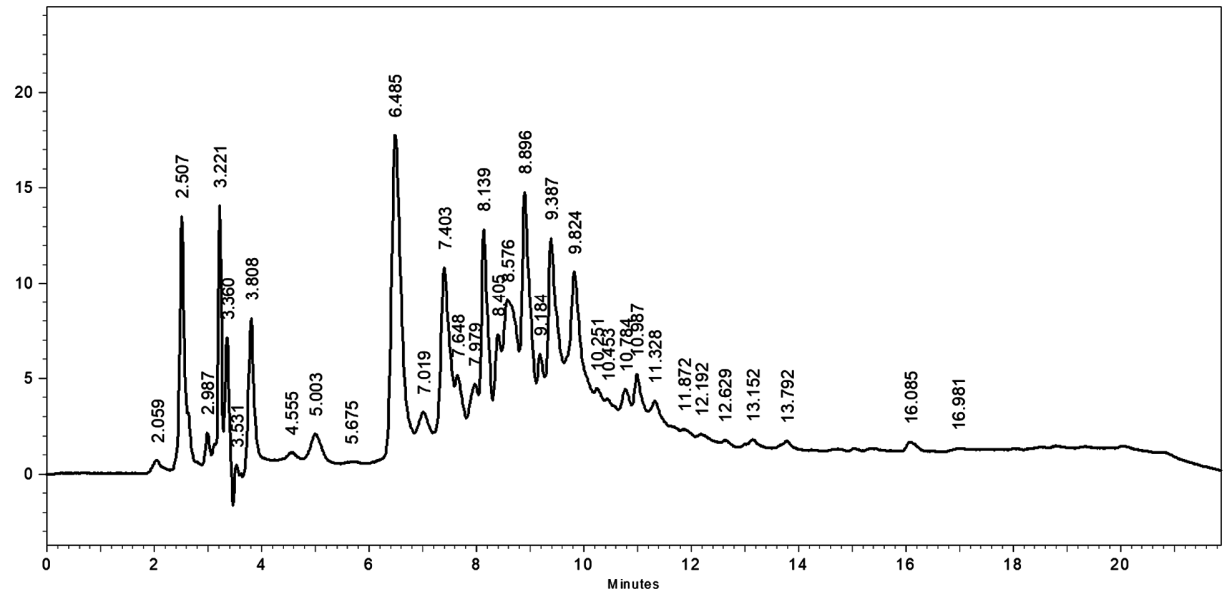

Fig. 1 — HPLC chromatogram of extracted dry moringa leaves

Physical Test

Proximate analysis

The analysis found that Moringa leaves have 68.09% moisture, 3.68% ash, 7.29% fiber, 11.12% protein, 1.89% fat, and 7.93% carbohydrates. In contrast, Moringa spice mix contains 1.43% moisture, 14.28% ash, 18.59% fiber, 26.88% protein, 17.36% fat, and 21.46% carbohydrates.

Compared to USDA data (78.66% moisture, 8.28% carbohydrates, 1.4% fat, 9.4% protein, and 4.9% ash), Moringa leaves show lower moisture, carbohydrate, and ash content but higher protein and fat content, possibly due to drying and variations in leaf quality. The lower moisture suggests limited suitability for long-term storage. Ash content indicates mineral levels, while fat content impacts energy availability and excessive consumption risks.

Colour measurement

The LAB colour mode, a three-axis system, ensures consistent colour representation. It's device-independent, serving as a standard for colour communication across devices. The L axis represents lightness from black to white, an axis spans cyan to magenta/red, and the b axis ranges from blue to yellow. Colours are plotted based on device profiles. dE measures colour difference in LAB space, ranging from 0 to 100. D65 represents average daylight with a correlated colour temperature of about 6500 K. The data are shown in Table 2. The PCA score plot of Dry *Moringa* leaves and Moringa Spice Mix are shown in Fig. 2.

Table 2 — Colour Measurementof Moringa leaves and Moringa Spice mix

Sample	dE (D65)	L* (D65)	a* (D65)	b* (D65)
Dry Moringa leaves	62.11	38.82	- 4.56	20.92
Moringa Spice mix	64.36	38.95	- 0.99	27.44

Electronic Nose (E-Nose)

The PCA scores in the Fig. 3 illustrate fragrance patterns for dry Moringa leaves (Fig. 3a) and Moringa spice mix (Fig. 3b). PC1 contributes 68.888% to total dispersion, while PC2 contributes 25.433%. In dry Moringa leaves, aroma components decrease as PC1 shifts from the first quadrant to the second, while in Moringa spice mix, an increase in PC2 is observed in the third quadrant, indicating the presence of different aroma compounds. Tables 3 and 4 below lists retention times, formula names used in the sensory test, and their corresponding sensory attributes obtained from Hercules Neo.

Sensory Evaluation

The sensory evaluation was done on the basis of colour, taste, texture and flavour and the results are shown in Fig. 4. Consumer acceptance study was done by providing samples to 10 people. After sensory evaluation, Sample 101, containing equal parts of dry Moringa leaves and other spices, showed an overwhelming, non-tolerable strong flavour, with Moringa leaves not being the dominant taste. Sample 301 exhibited a pungent and bitter taste, overshadowing the flavours of other spices. Conversely, Sample 201 received high acceptance among sensory evaluators, indicating a well-balanced

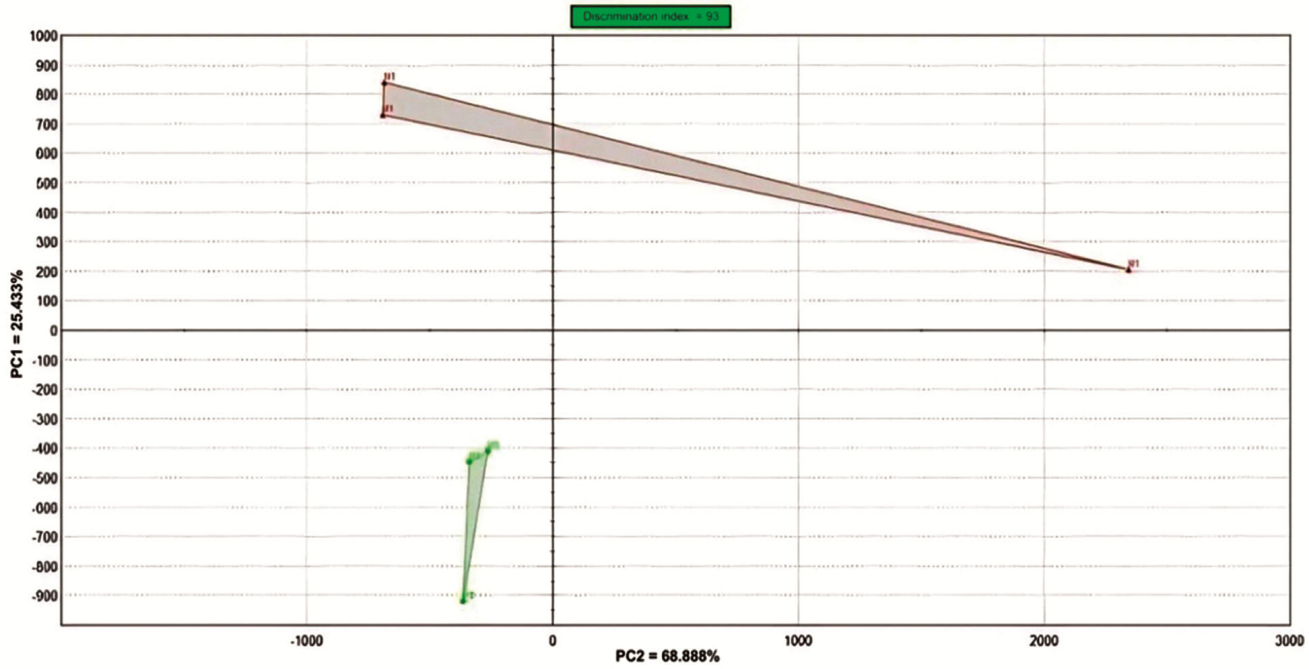

Fig. 2 — PCA score plot of Dry *Moringa* leaves (red) and Moringa Spice Mix (green)

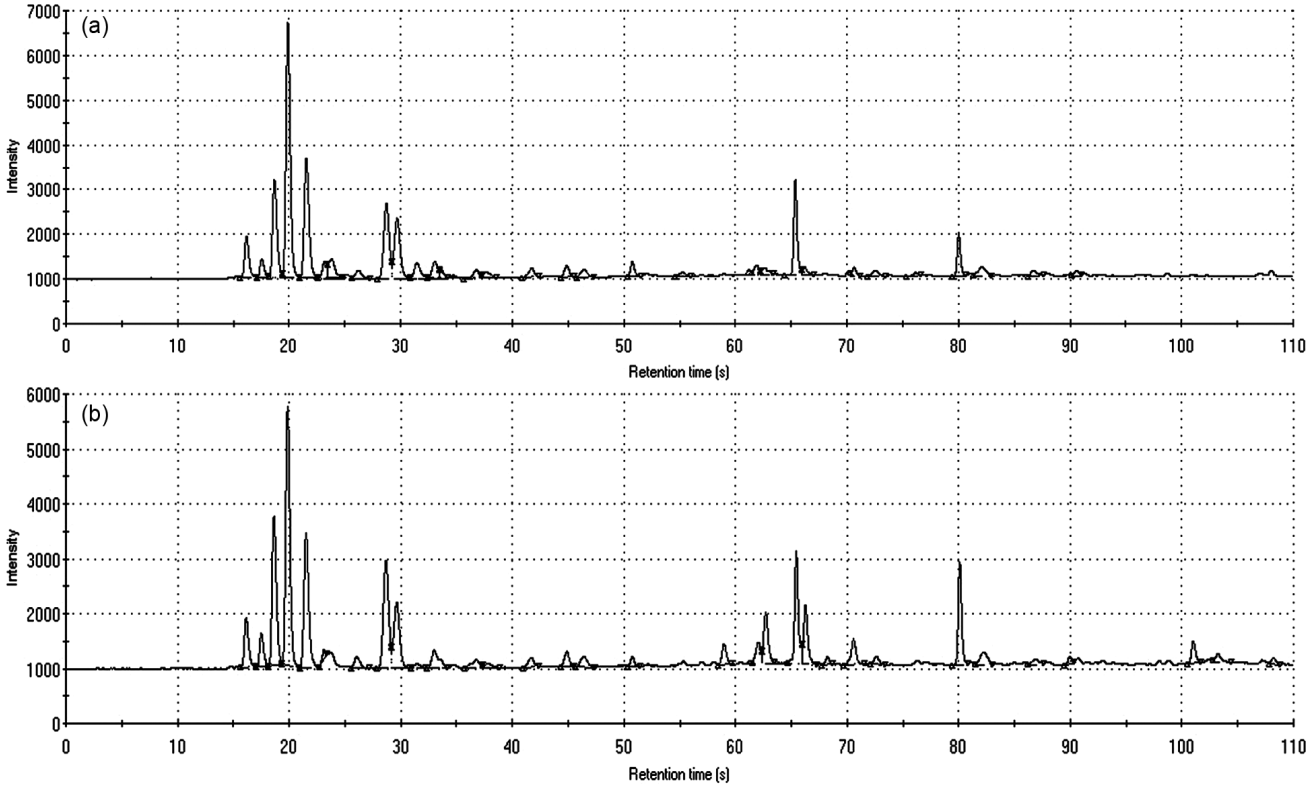

Fig. 3 — Retention time of (a) Dry Moringa leaves and (b) Moringa spice mix in E- Nose

Table 3 — Volatile compounds in Dry Moringa Leaves using E- nose

Compound	Retention time	Sensory attributes
dimethyl sulfide	18.68, 19.88	Vegetable, Cabbage
2-propanol	18.68, 19.88	Woody, Pleasant
ethyl formate	18.68, 19.88	Pungent, Etheral
2-methylpropanal	19.88, 21.54	Toasted, Burnt
butane-2,3-dione	21.54	Strong, Caramelized
butanal	21.54	Green, Malty

Table 4 — Volatile compounds in Moringa Spice Mix using E- nose

Compound	Retention time	Sensory Attributes
dimethyl sulfide	18.63, 19.85	Vegetable, Cabbage
2-propanol	18.63, 19.85	Woody, Pleasant
ethyl formate	18.63, 19.85	Pungent, Etheral
2-methylpropanal	19.85, 21.53	Toasted, Burnt
3-methylbutanal	28.67	Herbaceous, Fatty
Isopropyl acetate	28.67	Chemical, Fruity
Isobutanol	28.67	Bitter, Fusel
n-butanol	28.67	Medicinal, Rancid

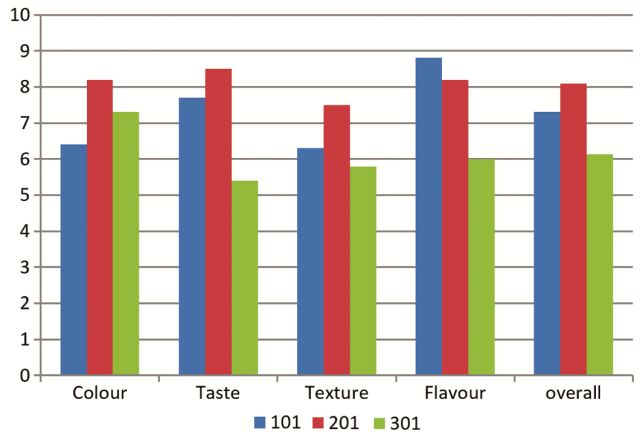

Fig. 4 — Sensory attributes of Moringa Spice mix Samples., here, 101= Moringa spice mix with 50% Moringa leaves from total composition, 201= Moringa spice mix with 60% Moringa leaves from total composition and 301= Moringa spice mix with 70% Moringa leaves from total composition

and appealing flavour profile. Therefore, Sample 201 was selected for subsequent biochemical and physical assessments.

Conclusion

The development and evaluation of the Moringa Spice mix rich in bioactive compounds was attempted. The substantial presence of essential nutrients and bioactive compounds, coupled with favourable sensory attributes, positions the Moringa Spice mix as a nutraceutical product. Further research into its long-term effects is warranted.

Acknowledgement

The authors thank the Director, CSIR-CFTRI, Mysuru for providing the infrastructure for the research.

Ethical Permission for sensory panel

The ethical permission for the sensory panel (individual) is not required as per the institute norms.

Consent by panelists

The consent of the sensory panelists was taken individually before the experiments.

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