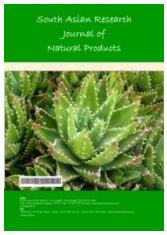

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Development of Healthy Fiber-Rich Herbal Crackers from Whole Wheat, Finger Millet, Rice Bran, and *Gymnema sylvestre* Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Crackers are considered as healthy snacks as they have low level of salt, sugar, and moderate level of fat. This study aimed to develop healthy fiber-rich herbal crackers from whole wheat, finger millet, rice bran, and *Gymnema sylvestre* leaves. Crackers were made with four different formulations by incorporating *G. sylvestre* leaves at different proportions (0%, 1%, 5%, and 8%). Crackers without the incorporation of *G.sylvestre* leaves were considered as control. This investigation evaluated the proximate composition, phenolic, flavonoid, carotenoid content, antioxidant, anti-diabetic, and anti-inflammatory properties of raw ingredients and crackers. Further, sensory profile, invitro-gastrointestinal digestion, and physical properties of crackers were also analyzed and compared with control. The results showed that among raw ingredients rice bran had a higher amount of fiber (18.74± 0.73%), ash (9.38± 0.01%), and flavonoid content (9.25±0.14 mg RE/g) and *G. sylvestre* leaves had higher phenolic (51.89±0.28 mg GAE/g), carotenoid (0.275±0.004 mg/g), and anti-diabetic properties (70.37±0.68% inhibition). Based on sensory evaluation, it was concluded that *G. sylvestre* leaves can be substituted up to 5% in crackers without adversely affecting quality attributes. When comparing 5% leaf substituted crackers with control, the 5% cracker had a slightly higher percentage of phenolic, flavonoid, carotenoid, antioxidant, anti-diabetic, and anti-inflammatory properties than the control. Accordingly, phenolic, flavonoid, carotenoid, antioxidant, anti-diabetic, and anti-inflammatory property of crackers with 5% *G. sylvestre* leaves are 4.66±0.02 mgGAE/g, 1.4±0.04 mgRE/g, 0.214±0.003 mg/g, 32.77±0.64%,

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48.3±0.68%, and 71.42±2.38% respectively. Further, the physical properties of control and crackers with 5% leaves are almost the same. So, these results concluded that *G. sylvestre* leaves can be incorporated up to 5% in crackers preparation along with whole wheat flour, finger millet flour, and rice bran to enhance the functional properties of crackers.

Keywords: Anti-diabetic property; dietary fiber; proximate composition.

1. INTRODUCTION

People in the modern world are at a higher risk of chronic diseases such as diabetes mellitus due to an unfit diet. Diabetes Mellitus (DM) is considered as the condition of elevated blood sugar levels in the body, and can damage multiple organ systems mediated by free radicals [1]. Nowadays, Diabetes Mellitus is the leading non-communicable metabolic disorder in the world. Based on International Diabetes Federation (IDF) data, about 415 million adults suffer from DM worldwide, and it will be increased to 642 million by 2040 [2]. When considering Sri Lanka, the prevalence of DM in Sri Lanka was 7.9% in 2016 [3] however, it was 10.7% in 2019. One in twelve adults (1.16 million) in Sri Lanka suffer from DM, and within the last five years, about 1-1.4% of total diabetes patients have lost their lives due to DM [2]. The reasons behind the increase of DM are unhealthy diets, insufficient physical activity and mental stress. Among all these, unhealthy diets have a significant role in increasing DM among people.

According to the previous reports, following a healthy diet plan will help to control DM. There are plenty of plant sources, such as cereals, medicinal plants, fruits, and vegetables, that have anti-diabetic properties. Consumption of those sources will help to control DM. For individuals with DM, cereals such as whole-wheat flour, finger millet, and rice [4], medicinal plants such as *Gymnema sylvestre* leaves [5] are recommended to control their blood sugar level. In these sources, finger millet has a low Glycemic Index, and it slowly releases glucose into the body. Furthermore, it takes a longer time for digestion and gives a feeling of fullness in the stomach [6]. These functions of finger millet will support to control DM. Whole-wheat flour is a rich source of dietary fiber. This dietary fiber can control the blood glucose level in the body. Rice bran is a by-product obtained during the milling process of rice. The milling process yields about 8-12% of rice bran, and it depends on the milling degree and type of rice [7]. In Sri Lanka, rice bran is commonly used as animal feed. However, it can add value to the food product as it is a rich source of phytochemicals such as γ-oryzanol, vitamin E (mainly tocotrienols), and dietary fiber [7]. The incorporation of locally available cereals such as finger millet and byproducts such as rice bran into food products adds value to the product by increasing its functionality, and this encourages local cereal cultivation.

Nowadays, plants leaves, and flowers have great potential in maintaining people's health as they have therapeutic properties. *Gymnema sylvestre* is a medicinal plant known as "Periploca of the woods" in English, *Masbadda* in Sinhala, and *Chirukurinja* in Tamil. This has the ability to suppress sweet taste, reduce weight gain and fat accumulation as it has multiple enzyme inhibiting properties [1]. Further, gymnemic acid and gurmarin are the major chemical constituents of *G. sylvestre,* which can block sweet tastes in humans. With these, *G. sylvestre* can increase insulin secretion, promote regeneration of islet cells, decrease gluconeogenic enzymes, and inhibit sorbitol dehydrogenase activity [8]. So, incorporating these sources (whole-wheat flour, finger millet, rice, and *Gymnema sylvestre)* into food will reduce the food's Glycemic Index and increase the anti-diabetic property of the food.

Accordingly, in developing countries, with increasing urbanization, the demand for convenient, functional food is increasing rapidly [9]. This has encouraged food industries to develop foods such as snacks, which are a convenient and good source of nutrition [10]. Crackers are a kind of snack which are considered as convenient functional food. It is most common among people because of its better shelf-life, easy distribution, and very low sugar and fat content [11]. So, introducing anti-diabetic properties containing raw ingredients such as whole wheat flour, finger millet flour, rice bran, and *G. sylvestre* leaves in crackers production will result in a convenient functional food that has healthy fiber-rich anti-diabetic properties.

2. MATERIALS AND METHODOLOGY

2.1 Materials and Reagents

Whole wheat flour was purchased from a grocery shop in Jaffna, finger millet was collected from the local farmers, rice bran was obtained from the rice mill in Jaffna, and *G. sylvestre* leaves were collected in the home garden.

2.2 Methodology

2.2.1 Proximate composition of raw materials

The raw materials (whole wheat flour, finger millet flour, rice bran and *G. sylvestre* leaves) were analyzed for protein, fat, ash, fiber, and moisture using the standard procedures of AOAC (2000).

Moisture Content: Moisture content of wheat flour, finger millet flour, rice bran, and *G. sylvestre* leaves were determined using oven drying method. Initially, moisture cans were oven dried at 105^0C for 3 hours. Then the moisture cans were cooled inside the desiccator. Initial weights of the moisture cans were recorded (W1). Approximately five grams of substance was weighed (W2) into the moisture cans. Then moisture cans were placed inside the air-drying oven at 105^0C and dried to constant weight. Subsequently, the moisture cans which contain samples were placed in a desiccator with partially covered lid for 10 minutes. Finally, the final weights of the moisture cans with samples (W3) were weighed.

$$\text{Moisture content \% (wet basis)} = \frac{\text{Weight of the initial sample} - \text{weight of the dried sample}}{\text{Weight of initial sample}} \times 100$$

$$= \frac{(W2-W1) - (W3-W1)}{(W2-W1)} \times 100$$

Fat Content: All glass apparatuses were rinsed by petroleum ether and dried in an oven at 105^0C to ensure weight were stable. Three grams of sample was weighed and put into the thimble. Thimble was covered with cotton plug. After that, thimbles were placed in the Soxhlet extractor. About 200 mL petroleum ether was poured into the round bottom flask. The whole setting was placed on a heating mantle and the petroleum ether was allowed to boil. The extraction process was continued for 12 hours. The residual content in the round bottom flask was transferred into an empty clean beaker of known weight. Then the beaker was placed in the oven at 70^0C until it reached constant weight. Finally, the weight of the beaker with fat was taken.

$$Fat\ \% = \frac{Weight\ of\ fat}{Initial\ weight\ of\ the\ sample} \times 100$$

Protein Content Protein content of the raw materials and crackers were determined by using Kjeldahl method. It was carried out by three steps namely digestion, distillation, and titration.

Digestion: Nearly 1 g of sample was weighed into a digestion flask (BUCHI-USA). Then the sample was digested with 25 ml of concentrated H_2SO_4 in the presence of catalyst (K_2SO_4 + 17 $CuSO_4$) at 440^0C in the digestion system until the solution became colorless. Samples were allowed to cool down to room temperature. A flask containing above mentioned chemicals except the sample was kept as blank. Same procedure was followed for all the samples.

Distillation: Adequate amount of 40% NaOH and distilled water (for neutralization) were filled into the containers separately and the tubes containing digested samples were fixed into Kjeldahl semi-automated distillation unit. Tubes were diluted with 50 mL of distilled water and 80mL of 40% NaOH solution was added. The neutralized samples were subjected to steam distillation and liberated ammonia was trapped in 25 mL of 4% boric acid solution with 2-3 drops of Methyl Red Bromocresol green indicator.

Titration: Distillate was titrated with 0.1M hydrochloric acid until the color changed to pink.

$$Protein\ \% = \frac{1.4 \times (V1-V2) \times N}{P} \times 6.25$$

V_1: Volume (mL) of 0.1M HCL used for the titration of sample
V_2: Volume (mL) of 0.1M HCL used for the titration of blank
N: Normality of HCL
P: Weight of sample (g)
6.25: The protein nitrogen conversion factor

Ash Content: Initially, crucibles and lids were placed overnight in the furnace at 550^0C to ensure that impurities on the crucible were burnt off. Then the crucibles and the lids were cooled inside the desiccator. Initial weight of empty crucibles was measured to three decimal points. Around 5g of sample was weighed into the each weighed crucible and samples were ignited in muffle furnace at 550^0C for 5 hours until constant weight and ash color of the sample was achieved. Finally, crucibles were cooled inside the desiccators and reweighed.

$$Ash\ content = \frac{Weight\ of\ the\ ash}{Initial\ weight\ of\ the\ sample} \times 100$$

Crude Fiber Content: Initially, around 2.0 g of sample was taken (W1) into the conical flask. Then about 200ml of 0.1M HCl was added into it and boiled on a heating plate. Then the mixture was filtered using muffling clothe and the residue was transferred into a conical flask. About 200mL of 0.1M NaOH was added into the residue and boiled on a heating plate. Then the mixture was filtered using muffling clothe and the residue was transferred into the crucibles. After that the crucibles were placed inside the oven at 120^0C for two hours. Then the weight of the crucible with sample was measured (W1). After that, the crucible was placed inside the muffle furnace at 550^0C for 4 hours. Then it was placed inside the desiccator for cool down and its final weight was measured (W2).

$$Crude\ fiber\ \% = \frac{(W1-W2)}{Initial\ weight\ of\ the\ sample} \times 100$$

Carbohydrate Content: Carbohydrate content was determined using the following equation.

$$Carbohydrate\ \% = 100 - (\text{moisture}\% + \text{protein}\% + \text{fat}\% + \text{ash}\% + \text{fiber}\ \%)$$

2.2.2 Methanolic extraction of samples

Methanolic extraction of samples were prepared according to the methods described in Janarny *et al.*, 2020. Briefly, 2g of sample was mixed with 20 ml of 80% methanol. Mixed sample was continuously stirred for 30 minutes at room temperature using magnetic stirrer. Then the mixture was centrifuged at 1000 rpm for 10 min and the supernatant was collected. The residual of the sample was extracted again twice, and all the supernatant was combined. Then the solvent was evaporated at 35℃ using a rotary evaporator (Hahn Shin Scientific, Korea). The concentrated mixture was collected into a brown color bottle, and it was stored at -18℃ until further analysis. The extract was diluted by 5 times. This diluted sample was used for the analysis of phenolic content, flavonoid content, carotene content, anti-inflammatory property, and anti-diabetic property of the sample.

2.2.3 Functional properties of raw materials

2.2.3.1 Determination of total phenolic content

The total phenolic content of the extract was determined using the Folin- Ciocalteu method described in [12]. Accordingly, 0.5 mL of diluted methanolic extracts was mixed with 2 mL distilled water and 0.5 mL of Folin- Ciocalteu reagent. The reaction mixture was mixed well. After 8 minutes, 0.5 mL of Na_2CO_3 was added into the Gerber tube and marked up to 10 mL using distilled water. Then it was incubated at 27^0C for 30 minutes. Finally, the absorbance of the mixture was measured at 765 nm using a UV/VIS spectrophotometer (JP Selecta, Spain). The blank was prepared with all the chemicals above expect the sample. Gallic acid was used for the standard curve preparation and the total phenolic content was expressed as mg gallic acid equivalents (GAE) per g.

2.2.3.2 Determination of total flavonoid content

The total flavonoid content of the extract was determined using the aluminum chloride method described in [13]. Accordingly, 0.5 mL of diluted methanolic extracts was mixed with 3 mL of distilled water and 0.3 mL of 5% $NaNO_2$. After 5 min 0.3 mL of 10% $AlCl_3$ was added to the mixture and mixed well. The reaction mixture was allowed to stand for 6 min. Then 2 mL of 1 M NaOH was added to the mixture. The reaction mixture was volume up to 10 mL with distilled water and mixed well. Finally, the absorbance of the mixture was measured at 510 nm using a UV/VIS spectrophotometer. The blank was prepared with all the chemicals above expect the sample. The total flavonoid content was expressed as mg rutin equivalents (RE) per g.

2.2.3.3 Determination of carotenoid content

The total carotenoid content was determined according to the method described by [12]. On briefly test samples of 0.5 mL were separated from the diluted extracts and the absorbance was read at 470, 653, and 666 nm on UV/VIS Spectrometer. Total carotenoid content was calculated according to the formulas mentioned below,

$$Chlorophyll\ a\ (Ca) = 11.75(A666) - 2.350(A653)$$
$$Chlorophyll\ b\ (Cb) = 18.61\ (A653) - 3.960\ (A666)$$
$$Carotene = 1000\ (A470) - 2.270\ (Ca) - \frac{81.4\ (Cb)}{22}$$

2.2.3.4 DPPH radical scavenging activity

Initially 1g of sample was mixed with 25mL methanol inside a conical flask. Then it was kept

inside a shaking water bath at room temperature, 100 rpm for 2 ½ hours. The sample was centrifuged for 15 minutes at 6000 rpm. Then it was filtered using filter paper and the extracted solution was collected. About 1mL from the extracted solution was taken inside a Gerber tube and markup with methanol. Then, 1mL from this solution was taken into another Gerber tube and mixed with 3mL DPPH solution. It was markup with methanol and kept inside a dark room for 30 minutes. Finally, the absorbance was measured at 517nm.

2.2.3.5 Anti-inflammatory properties

Anti-inflammation property was determined using protein denaturation assay described by [14] with some modification. Accordingly, 0.2 mL of 1% egg albumin, 4.78 mL of phosphate-buffered saline (PBS, pH 6.4) and 0.02 mL methanolic extract were mixed and incubated at 37˚C for 15 min in a water bath. Then, the reaction mixture was heated at 70˚C for 5 min. After cooling the heated reaction mixture, its absorbance was measured at 660 nm using a UV/Visible spectrophotometer. PBS without sample was used as the control and the percentage inhibition of protein denaturation was calculated.

2.2.3.6 Anti-diabetic properties

Alpha-amylase inhibition assay was performed to evaluate the anti-diabetic properties based on the method explained in [12]. Briefly, 100 µL of the sample was mixed with 200 µL of α-amylase enzyme and 100 µL of 2 mM of phosphate buffer (pH-6.9) and incubated for 20 minutes. After that, 100 µL of 1% starch solution was added and incubated for 5 minutes. Then, 500 µL of 3, 5-dinitrosalicylic acid reagent was added and kept in a boiling water bath for 5 min. Finally, the absorbance was recorded at 540 nm using UV/Visible spectrophotometer and the percentage inhibition of the α-amylase enzyme was calculated. Control was prepared by the same method where the addition of sample was replaced with methanol.

2.2.4 Crackers preparation

2.2.4.1 Composite flour preparation

The whole wheat flour was substituted with the finger millet and rice bran in the fixed ratio of;

Whole wheat flour: Finger millet: Rice bran = 5: 3: 2.

2.2.4.2 Substitution of flour blend with G. sylvestre leaves

Flour blend (F) was partially substituted with 1%, 5%, and 8% of *G. sylvestre* leaves (G). This made three different formulations for cracker preparation (F: G = 99:1, 95:5 and 92:8).

2.2.4.3 Crackers preparation

Bake trials of crackers were conducted under laboratory conditions. Weighing the ingredients, processing, and baking were performed on laboratory-scale equipment. Cracker samples were prepared in a straight dough process. Briefly, all the dry ingredients, including flour blend, leaf powder, salt, and baking powder, were mixed well. Then water was added slowly and mixed properly to form a dough. The formed dough was allowed to rest for 10 minutes at room temperature to ensure uniform distribution of the liquids. The dough was manually sheeted into large sheets, and circular crackers were cut from the dough sheet using a dough cutter. The crackers were placed in a lightly greased and floured oven tray. Then the cracker-containing tray was placed in an oven at 165^0C for 20-30 minutes. Baked crackers were then cooled at an ambient temperature for 15 minutes. Flour blend without leaf powder was used for the preparation of control crackers and flour blend was replaced with leaf powder at varying amounts as follows: 1%, 5%, and 8% to form crackers with different formulations.

Fig. 1. Final product

2.2.5 Sensory analysis

Sensory analysis was done using a 7-point hedonic scale. Twenty-five semi-trained panelists were used for this analysis.

2.2.6 Proximate composition & functional properties of crackers

The proximate composition and functional properties of crackers were determined using the same procedure used for raw materials.

2.2.7 Physical properties of crackers

2.2.7.1 The stack height of the crackers

The stack height of the crackers with 5% *G. sylvestre* leaf substitution and control crackers was measured using seven sample pieces. The stack height was measured with a vernier caliper. The height was measured once. Then the crackers were turned at 90^0 and measured again to obtain the average value. Seven crackers were stacked one above another and restacked four times. The average of the heights obtained was taken as the final thickness of a cracker.

2.2.7.2 Stack weight of crackers

The stack weight of the crackers with 5% *G. sylvestre* leaf substitution and control crackers was measured using seven sample pieces. Seven crackers were selected randomly and weighed by using a calibrated analytical balance.

2.2.7.3 Spread ratio

The spread ratio of the crackers with 5% *G. sylvestre* leaf substitution and control crackers was measured using seven sample pieces. Briefly, seven crackers were placed one by one in a line and their lengths were measured. This was performed three times, and the average value was taken.

2.2.8 *In-vitro* gastrointestinal digestion

Crackers with 5% *G. sylvestre* leaf substitution and control crackers were subjected to *invitro* gastrointestinal digestion according to the method described by [13]. This was done in two phases, namely the gastric phase and the intestinal phase.

2.2.8.1 Gastric phase

About 10 g of fine ground crackers with 5% *G. sylvestre* leave substitution and control crackers were mixed with 50 mL of 0.9% NaCl solution and 4.0 mL of pepsin solution (40 mg/mL in 0.1 M HCl). The pH of the mixture was adjusted and maintained at 1 by adding 0.1 M HCl. Then the mixture was incubated at 37˚C for 1 h at 100 rpm using a shaking water bath (Daihen Lab tech, Korea). Aliquots of digested samples were separated and filtered. It was stored at -18℃ until further analysis. Aliquots were used for the bioactive (phenolic, flavonoid, and carotene content) and antioxidant activity analysis.

2.2.8.2 Intestinal phase

In the intestinal phase, the pH of the filter obtained from the gastric phase was brought to 6.5 with $NaHCO_3$ before adding pancreatin and bile extract into the digesta. Then, 18mL of a mixture of pancreatin and bile extract (prepared by dissolving 2 mg/mL pancreatin and 12mg/mL bile extract in 0.1M $NaHCO_3$) were added into the filter obtained from the gastric phase. After that, the mixture was incubated at 37˚C for 2 h at 100 rpm using a shaking water bath (Daihen Lab tech, Korea). Aliquots of digested samples were separated and filtered. It was stored at -18˚C until further analysis. Aliquots were used for the bioactive (phenolic, flavonoid, and carotene content) and antioxidant activity analysis.

2.2.9 Statistical analysis

All experiments were carried out in triplicates and data were reported as mean ± standard deviation. Significant differences between the average values were calculated by analysis of variance using SPSS 16.0 software and MS Excel 2013. A level of confidence of 95% ($p < 0.05$) was used for this statistical analysis.

3. RESULTS

3.1 Proximate Composition of Raw Ingredients

The proximate composition of the raw ingredients such as whole wheat, finger millet, rice bran, and *G. sylvestre* leaves is shown in Table 1. The mean moisture, crude protein, crude fat, crude fiber, crude ash, and total carbohydrate contents of raw ingredients varied from (11.1± 0.86-7.77±0.18), (11.08±0.09 - 7.48 ±0.19), (18.52±0.35 - 1.25± 0.02), (18.74±0.73 - 1.41± 0.19), (9.38± 0.01 - 1.27± 0.01), and (77.38±0.01 - 32.10±0.01) g/100 g dry weight basis respectively.

Table 1. Proximate composition of raw ingredients (g/100g dry weight basis)

Sample	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
Finger millet	11.1± 0.86^{a}	7.48 ±0.19^{d}	1.25± 0.02^{d}	4.21±0.18^{c}	2.33±0.12^{c}	73.57±0.03^{b}
Rice bran	10.18±1.82^{ab}	11.08±0.09^{a}	18.52±0.35^{a}	18.74±0.73^{a}	9.38± 0.01^{a}	32.10±0.01^{d}
Whole wheat	8.11±0.29^{c}	9.79± 0.17^{c}	2.05± 0.01^{c}	1.41± 0.19^{d}	1.27± 0.01^{d}	77.38±0.01^{a}
Leaf	7.77±0.18^{cd}	10.28±0.02^{b}	6.07±0.23^{b}	15.57± 0.23^{b}	8.12± 0.02^{b}	52.19±0.03^{c}

Values are expressed as mean ± SD values followed by different letters for each assay in the same column are significantly different ($p < 0.05$)

Table 2. Table of functional properties of raw ingredients

Sample	TPC (mg *GAE/g)	TFC (mg *RE/g)	TCC (mg/g)	Antioxidant (DPPH % inhibition per g)	Anti-inflammatory (% inhibition)	Anti-diabetic (% inhibition)
Finger millet	3.96±0.016^{c}	0.31±0.03^{d}	0.033±0.003^{c}	28.87±1.83^{d}	60.32±4.96^{b}	49.63±0.26^{c}
Rice bran	19.57±0.4^{b}	9.25±0.14^{a}	0.118±0.003^{b}	76±0.87^{a}	57.94±5.99^{c}	51.85±0.51^{b}
Whole wheat	1.18±0.01^{d}	0.94±0.07^{c}	0.017±0.003^{d}	34.59±0.87^{c}	79.36±3.63^{a}	45.48±1.12^{d}
Leaf	51.89±0.28^{a}	6.58±0.15^{b}	0.275±0.004^{a}	58.99±1.67^{b}	56.35±3.64^{c}	70.37±0.68^{a}

**GAE- Gallic acid equivalent. *RE- Rutin equivalent. TPC- Total phenolic content. TFC- Total flavonoid content. TCC- Total carotene content. Average quantities ± SD of three independent samples data is presented in this table. Different letters within each assay represent there is a statistically significant difference ($p < .05$)*

Table 3. Proximate composition of crackers (g/100g dry weight basis)

Sample	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
5%	3.89 ±0.19^{a}	11.19±0.1^{a}	5.2±0.17^{a}	4.54± 0.04^{a}	6.6±0.01^{a}	68.6±0.03^{b}
Control	2.67 ±0.58^{b}	10.19±0.35^{b}	1.01± 0.02^{b}	3.4±0.51^{b}	5.46± 0.22^{b}	77.26±0.01^{a}

Values are expressed as mean ± SD values followed by different letters for each assay in the same column are significantly different ($p < 0.05$)
5%- Crackers substituted with 5% G. sylvestre leaves
Control- Crackers with 0% G. sylvestre leaves

Table 4. Table of functional properties of crackers

Sample	TPC (mg *GAE/g)	TFC (mg *RE/g)	TCC (mg/g)	Antioxidant (DPPH % inhibition per g)	Anti-inflammatory (% inhibition)	Anti-diabetic (% inhibition)
Cracker (5%)	4.66±0.02[a]	1.4±0.04[a]	0.214±0.003[a]	32.77±0.64[a]	71.42±2.38[a]	48.3±0.68[a]
Control	4.44±0.01[b]	0.94±0.01[b]	0.116±0.001[b]	28.31±0.24[b]	65.88±1.37[b]	40.15±1.12[b]

**GAE- Gallic acid equivalent. *RE- Rutin equivalent. TPC- Total phenolic content. TFC- Total flavonoid content. TCC- Total carotene content. Average quantities ± SD of three independent samples data is presented in this table. Different letters within each assay represent there is a statistically significant difference (p < .05)*

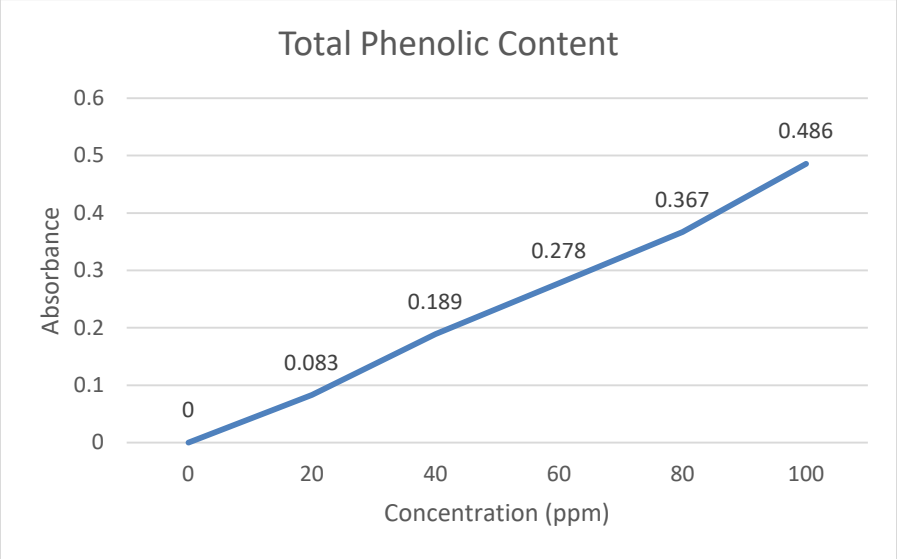
Table 5. Table of physical properties of crackers

Sample	Stack Height (for 7 samples) (mm)	Stack Weight (for 7 samples) (g)	Spread Ratio (for 7 samples) (cm)
Cracers 5%	7.43 ± 0.6[a]	18.61 ± 0.81[a]	30.83 ± 1.72[a]
Control Crackers	7.37 ± 0.45[ab]	18.6 ± 2.48[ab]	30.13 ± 0.93[ab]

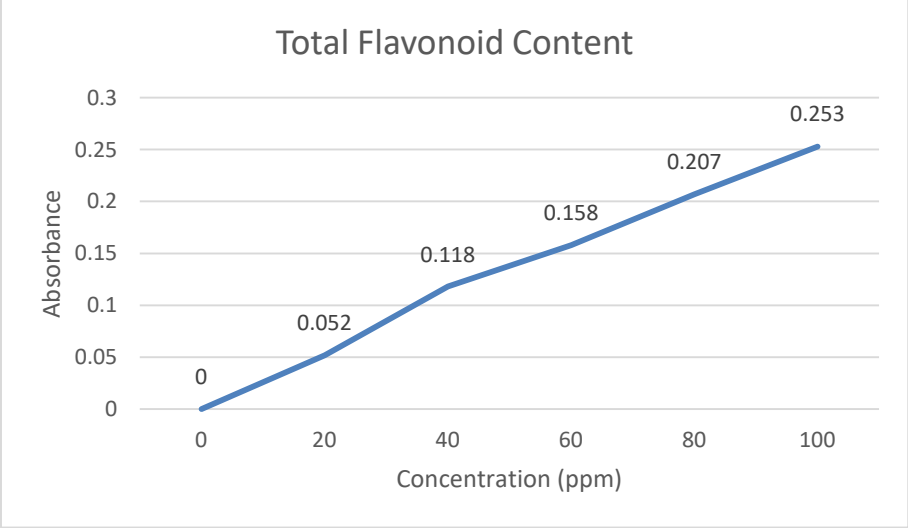
Average quantities ± SD of three independent samples data is presented in this table. Different letters within each assay represent there is a statistically significant difference (p < .05)

3.2 Functional Properties of Raw Ingredients

Table 2 shows the functional properties of raw ingredients whole wheat, finger millet, rice bran, and *G. sylvestre* leaf extract. Gallic acid was used as a standard compound in total phenolic content analysis and the total phenols were expressed as mg/g Gallic Acid Equivalent (mg GAE/g) using the standard curve, where absorbance at 765 nm expressed in Y-axis and total phenolic content in the methanolic extracts of the sample expressed in X-axis. Rutin was used as a standard compound in total flavonoid content analysis and the total flavonoids were expressed as mg/g Rutin Equivalent (mg RE/g) using the standard curve, where absorbance at 510 nm expressed in Y-axis and total flavonoid content in the methanolic extracts of the sample expressed in X-axis. Based on the results obtained total phenolic content, total flavonoid content and total carotenoid content of the raw ingredients varied in the range of (51.89±0.28 - 1.18±0.01 mg GAE/g), (9.25±0.14 - 0.31±0.03 mgRE/g), and (0.275±0.004 - 0.017±0.003 mg/g). While antioxidant property, anti-inflammatory property, and anti-diabetic property of raw ingredients varied in the range of (76±0.87 - 28.87±1.83), (79.36±3.63 - 56.35±3.64), and (70.37±0.68 - 45.48±1.12) % inhibition respectively.



Standard curve for TPC



Standard curve for TFC

3.3 Sensory Evaluation Results of Final Products

Sensory evaluation was conducted after designing the three different formulations by adjusting the percentage of *G. sylvestre* leaves to determine the consumer preference of the final product (crackers). A seven-point hedonic scale test was used to evaluate the preference. Based on the results, crackers substituted with 5% *G. sylvestre* leaves were accepted as the most preferred crackers. Fig. 1 shows the average ranking score values for three crackers samples considering their sensory attributes: appearance, flavor, taste, color, texture, and overall preference.

3.4 Proximate Composition of Crackers

The proximate composition of the crackers with 5% leave substitution and control crackers is shown in the table 03. The mean moisture, crude protein, crude fat, crude fiber, crude ash, and total carbohydrate contents of crackers varied in the range of (3.89 ±0.19 - 2.67 ±0.58), (11.19±0.1 - 10.19±0.35), (5.2±0.17 - 1.01± 0.02), (4.54± 0.04 - 3.4±0.51), (6.6±0.01 - 5.46± 0.22), and (77.26±0.01 - 68.6±0.03) respectively.

3.5 Functional Properties of Raw Ingredients

Table 4 shows the functional properties of crackers sample. Based on the results obtained total phenolic content, total flavonoid content and total carotenoid content of the crackers sample varied in the range of (4.66±0.02 - 4.44±0.01), (1.4±0.04 - 0.94±0.01), and (0.214±0.003 - 0.116±0.001). While antioxidant property, anti-inflammatory property, and anti-diabetic property of crackers sample varied in the range of (32.77±0.64 - 28.31±0.24), (71.42±2.38 - 65.88±1.37), and (48.3±0.68 - 40.15±1.12).

3.6 Physical Characteristics of Crackers

Physical characteristics of crackers were measured to determine the effect of supplementation of *G. sylvestre* leaves on stack weight, stack height, and specific volume of crackers. The result obtained for physical characteristics of crackers is mentioned in Table 5. Based on the results obtained stack height, stack weight, and spread ratio of crackers in the range of (7.43 ± 0.6 - 7.37 ± 0.45), (18.61 ± 0.81 - 18.6 ± 2.48), and (30.83 ± 1.72 - 30.13 ± 0.93) respectively.

3.7 *In-vitro* Gastro-Intestinal Digestion

3.7.1 Total phenolic content

In-vitro bio-accessibility and the potential uptake of polyphenols after simulated *invitro* gastrointestinal digestion of crackers are shown in Fig. 2. Total polyphenol content in methanolic extracts of crackers was in the range of 0.2- 4.7 mg GAE/ g. The results showed that the phenolic

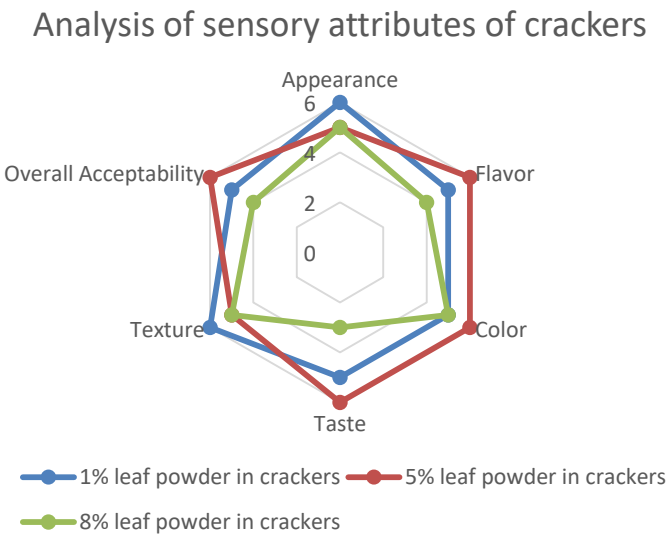


Fig. 2. Spider web analysis of sensory attributes median score for cracker formulations to select the appropriate percentage of leaf powder in cracker

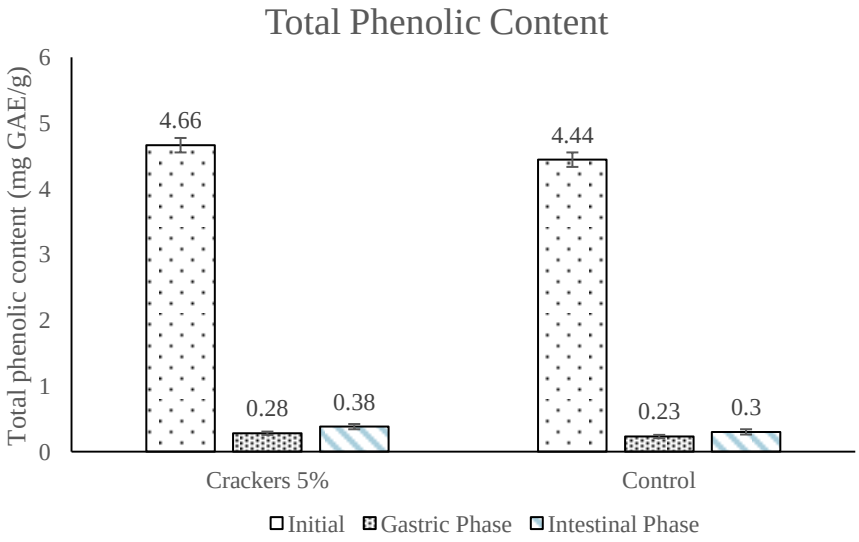


Fig. 3 .Total phenolic content of crackers subjecting to simulated invitro gastric and intestinal digestion and initial-methanolic extract of crackers. Average quantities ± SD of three independent samples data is presented in this table. Different letters within each assay represent there is a statistically significant difference (p < .05)

content of crackers in the initial stage was higher than the values obtained in the gastric and intestinal phases. Further the phenolic content of crackers with 5% leaves substitution was higher than the control crackers in all three stages (initial stage, gastric phase, and intestinal phase).

3.7.2 Total flavonoid content

In-vitro bio-accessibility and the potential uptake of flavonoids after simulated *invitro* gastrointestinal digestion of crackers are shown in Fig. 3. The results showed that the flavonoid content of crackers in the initial stage was higher than the values obtained in the gastric and intestinal phases. Further the flavonoid content of crackers with 5% leaves substitution was higher than the control crackers in all three stages (initial stage, gastric phase, and intestinal phase).

3.7.3 Total carotenoid content

Total carotenoid content was analyzed to determine the bioavailability of carotenoid at gastric and intestinal phase. Fig. 4 shows the bioavailability of total carotenoid content at initial stage, gastric phase, and intestinal phase. Based on the results obtained, total carotenoid content was higher in intestinal phase when compared to initial stage and gastric phase. Further crackers with 5% leave substitution have high bioavailable carotenoid when compared to control crackers.

3.7.4 DPPH (Inhibition % per gram of fresh weight)

DPPH radical scavenging assay was analyzed to determine the antioxidant property of crackers at gastric and intestinal phase. Fig. 5 shows the DPPH radical scavenging activity at initial stage, gastric phase, and intestinal phase.

4. DISCUSSION

Recently many researchers have attempted to understand the bioavailability of bioactive components in the food matrices and did many studies to enhance the functionality of food products by substituting the food products with bioactive components. Crackers is one of the food products which has been studied by various researchers to enhance their functional properties by substituting different food matrices such as leaves, by-products of cereals, etc. Accordingly, crackers prepared with *Carica papaya* leaves [15] with green and red spinach [16] and crackers enhanced with Kelor (*Moringa Oleifera*) leaves in [17] are some evidence for the development of crackers with different herbs to enhance their functional property. Furthermore, because of their improved nutrient profile, these nutritionally, and functionally enhanced food

items may play a role in the growing interest in healthy processed foods among people who lead sedentary lifestyle. So, the present study was conducted to develop crackers by substituting the *G. sylvestre* leaves at different percentages and analyzing the chemical composition, physical characteristics, and sensory profiles of the raw ingredients and crackers.

4.1 Proximate Composition of Raw Materials

Proximate composition of raw ingredients such as whole wheat flour, finger millet flour, rice bran, and *G. sylvestre* leaves was done to determine the nutritional value of each raw ingredient. Based on the results mentioned in Table 1, the

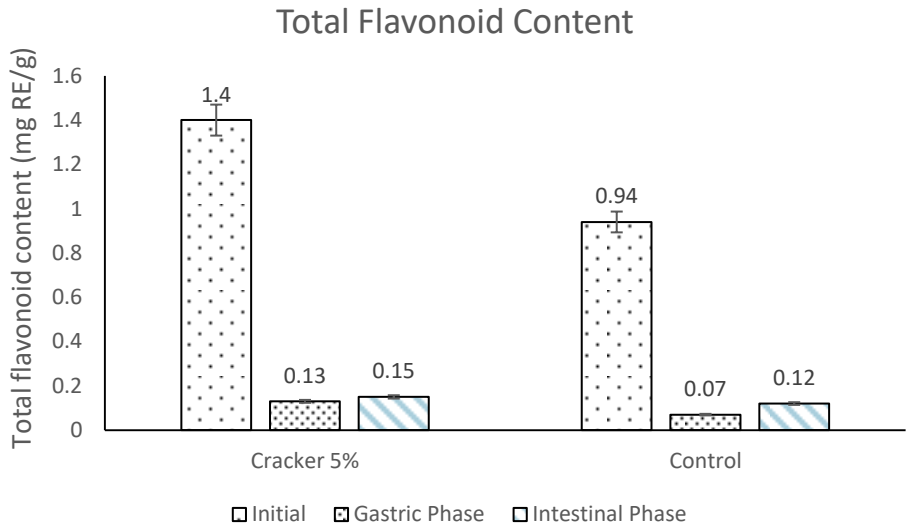


Fig. 4. Total flavonoid content of crackers subjecting to simulated invitro gastric and intestinal digestion and initial-methanolic extract of crackers. Average quantities ± SD of three independent samples data is presented in this table. Different letters within each assay represent there is a statistically significant difference (p < .05)

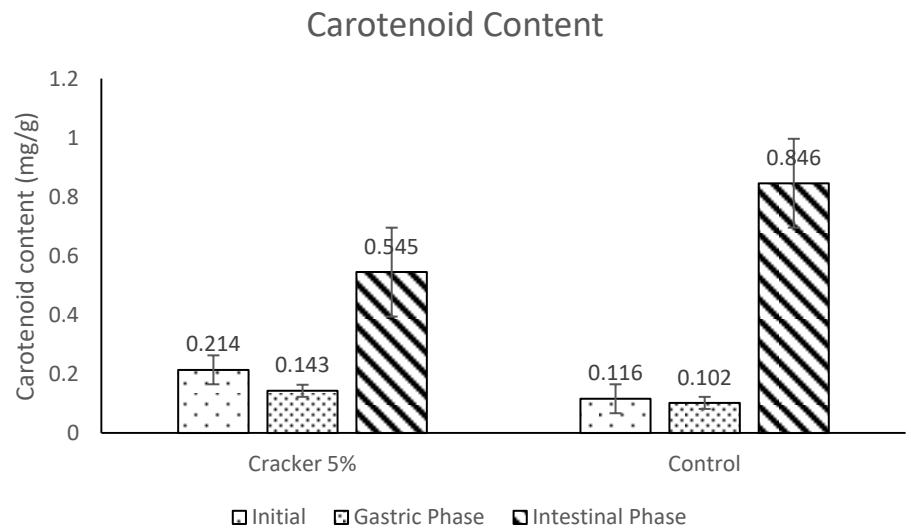


Fig. 5. The total carotenoid content of crackers subjecting to simulated invitro gastric and intestinal digestion and initial-methanolic extract of crackers. Average quantities ± SD of three independent samples data is presented in this table. Different letters within each assay represent there is a statistically significant difference (p < .05)

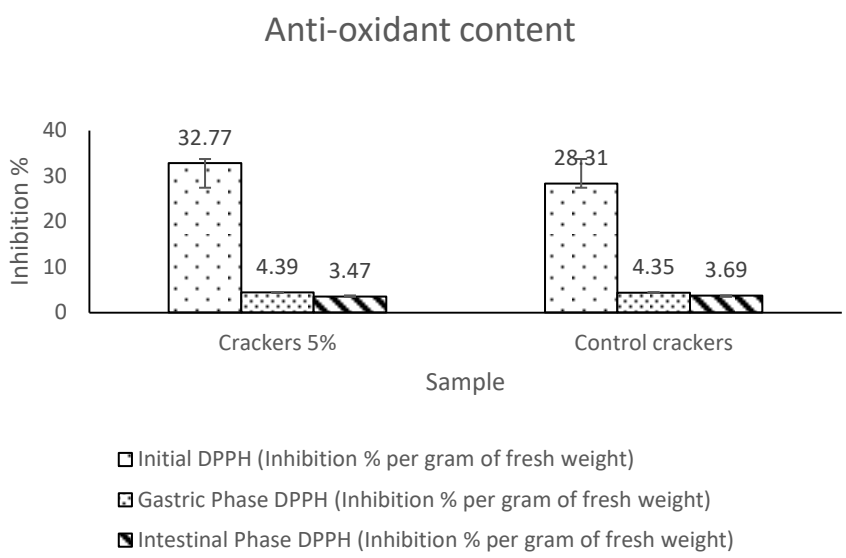


Fig. 6. The total antioxidant content of crackers subjecting to simulated invitro gastric and intestinal digestion and initial-methanolic extract of crackers. Average quantities ± SD of three independent samples data is presented in this table. Different letters within each assay represent there is a statistically significant difference ($p < .05$)

values of finger millet proximate composition were matched with the proximate composition of finger millet obtained in [18], and [19] research studies. The proximate composition values obtained for whole wheat were almost same as the values obtained in the [20] study. The values obtained for rice bran were present in-between the range of values mentioned in the [21] study and the values obtained for the proximate composition of dried *G. sylvestre* leaves were same as the values mention in the [22] research study.

Carbohydrates are the major component found in cereals. These carbohydrates support the provision of enough energy and calories by the cereals. Accordingly, cereals such as whole wheat (77.38±0.01) and finger millet (77.38±0.01) have comparatively high carbohydrate content when compared with rice bran and *G. sylvestre* leaves. Further, when considering these raw ingredients, whole wheat flour has a higher carbohydrate content (77.38±0.01), and rice bran has a lower carbohydrate content (32.10±0.01).

The protein content of the raw ingredients was in the range of 11.08±0.09 to 7.48 ±0.19. Whole wheat had 9.79± 0.17 % of protein. Whole wheat has two major proteins, namely gliadin and glutenin, which are responsible for forming the gluten protein. This gluten protein is the major reason for the elasticity and extensibility of the dough. Further gluten support to form the proper texture of crackers as the final product. Rice bran has a high crude fat content (18.52±0.35%) when compared with other raw ingredients. Presence of high fat content in rice bran is the major reason for the shorter shelf life of rice bran. Because fat present in the rice bran easily undergo rancidity reaction and by that it affects the quality and shelf life of the rice bran.

Rice bran is the outer covering of the rice. This is a rich source of fiber when considering other parts of the rice kernel. The result also shows that among the four raw ingredients, rice bran has the highest fiber content (18.74±0.73). *G. sylvestre* leaves have the next higher percentage of fiber content (15.57± 0.23). Fibers present in plant material such as leaves, fruits, and bran decrease the absorption of glucose in the intestine. Due to this, food substance with higher fiber content supports the control of non-communicable diseases such as diabetes mellitus and high cholesterol levels.

4.2 Functional Properties of Raw Materials

Phytochemicals are rich in plant species, therefore plant-derived products are considered as functional foods. Each raw ingredient was analyzed for its total phenolic content (TPC), total

flavonoid content (TFC), and total carotenoid content (TCC), antioxidant property, anti-inflammatory property, and anti-diabetic property to determine the functional properties of each raw material. The results obtained are summarized in Table 2. According to the results, *G. sylvestre* leaves had high TPC (51.89±0.28), TCC (0.275±0.004), and anti-diabetic property (70.37±0.68). Rice bran had a high TFC (9.25±0.14), and antioxidant property (76±0.87). Whole wheat had a high anti-inflammation property (79.36±3.63). Further, rice bran had high TPC (19.57±0.4) and TCC (0.118±0.003) next to *G. sylvestre* leaves. Studies by [12] showed that red rice bran varieties have high TPC, TFC, and TCC when compared to white rice bran. The presence of high TPC, TFC, and TCC shows that *G. sylvestre* leaves have high bioactive compounds compared to other raw materials. Presence of high bioactive components such as phenol, flavonoid and carotenoid contribute to the enhancement of food substance antioxidant property.

There are several methods used in antioxidant assays, including Ferrous Chelating Activity, Trolox Equivalent Antioxidant Capacity (TEAC), and 2,2 Diphenyl 1-1-picrylhydrazl (DPPH). DPPH is an extensively used substrate to evaluate antioxidant activity. This study used the DPPH radical scavenging assay to evaluate the free radical scavenging ability of each raw material. A higher percentage of DPPH radical scavenging activity of extracts indicates the higher antioxidant activity of samples in terms of hydrogen donating capacity [12]. Based on the results, rice bran (76±0.87) and *G. sylvestre* leaves (58.99±1.67) had high antioxidant properties when compared to other raw ingredients.

The anti-inflammatory property of the raw materials was evaluated using a protein denaturation assay. The anti-inflammatory property is present in food substances to support the defense mechanism of the body against pathogenic entry, injuries, and diseases. Based on the results, whole wheat has a high anti-inflammatory property (79.36±3.63) when compared to others. Furthermore, the anti-inflammatory properties of each ingredient are higher than 50%. This shows that all four ingredients have high anti-inflammatory properties.

The anti-diabetic property of each raw ingredient was evaluated using an alpha-amylase inhibition assay. Alpha-amylase is one of the key enzymes that is involved in starch digestion, which breaks down polysaccharides into monosaccharides and disaccharides and increases the postprandial blood glucose level. The plant materials have different levels of anti-diabetic properties. Plant material with high anti-diabetic properties inhibits the activity of alpha-amylase at a high level. By that, it controls the breakdown of starch into its monomers and prevents the increase of postprandial glucose levels. So, if a plant material has high anti-diabetic properties, then it will support to control the blood glucose level and by that, control the diabetic condition. Accordingly, the anti-diabetic property of these four ingredients was in the range of 45.48 – 70.37%. This indicates that all these four ingredients have high anti-diabetic properties and among these four raw materials, *G. sylvestre* leaves had the highest anti-diabetic properties (70.37±0.68). Different studies show that *G. sylvestre* leaves have different components to control the postprandial glucose level [23]. Because of those components, it shows high alpha-amylase inhibition.

4.3 Sensory Evaluation of Crackers

Sensory evaluation of crackers that were replaced with different percentages of *G. sylvestre* leaves, was shown in Fig. 1. Crackers had three different percentages of *G. sylvestre* leaves such as 1%, 5%, and 8%. The appearance, texture, taste, flavor, color, and overall acceptability of crackers were evaluated in the sensory evaluation. Results obtained for the sensory evaluation show that the addition of *G. sylvestre* leaves to the cracker preparation strongly influenced panelists' acceptance of the crackers. Accordingly, crackers substituted with 1% and 5% provide almost the same responses in appearance, texture, taste, flavor, and color. While crackers substituted with 8% leaves obtained a relatively lower score when compared with the other two substitutions. Further, the highest overall acceptance was given to the crackers incorporated with 5% *G. sylvestre* leaves. So, based on the sensory evaluation, crackers substituted with 5% *G. sylvestre* leaves were considered the most acceptable crackers with high sensory qualities.

4.4 Proximate Composition of Crackers

The proximate composition results for crackers with 5% substituted leaves and control crackers are mentioned in Table 3. Based on the results,

the nutritional qualities of the formulated products were enhanced due to the incorporation of *G. sylvestre* leaves when compared with control crackers.

Accordingly, moisture content can serve as an indicator of the shelf life of products because presence of high moisture content means the presence of high free water in the food substance. Presence of free water support for the several deteriorate biological, chemical, and microbial reactions in the food which affects the shelf life of the food products. It is considered that, moisture content at 3-7% can reduce the possibility of microorganism growth and damaging chemical reactions such as hydrolysis and fat oxidation [17]. Since the cracker is a dried product, it is expected to have a lower moisture content. All the samples recorded a moisture loss of less than 13%, which is good for preventing mold growth, spoilage, and poor quality of these crackers [15]. Based on the results obtained, both control crackers and crackers with 5% leaves have a less moisture content. This shows that both crackers have better shelf life. But the moisture content of crackers with 5% leave substitution (3.89 ±0.19) is slightly higher than the control crackers (2.67 ±0.58). This increase in moisture content may be attributed to the presence of hydroxyl groups in the fiber of leaves, which allows more water interaction through hydrogen bonding [16].

Ash content indicates the mineral content in food products. Ash content of the crackers with 5% leave substitution (6.6±0.01) is higher than the control (5.46± 0.22). The increase in the amount of ash content in crackers was caused by the addition of minerals originating from the *G. sylvestre* leaves powder which was added to the formulation. Shelbaya, [24] also indicated that crackers were a higher score in fiber due to the high content of its additives (*G. sylvestre* leave powder). This shows that the cracker samples had high mineral and fiber content than the control crackers.

Protein is one of the macronutrient groups besides fats and carbohydrates. Protein plays a role in forming biomolecules rather than as a source of energy [17]. The protein content of crackers with 5% leave substitution (11.19±0.1) is slightly higher than control crackers (10.19± 0.35). This show that the crackers sample have higher biomolecules when compared to control. With these cracker sample had high fiber content and less carbohydrate content when compared to control crackers. Presence of less carbohydrate and higher amount of fiber and ash will support to control the non-communicable diseases. So crackers sample with 5% leave substitution has higher chances of controlling non-communicable diseases than the control when comparing the proximate composition of both samples.

4.5 Functional Properties of Crackers

High levels of bioactive compounds can influence glucose metabolism. Incorporation of bioactive components like *G. sylvestre* leaves and rice bran into cereal flour for crackers formulation can contribute to the bioactivity of the crackers. Accordingly, functional properties of crackers with 5% leave substitution and control crackers were determined by the analysis of TPC, TFC, TCC, antioxidant property, anti-diabetic property, and anti-inflammatory property. The results obtained are summarized in Table 4. According to the result, crackers with 5% leave substitution had a slightly high levels of TPC, TFC, TCC, antioxidant property, anti-diabetic property, and anti-inflammatory properties when compared to control crackers. When considering the bioactive components such as phenol, flavonoid and carotenoid present in the cracker sample, crackers with 5% *G. sylvestre* leaves substitution have high bioactive components when compare with control crackers. This result shows that incorporation of *G. sylvestre* leaves powder significantly increases the functional properties of crackers.

Antioxidants play a vital role in defending the human body against generated free radicals. Based on the results, the antioxidant property of crackers with 5% leaves is 32.77±0.64% which is slightly higher than the antioxidant property of control 28.31±0.24%. This shows that the addition of *G. sylvestre* leaves significantly increases the antioxidant property of crackers. These antioxidant activities of the cracker biscuits suggest that the formulated snacks may be practical radical scavengers, capable of combating key degenerative diseases associated with free radicals as well serving as a functional snack for dietary intervention [25].

Anti-inflammatory property of a food product is supported to control the inflammation in the body. Based on the result obtained, anti-inflammatory property of crackers with 5% leave substitution and control crackers are (71.42±2.38), and (65.88±1.37) respectively. This

show both crackers with 5% leave substitution and control crackers have high anti-inflammatory properties.

The anti-diabetic property of crackers was determined using an alpha-amylase inhibition assay. Percentage α-amylase inhibition increased with an increase in supplementation with *G. sylvestre* leaves. That means, control crackers showed inhibitory activity of 40.15(±1.12) % while crackers with 5% leave exhibited the highest α-amylase inhibitory activity 48.3(±0.68) %. This shows that crackers with 5% leaves substitution have nearly 50% anti-diabetic properties and can have the ability to control Diabetic Mellitus. With that, the high percentage of α-amylase inhibition may help to slow down the absorption of carbohydrates after food intake. The supplementation of the biscuits with *G. sylvestre* leaves may be responsible for the good digestive enzyme inhibitory activity of the biscuits. Furthermore, the functional properties like flavonoids and phenols fractions for raw *G. sylvestre* leaves, finger millet, and rice bran were higher than their mixtures crackers. These results are due to the high instability of polyphenols that cause numerous reactions in the course of food processing [26].

4.6 Physical Characteristics of Crackers

Physical characteristics of crackers were measured to determine the effect of supplementation of *G. sylvestre* leaves on stack weight, stack height, and spread ratio of crackers. The results obtained for physical characteristics of both crackers with 5% leaves substitution and control were summarized in Table 5. According to the results obtained, the stack height, weight, and spread ratio of crackers with 5% leave substitution are almost equal to the stack height, weight, and spread ratio of control. This showed that the addition of *G. sylvestre* leaves to crackers was not much affected the physical characteristics of crackers.

4.7 *In-vitro* Gastro-Intestinal Digestion of Crackers

In this study, an *invitro* gastrointestinal digestion analysis was done to identify the bio accessibility of total polyphenols, total flavonoids, and carotenoids present in the crackers. Further, changes in antioxidant activities of antioxidant compounds in crackers at different digestion phases also have been analyzed. Biomolecules such as polyphenols, flavonoids, and carotenoids which are in crackers want to be extracted to be bio accessible and bioavailable. In the digestive system, the gastrointestinal tract act as an extractor because mechanical and chemical actions take place during digestion at gastrointestinal tract support to extract the bioactive compounds from the food [27]. The bioavailability of phenols, flavonoids, and carotene content in the gastric phase is less when compared to the initial stage and intestinal phase. This may be due to the presence of low pH around 2.0 in the simulated gastric digestion which could affect the stability of low molecular weight polyphenols. Fig. 2 shows the total phenolic content of the *in-vitro* gastrointestinal digestion. Based on the results, total polyphenol content in all digested fractions of both crackers with 5% leave substitution and control was lower when compared to their methanolic extracts. This may be due to the partial release or degradation of polyphenols during the gastric and intestinal digestion process [13]. The total phenolic content of crackers with 5% leave is higher than the control in all three situations (initial stage, gastric phase, and intestinal phase). This shows the bioavailability of phenol in sample crackers is slightly higher than in the control crackers.

The total flavonoid content of the sample crackers and control at the initial stage, gastric phase, and intestinal phase was summarized in Fig. 3. Same as phenolic content, flavonoid content of crackers with 5% leave and control was high in the initial stage when compared to gastric and intestinal phase. Further, the total flavonoid content at the initial stage, gastric phase, and intestinal phase were high in crackers with 5% leave when compared to control. So, the bioavailability of flavonoid content in crackers with 5% leaves is high than the control crackers.

The total carotene content of the sample crackers and control at the initial stage, gastric phase, and intestinal phase was summarized in Fig. 4. Based on the result, carotene content was high in the intestinal phase for both sample cracker and control when compared to the initial stage and gastric phase. Further in the initial stage and gastric phase carotene content was high for crackers with 5% leaves but in the intestinal phase, carotene content is high for control when compared to crackers with 5% leaves.

Based on the results obtained for *invitro* gastrointestinal digestion, crackers with 5% leaves provide comparatively high bioactive components when compared to control. Due to these bioavailable components, crackers with 5% leaves had high antioxidant properties than the control.

The antioxidant property of sample crackers and control at the initial stage, gastric phase, and intestinal phase was determined using DPPH radical scavenging activity. The values obtained for DPPH inhibition % were summarized in Fig. 5. Based on the results, DPPH inhibition % was high for crackers with 5% leaves when compared to control at the initial stage, and gastric phase. But for the intestinal phase, DPPH inhibition % was nearly equal for both control and crackers with 5% leaves. This result shows that crackers with 5% leaves had high antioxidant property at the initial stage and gastric phase. While control and crackers with 5% leaves substitution had nearly equal antioxidant properties at the intestinal phase.

5. CONCLUSION

The aim of this study was developing crackers using whole wheat, finger millet, rice bran and *G. sylvestre* leaves and analysis the nutritional and functional properties of both raw ingredients and crackers. Based on this research study, it can be observed that all the raw ingredients namely whole wheat, finger millet, rice bran and *G. sylvestre* leaves have higher nutritional and functional properties. Among them *G. sylvestre* leaves have higher phenolic, carotenoid content, and rice bran have higher flavonoid content. Further *G. sylvestre* leaves have high anti-diabetic property, whole wheat has high anti-inflammatory property and rice bran has high antioxidant property.

Based on the sensory evaluation, crackers substituted with 5% *G. sylvestre* leaves is considered as the best formulation among those different formulation. Further increasing the amount of *G. sylvestre* leaves in crackers preparation increases the nutritional, functional properties, and bio availability of bio active components such as phenol, flavonoid, and carotenoid compounds. But addition of *G. sylvestre* leaves in crackers preparation does not affect the physical characteristics of crackers. So, *G. sylvestre* leaves can be incorporated along with whole wheat, finger millet and rice bran to enhance the nutritional and functional properties of crackers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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