Italian Journal of Food Science, 2022; 34 (2): 50–66

Exploring the potential of bottle gourd (*Lagenaria siceraria*) flour as a fat mimetic in biscuits with improved physicochemical and nutritional characteristics and anti-diabetic properties

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Received: 20 October 2020; Accepted: 12 May 2021; Published: 28 April 2022

PAPER

Abstract

Bottle gourd (*Lagenaria siceraria*) is a naturally rich source of several phytochemicals that play a vital role in the prevention of diseases. In the present study, the effect of bottle gourd flour (BGF) as a fat mimetic in biscuits at different concentrations (10, 15, 20, 25, and 50% w/w) was explored**.** Rheological properties, the microstructure of dough, bioactive compounds, anti-diabetic potential, storage stability, and the nutritional, physical, and sensory properties of samples were investigated. The addition of BGF in wheat flour (WF) showed increased water absorption (58.70–72.43%) and reduced dough stability time (11.71–5.01 min). Trough (1165.11–1096.10 Torque) and set back viscosities (471.21–448.09 Torque) decreased significantly ($P \leq 0.05$) with the increased level of BGF in WF. Furthermore, gluten content was also reduced. The baking process decreased the IC_{50} values, although total phenol content and total flavonoid content decreased. Shelf life study of 2 months revealed no significant ($P \leq 0.05$) difference in bioactive compounds, and alpha-amylase inhibition activity of fat-replaced biscuits. Moreover, the anti-hyperglycemic activity of BGF-WF biscuits in human subjects also showed positive results. The percentage increase in both peroxide value (0.2–2.87 milliequivalent O_2/kg of fat) and free fatty acid (0.1–0.9%) during storage was significantly ($P \leq 0.05$) lower than control biscuits (0.9–5.53 mill-equivalent O_2/kg of fat and 0.2–1.1%), which correlates to the enhanced activity of bioactive compounds in BGF-WF biscuits, which in turn relates to decreasing the rate of oxidation. The crude fiber and protein contents of BGF-WF biscuits were improved from 0.21 to 17.30% and 11.20 to 18.70%, respectively. Sensory and textural performance exhibited that the biscuits were acceptable after fat replacement of up to 15% BGF. Based on this study, BGF may be suggested as an excellent natural fat replacer to be used in nutraceuticals and functional foods.

Keywords: anti-diabetic potential; antioxidant; bottle gourd flour; dough rheology; physical properties; sensory profile

Introduction

Obesity is one of the biggest challenges of the present world. People are becoming highly conscious of their body weight due to the increased risk of obesity-linked diseases like diabetes mellitus, hypertension, cardiovascular disease, and cancer. Possible ways to control obesity are reducing the intake of fat, sugar, salt, limiting kilocalories in the daily diet, and increasing the consumption of high fiber vegetables and fruits. That's why ingredients

ISSN 1120-1770 online, DOI 10.15586/ijfs.v34i2.1954

that could replace fats and sugars are being extensively explored for applications in food industries.

Lagenaria siceraria is the most popular fruit vegetable throughout the world. It belongs to the family Cucurbitaceae and is commonly called as Bottle gourd (BG) or Lauki. Among the different vegetables grown in India and Pakistan, BG is more commonly consumed because it is inexpensive and well-known for its medicinal properties (Attar and Ghane, 2019). Several studies have revealed that BG served as an antidiabetic and anticancer agent. Besides, BG is also used to treat various neurological disorders like Alzheimer's disease. Nutritional evaluation reveals that it is a rich source of various nutrients such as vitamin B, C, pectin, fibers, beta-carotene, amino acids, proteins, and glycosides (Attar and Ghane, 2019). Its pulp and seeds have been used to manufacture different food products; high fiber and protein-rich biscuits were prepared by incorporating BG seed powder in wheat flour (Patel and Pradhan, 2015). In another study, BG pulp was used for the preparation of BG sweet meat and was evaluated for sensorial and textural properties (Saini and Sharma, 2018). On the basis of its diverse functional properties, bottle gourd flour (BGF) can be considered as a great source of fat mimetic. For instance, the presence of the nonpolar compounds confers lipid-soluble flavor-carrying capacity while polar groups facilitate water-binding, which together help to generate creaminess and lubricity in foods similar to that found in full-fat products (Saeed *et al.*, 2020a).

Numerous studies have been conducted based on the utilization of dietary fiber or inulin as a fat replacer in biscuits and analyzed for its chemical, physical, and sensory properties. Saeed *et al.* (2021) studied the effect of fat replacement by date pit flour on the rheological and other quality parameters of the biscuits. Furthermore, Saeed *et al.* (2020c) studied the utilization of lotus root flour as a fat replacer in biscuits, while Roman *et al.* (2015) proved that extruded wheat flour paste with the addition of emulsifier could be an active fat replacer in cakes formulation.

Although some studies have demonstrated favorable health effects of bakery products that contain fat replacer or fat mimetic components, no information is available on the use of BGF as a fat mimetic. Therefore, this study was proposed to evaluate the functional properties of BGF and the effect of its incorporation at different levels (10–50%) on the physicochemical properties, rheological properties, microstructure of dough, nutritional profile, physical parameters (dimensional, color, and textural), and sensorial attributes of biscuits. Furthermore, the dough and biscuits were evaluated for their antioxidant activity, bioactive compounds, antidiabetic properties, and storage stability, so that new type of functional biscuits with optimum sensory and nutritional benefits could be introduced at the industrial level.

Materials and Methods

Raw materials

Commercial wheat flour (WF) made from wheat *Triticum aestivum* sp. *vulgare* (hexaploid) was received from Graib Sons Private Limited, Karachi. Icing sugar, BG, whole fresh egg, and salt were purchased from the local market of Karachi. Semi-solid fat (partially unsaturated) was purchased from Paracha Mills Ghee Textile Unit, Karachi. Glucose, sodium bicarbonate, and soy lecithin were obtained from Sulop Chemicals located in Karachi. All chemicals used for the study were of analytical reagent grade procured from Dae-Jung Chemicals, South Korea and Sigma-Aldrich, Germany.

Preparation of BGF

The BGs were thoroughly washed in distilled water, peeled, and then deseeded. BG was manually cut into thin slices of thickness 0.002 cm and dried in a hot air oven (DSO-300 D, digisystem laboratory instruments, Taiwan) at 60°C for 8 h. Dried BG flakes were milled by a laboratory miller (3100, Perten Instruments) and then passed through a sieve of 60 μm. The fine bottle gourd flour (BGF) was stored in airtight glass bottles at 4°C. Varying concentrations of BGF [10, 15, 20, 25, and 50% (w/w)] were added to wheat flour as a fat mimetic to have sample flours of varying wheat to BGF ratios, represented as BGF-WF.

Proximate composition

Proximate profiles of BGF-WF blends were analyzed according to the method of Ali *et al.* (2018). Moisture was determined by moisture analyzer (Brabender 51–55, CW Brabender, Duisbury, NJ, USA). Protein and ash contents were estimated by Brabender Kernelyzer (Brains Instruments, Germany). Fat content was estimated by AACC Method 30-25 (AACC, 1999). Carbohydrate content was calculated by subtracting the contents of protein, fat, ash, and moisture from 100. Wet gluten (WG), dry gluten (DG), gluten index (GI), and total gluten contents (TGC) were estimated by Glutomatic (2200, Perten Instruments). Each sample was tested in triplicates for proximate analysis.

Dough rheological properties

The effect of the incorporation of BGF in wheat flour in various concentrations on rheological properties was studied by using Farinograph (mixer bowl 300g, Brabender OHG, Duisburg, Germany) by the AACC

Method 54-21(AACC, 2000). The parameters measured include water absorption (WA), dough development time (DDT), the degree of softening (DoS), dough stability time (DST), and farinograph quality number (FQN).

Pasting properties of flour

The pasting properties were studied by Microvisco-amylo-graph (Brabender, Duisburg, Germany) according to the AACC Method 22-10 (AACC, 2000). The parameters measured for each sample included the average values for peak viscosity (PV), final viscosity (FV), and breakdown and setback viscosities.

Functional properties

Water absorption capacity (WAC) and oil absorption capacity (OAC) of flour samples were determined by the method of Saeed *et al.* (2020a). Briefly, the WAC was determined by taking 1 g of the flour sample in a 20 mL centrifuge, and 10 mL of distilled water was added to it; the mixture was vortexed for 2 min. The tube was centrifuged at 2200×g for about 20 min and the supernatant was decanted while the residual pellets were weighed and analyzed for WAC. The same procedure was used to determine OAC using 10 mL soya bean oil.

Micromorphology of biscuits dough

The microstructure of biscuit dough was evaluated using Scanning Electron Microscope (JOEL, Analysis system, Model # JSM-6380, Japan), as described by Ali *et al.* (2018). Briefly, the samples were first frozen at −20°C, then transferred into a freeze dryer (Laboratory Freeze Dryer VaCo 2, Germany), which operated at −50°C and 0.1m Pa. Freeze-dried dough samples were cut transversally into slices with a sharp blade without damaging the structure. Samples were mounted on the sample holder and sputter-coated with gold (2 min, 2 mbar). Scanning electron microscopic (SEM) studies were carried out on an applied voltage of 15 KV at 500´ magnification.

Biscuit preparation

Formulations of biscuits samples are presented in Table 1. Control and BGF-WF biscuits were prepared by incorporating different levels of fat. For the preparation of biscuit samples, fat was replaced from the recipe by BGF at concentrations of 10, 15, 20, 25, and 50%. The standard biscuits recipe was followed, which consists of flour 100 g (VMF and wheat flour mixed according to different ratios of fat replacement), sugar 40 g, fat 40 g, salt 0.5 g, egg 13.4 g, glucose 0.5 g, sodium bicarbonate1 g, soya lecithin 0.25 g, and water 20±5 mL. Biscuit samples were prepared, as reported by Ali *et al.* (2018). Briefly describing, the dough was sheeted at a fixed thickness of 7.2 mm and was cut into circular shapes using a biscuit cutter having a fixed diameter of 36.3 mm. Biscuits were baked at 180°C for 15 min in an oven. About 25 biscuits were baked per batch, and each batch was prepared in triplicates.

Antioxidant activity and phytochemicals

Antioxidant activity of BGF-WF blends, BGF-WF biscuits, and control biscuits was determined by free radical scavenging activity and ferric reducing power. Extracts of BGF-WF blends, control, and BGF-WF biscuit samples were prepared by the method of Saeed *et al.* (2021). The blends of BGF-WF and biscuit powder (control and BGF-WF) were added to methanol at a concentration of 60 mg/mL, 125 mg/mL, 185 mg/mL and 250 mg/mL, and the mixtures were analyzed.

2,2-diphenyl-1-picrylhydrazyl-DPPH radical scavenging activity

The technique mentioned by Saeed *et al.* (2020c) with slight modification was utilized for determining the

Table 1. Composition of biscuits prepared from bottle gourd flour (BGF).

S.No.	Ingredients (g)	Control	BGF (10%)	BGF (15%)	BGF (20%)	BGF (25%)	BGF (50%)
1	Flour	100	100	100	100	100	100
2	Fat	40	36	34	32	30	20
3	Icing Sugar	40	40	40	40	40	40
4	Egg	13.4	13.4	13.4	13.4	13.4	13.4
5	Soya Lecithin	0.25	0.25	0.25	0.25	0.25	0.25
6	Baking Powder	1	1	1	1	1	1
7	Salt	0.5	0.5	0.5	0.5	0.5	0.5
8	Glucose	0.5	0.5	0.5	0.5	0.5	0.5
9	Water	20±5	20±5	20±5	20±5	20±5	20±5
10	BGF	–	4	6	8	10	20

antioxidant activity of extracts of BGF-WF blends, control, and BGF biscuits. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution was prepared by dissolving 33.9 mg of DPPH in 100 mL of methanol. One milliliter of each of the prepared samples (having concentrations of 60 mg/mL, 125 mg/mL, 185 mg/mL, and 250 mg/mL) was mixed with 1 mL of DPPH solution in a test tube and was placed in the dark for about 30 min. Absorbance (Abs) values were estimated at the wavelength of 517 nm using a spectrophotometer (Perkin Elmer, Lambda 25, and UV-Vis Spectrophotometer). The percentage scavenging activity was determined as follows.

$$\text{Scavenging activity \%} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100 \qquad (1)$$

Ferric/Ferricyanide (Fe^{3+}) reducing antioxidant power

Ferric/Ferricyanide reducing antioxidant power (FRAP) values of the samples were analyzed according to the method of Gawlik-Dziki *et al.* (2014) with slight modification adapted by Saeed *et al.* (2021). Perl's Prussian color was measured at an absorbance of 700 nm, where the increase in Abs is an indication of increased antioxidant activity.

Total phenolic content

Total phenolic content (TPC) of samples were determined by using Folin–Ciocalteu reagent, as described by Salar and Purewal (2017). The Abs of the extracts was recorded at 765 nm against a blank, and the results were expressed as milligram Gallic acid equivalent/100 g (mg GAE/100g) of the extract on dry weight (dw) basis obtained from the standard calibration curve.

Total flavonoid content

TFC of BGF-WF and biscuit samples were determined by the method of Gbenga *et al.* (2018). The Abs of the extracts was measured at 510 nm using Catechin as standard, and results were expressed as milligram Catechin Equivalent (mg CAE)/100 g of the extract on dry dw basis.

In vitro alpha-amylase inhibition in BGF biscuits

Alpha-amylase inhibition test by Elshibani *et al.* (2020) based on the starch–iodine technique was used to determine the *In-Vitro* enzyme inhibition in BGF biscuits. Abs was estimated at a wavelength of 630 nm .The alpha-amylase inhibitory activity was determined as follows:

$$\text{Inhibition of alpha} - \text{Amylase (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100 \qquad (2)$$

where Abs control is the Abs of all reagents except the test sample, and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Anti-hyperglycemic of BGF biscuits In-vivo

The anti-hyperglycemic activity of biscuits was determined in human subjects using the oral glucose tolerance test (OGTT) according to the method of Fombang and Saa (2016). Incremental area under the blood glucose response curve (IUAC) was calculated according to the method recommended by the Food and Agricultural Organization (1998).

Glycemic Index value was calculated as follows:

$$\text{Glycemic index (GI)} = \frac{\text{IUAC for the test food}}{\text{IUAC for the standard food}} \times 100 \qquad (3)$$

Evaluation of changes in biscuits during storage

BGF biscuits and control biscuits were stored at room temperature (27 ± 2) in an air tight container at relative humidity of 67%. After every 15 days, shelf life study was performed for 2 months. And, biscuits samples were analyzed for free fatty acid value (FFA), peroxide value (PV), antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC), and water activity (a_w)

Determination of free fatty acid (FFA) and Peroxide value (PV)

The FFA content of biscuit samples were analyzed by AOAC Method Cc 5a-40 (AOAC, 2001). The FFA content was calculated as the percentage of oleic acid according to the following equation:

$$\text{FFA as \%Oleic acid} = \frac{\text{ml NaOH} \times \text{NaOH normality} \times 28.2}{\text{Weight of sample (g)}} \qquad (4)$$

PV of biscuit samples was analyzed by the AOAC Method Cd 8-53, (AOAC, 2001)

Evaluating the effect of storage changes on antioxidants, alpha amylase inhibition, and phytochemicals

The impact of storage conditions on the antioxidant activity was determined by FRAP and DPPH tests, while alpha amylase inhibition, TPC, and TFC were determined as described earlier in the above sections (2.9 and 2.10).

Water activity to evaluate the possibility of microbial growth

Water activity (a_w) of biscuits samples was determined in two duplicates of each formulation, using a Decagon

Aqua Lab meter (Pullman, WA, USA) at room temperature (25 ± 2°C), calibrated with a saturated potassium acetate solution ($a_w = 0.22$) (Saeed *et al.*, 2020a). Sufficient amount of sample was taken in the sample holder, and precaution was taken so that the sample does not touch the sensor.

Evaluation of biscuits quality

Color analysis

Color was measured according to the method of Saeed *et al.* (2020b) by using NH3 Colorimeter (China). Color values L*, a*, and b* were recorded, each value being the average of four measurements at different points of the biscuits. L* value represents the lightness variable from 100 for perfect white to zero for black, while as a* and b* values are the chromaticity values that indicate (+) redness/(-) greenness and (+) yellowness/(-) blueness, respectively.

Textural analysis

Biscuits sample were studied for the effect of fat mimetic on its breaking strength (hardness) and fracturability using texture analyzer (UTM, Zwick/Roell, Germany) as per the method described by Kuchtová *et al.* (2018) utilizing three points bend rig technique (Load cell: 5 kg, pre-test speed: 1.0 mm/s, test speed: 5.0 mm/s, post-test speed: 10.0 mm/s, distance: 10 mm, trigger force: 50 g).

Dimensional analysis

The diameter or the width of biscuits was measured with the help of a venire caliper (twice by rotating the biscuit at 90°C). The thickness of biscuits was measured by stacking three biscuits on top of one another and the total height was divided by three to get the average value. The spread ratio of biscuits was calculated from the fraction of diameter and thickness (Kohajdová *et al.*, 2014).

Nutritional analysis

Nutritional analysis of samples included the analysis of protein, fat, carbohydrate, ash, moisture, crude fiber, and kilocalories. Protein and ash contents were determined by the Kjeldahl apparatus (Thermo Fisher Scientific) and Muffle furnace (Thermo Fisher Scientific), respectively, according to AACC Methods 08-01 and 46-10, respectively (AACC, 2000). Fat content and crude fiber were determined by the Soxhlet apparatus (Thermo Fisher Scientific) and Fiber digester (Marconi, MA-444, Brazil), respectively, by using AACC Methods 30-25 and 32-10, respectively (AACC, 2000). Moisture content was determined by moisture analyzer (Brabender 51–55, CW Brabender, Duisbury, NJ, USA). The total carbohydrate was determined by difference: Carbohydrate = 100 − (% moisture + % protein + % fat + % ash + % crude fiber). Calories were measured by applying the Atwater general factor system: carbohydrate (4 Kcal/g), lipid (9 Kcal/g), and protein (4 Kcal/g).

Sensory evaluation

Sensory examination was carried out in the baking laboratory following the method of Ali *et al.* (2018). Biscuit samples were evaluated by 40 trained panelists, male and female (age 24–45), comprising mainly of students and staff members of the Department of Food Science and Technology, University of Karachi (Karachi, Pakistan). The panelists were trained by utilizing the sensory profiles method (Lawless and Heymann, 2010) with the commercial biscuits and prototypes prepared in the baking laboratory. By means of this training, a specific terminology for the sensory characteristics and ranges for each attribute was agreed upon. The trained sensory panel passed the basic taste test, the odor test, and the color vision test, and their evaluation capacity were routinely verified by way of individual control cards. Panelists used 9 points hedonic scale (1 = extremely dislike to 9 = extremely like) for analyzing the desirability of biscuit samples for taste, color, appearance, texture, and overall acceptability

Statistical analysis

All the analyses were performed in triplicate and the average value was calculated. The results were expressed as mean ± standard deviation. The data were analyzed by analysis of variance (ANOVA) using SPSS (Version 17.0. Inc, Chicago, USA) statistical program. Duncan's multiple range test (DMRT) was applied to identify any significant differences among the treatments at $P \leq 0.05$. Furthermore, DMRT involves the computation of numerical boundaries that allow for the classification of the difference between any two treatment means as significant or nonsignificant. This requires calculation of a series of values each corresponding to a specific set of pair comparisons.

Results and Discussion

Proximate composition

Table 2 illustrates proximate compositions of BGF-WF blends. The moisture content of the refined wheat flour (14.1%) was slightly higher compared to the BGF-WF blends which ranged from 11.9 to 12.9%, and the decrease in moisture was due to less moisture content of raw BGF (8.01%) (Table 12). The increased ash content (0.93 to 2.02%) was due to the presence of a high amount of minerals in BGF than in the wheat flour. Similar results were reported by Saeed *et al.* (2020b) when black gram flour was incorporated in wheat flour as fat replacer. Since

Table 2. Effect of bottle gourd flour (BGF) addition on proximate and chemical properties of wheat flour.

Samples	Moisture content %	Ash %	Protein %	Gluten		
				Dry gluten	Wet gluten	Gluten index
Wheat flour	14.10 ± 0.13^{e}	0.43 ± 0.01^{a}	9.81 ± 0.10^{a}	9.20 ± 0.2^{d}	25.83 ± 0.26^{e}	95.00 ± 1.2^{d}
BGF 10%	11.91 ± 0.11^{a}	0.93 ± 0.03^{b}	13.82 ± 0.15^{b}	9.00 ± 0.18^{d}	24.00 ± 0.22^{d}	92.00 ± 1.42^{f}
BGF 15%	12.10 ± 0.12^{b}	0.98 ± 0.05^{b}	14.10 ± 0.18^{bc}	8.80 ± 0.16^{c}	24.20 ± 0.25^{d}	88.00 ± 1.21^{e}
BGF 20%	12.21 ± 0.10^{b}	1.05 ± 0.08^{c}	14.42 ± 0.19^{c}	8.40 ± 0.15^{bc}	23.60 ± 0.21^{c}	86.00 ± 1.08^{c}
BGF 25%	12.50 ± 0.13^{bc}	1.09 ± 0.09^{c}	14.91 ± 0.19^{d}	8.30 ± 0.13^{b}	18.70 ± 0.19^{b}	84.00 ± 1.06^{b}
BGF 50%	12.90 ± 0.12^{d}	2.02 ± 0.09^{d}	18.52 ± 0.21^{e}	7.70 ± 0.11^{a}	11.30 ± 0.14^{a}	78.00 ± 1.03^{a}

Values expressed are the mean ± standard deviation (n = 3). Means in the column with different superscripts are significantly (P ≤ 0.05) different.

protein in BGF has remarkable water holding property, the percentage moisture, WAC (%), and farinograph WA increased correspondingly (Table 3–4); the same results were observed by Saeed *et al.* (2020b). The wheat flour contained less protein content (9.8%) than the BGF as increasing the amount of BGF in wheat flour significantly increased (P ≤ 0.05) the protein content (13.8 to 18.5%). Gluten content (dry and wet) and gluten index also decreased as the level of incorporation of BGF increased in wheat flour, which might be due to dilution of gluten network which was also confirmed by micrograph scanning images (Figure 1). All these findings were in agreement with the study conducted by Saeed *et al.* (2020b).

Functional properties

Water absorption and oil absorption capacities

WAC and OAC are the critical functional properties of the food ingredients because they determine the texture, mouthfeel, and yield of the product (Ram and Singh, 2004). Table 2 represents the WACs of BGF-WF blends. The WAC ranged from 146 to 162%. The highest WAC was observed for BGF 50% (162%) followed by BGF 25% (155.43%) and lowest for BGF 10% (146%). The study revealed that more the amount of BGF incorporated, the more was the resultant WAC. In previous research, purple rice flour incorporated in wheat flour also showed similar behavior (Klunklin and Savage, 2018). Hence, result suggested that the addition of BGF to wheat flour affected the amount of water absorption, as BGF competes with wheat flour for water absorption, which may be due to the difference in chemical composition and structure of the BGF. Probably, carbohydrate structure and the polarity of amino acids are responsible for the WAC (Saeed *et al.*, 2021).

Table 3. Functional properties of wheat flour and different ratios of bottle gourd flour (BGF) incorporated in wheat flour.

Samples	WAC%	OAC%
Wheat flour	145.61 ± 2.08^{a}	153.03 ± 2.05^{g}
BGF	500.16 ± 3.52^{g}	100.21 ± 1.1^{a}
BGF 10%	150.26 ± 2.11^{b}	152.36 ± 2.25^{f}
BGF 15%	157.42 ± 2.15^{c}	150.22 ± 2.1e
BGF 20%	164.26 ± 2.05^{d}	148.36 ± 1.05^{d}
BGF 25%	170.26 ± 2.11^{e}	146.81 ± 1.17^{c}
BGF 50%	199.32 ± 2.57^{f}	137.34 ± 1.11^{b}

Values expressed are the mean ± standard deviation (n = 3). Means in the column with different superscripts are significantly (P ≤ 0.05) different. WAC, water absorption capacity; OAC, oil absorption capacity.

The OAC ranged from 100.2 to 137.3% in BGF-WF blends. OAC of the raw BGF was determined as 100%, whereas wheat flour showed maximum OAC, i.e., 153.03%. The data reported in Table 2 proved that OAC of BGF decreased with the increase in their concentration in wheat flour. Similar findings were reported by Klunklin and Savage (2018). Generally protein contains both hydrophobic and hydrophilic groups which are responsible for oil and water absorption (Saeed *et al.*, 2021), which is also proved by our results as the amount of BGF increased in wheat flour, degree of nonpolarity, and hydrophobicity decreased so did the OAC. Hence, BGF contains more water interactive proteins.

Dough rheological properties

Table 4 presents the dough mixing properties of BGF-WF blends. A continuous increase in WA (63.6 to 72.4%) and stability time was observed with increased amount of BG. Increased WA was probably due to the presence of dietary fiber in BGF. Similar findings of WA and stability were reported by Ahmad*et al.* (2015) when different ratios of green tea powder were incorporated in wheat flour. DDT and DoS also increased with the inclusion of BGF in wheat flour. The increase in DDT is due to the rise in WA of the dough. In general, high WA means excellent baking performance. On the other hand, wheat flour showed lower DDT, lower DoS, and higher DST (except BGF10%) than the BGF-WF blends. Inconsistency in the mixing properties of flour samples was attributed to an increase in fiber and protein content with the increased level of BGF (Bae *et al.*, 2014). Another reason could be the increased FQN, which gave a hardening effect to flour and strength to the dough and ultimately caused delay in DDT, DS, and DoS (Ali *et al.*, 2018).

Generally molecules of fat gives strength and elasticity to the dough matrix (Ali *et al.*, 2018). However, similar functions were performed by the protein moles of BGF in the absence of fat molecules.

Pasting properties of flour samples

The results of the pasting properties of wheat flour and BGF-WF blends are shown in Table 5. An increase in starch gelatinization was found with an increased amount of BGF in the wheat flour. It was due to the quick rupture of starch granules leading to lower pasting temperatures and higher paste consistency (Kuchtová *et al.,* 2018). Similar findings were reported when grape skin was added in the wheat flour *(*Kuchtová *et al.,* 2018). The highest peak viscosity was observed for BGF 10% and lowest for BGF 50% because of increment in nonstarch content (i.e., protein) upon addition of higher amount of BGF in wheat flour. The less breakdown viscosity was estimated for wheat flour which was related to the limited swelling of the starch granules. However, breakdown viscosity significantly ($P \leq 0.05$) increased when the level of BGF increased in wheat flour. The final viscosity decreased with an increased amount of BGF which resulted in less potential for the formation of viscous paste (Kuchtová *et al.*, 2018). In addition, trough and set back viscosities decreased significantly ($P \leq 0.05$) as the concentration of BGF increased in wheat flour. The decrease in trough and set back viscosities reflected the less retrogradation tendency and hence contributed to less staling rate of the biscuits (Saeed *et al.*, 2020b).

The microstructure of biscuits dough

The micrograph of the control biscuits dough showed (Figure 1A) a thin sheet representing protein matrix along with small and large starch granules embedded in it. SEM image of dough with BGF 10% (Figure 1B) was very similar to control biscuit dough; however, with the gradual increase in the concentration of BGF in dough

Table 4. Farinograph properties of wheat flour and different ratios of bottle gourd flour (BGF) incorporated.

Samples	WA (%)	DDT (min)	DST (min)	DoS (ICC)	FQN
Wheat flour	58.70 ± 0.2^{a}	1.67 ± 0.01^{a}	8.58 ± 0.18^{d}	35.12 ± 0.90^{a}	88.21 ± 1.12^{e}
BGF 10%	63.61 ± 0.20^{b}	2.21 ± 0.04^{b}	11.71 ± 0.18^{e}	78.11 ± 1.11^{b}	136.31 ± 1.52^{f}
BGF 15%	64.52 ± 0.33^{c}	5.20 ± 0.08^{c}	5.10 ± 0.15^{a}	129.10 ± 0.81^{c}	65.01 ± 1.10^{b}
BGF 20%	64.90 ± 0.51^{c}	5.71 ± 0.03^{d}	5.01 ± 0.11^{a}	143.09 ± 1.72^{d}	62.72 ± 1.11^{a}
BGF 25%	65.83 ± 0.58^{d}	5.82 ± 0.01^{e}	6.01 ± 0.13^{b}	153.32 ± 4.50^{e}	72.01 ± 1.12^{c}
BGF 50%	72.43 ± 0.81^{e}	5.80 ± 0.01^{e}	8.22 ± 0.16^{c}	168.11 ± 3.21^{f}	86.41 ± 1.14^{d}

Means with different letters in superscript within a column differ significantly, calculated by the Duncan method ($P \leq 0.05$). Each value was expressed as mean ± SD (n = 3). WA; water absorption, DDT; dough development time, DST; dough stability time, FQN; farinograph quality number.

Table 5. Microvisco-Amylo-Graph properties of wheat flour and different ratios of bottle gourd flour (BGF) incorporated.Means with different letters in superscript within a column differ significantly and are calculated by Duncan method ($P \leq 0.05$), each value is expressed as mean ± SD (n = 3).

Samples	Gelatinization (Torque)	Peak viscosity (Torque)	Trough (Torque)	Final viscosity (Torque)	Breakdown (Torque)	Setback viscosity (Torque)	Pasting temperature (°C)
Wheat flour	24.01 ± 0.11^{a}	1037.04 ± 10^{a}	717.08 ± 4.11^{a}	1542.32 ± 18.11^{f}	378.18 ± 4.11^{a}	479.21 ± 6.11^{e}	57.91 ± 0.23^{a}
BGF 10%	29.21 ± 0.32^{d}	1253.10 ± 14^{d}	1165.11 ± 11.01^{b}	1367.11 ± 16.21^{e}	456.14 ± 8.30^{f}	471.21 ± 7.42^{f}	76.01 ± 0.3^{d}
BGF 15%	30.01 ± 0.40^{e}	1222.01 ± 15^{e}	1116.15 ± 12.13^{d}	1299.12 ± 16.13^{d}	466.13 ± 7.50^{e}	493.41 ± 5.32^{d}	81.13 ± 0.34^{e}
BGF 20%	32.12 ± 0.60^{f}	1221.13 ± 18^{f}	1112.18 ± 13.10^{e}	1240.21 ± 13.01^{c}	485.13 ± 7.20^{d}	481.71 ± 5.10^{b}	81.13 ± 0.15^{e}
BGF 25%	33.31 ± 0.21^{b}	1217.30 ± 12^{c}	1099.11 ± 14.10^{f}	1120.91 ± 12.61^{b}	551.15 ± 6.42^{c}	457.14 ± 3.23^{c}	72.04 ± 0.17^{c}
BGF 50%	36.10 ± 0.36^{c}	1215.11 ± 12^{b}	1096.10 ± 10.00^{c}	1131.12 ± 11.21^{a}	613.19±5.11^{b}	448.09±2.21^{a}	68.03 ± 0.21^{b}

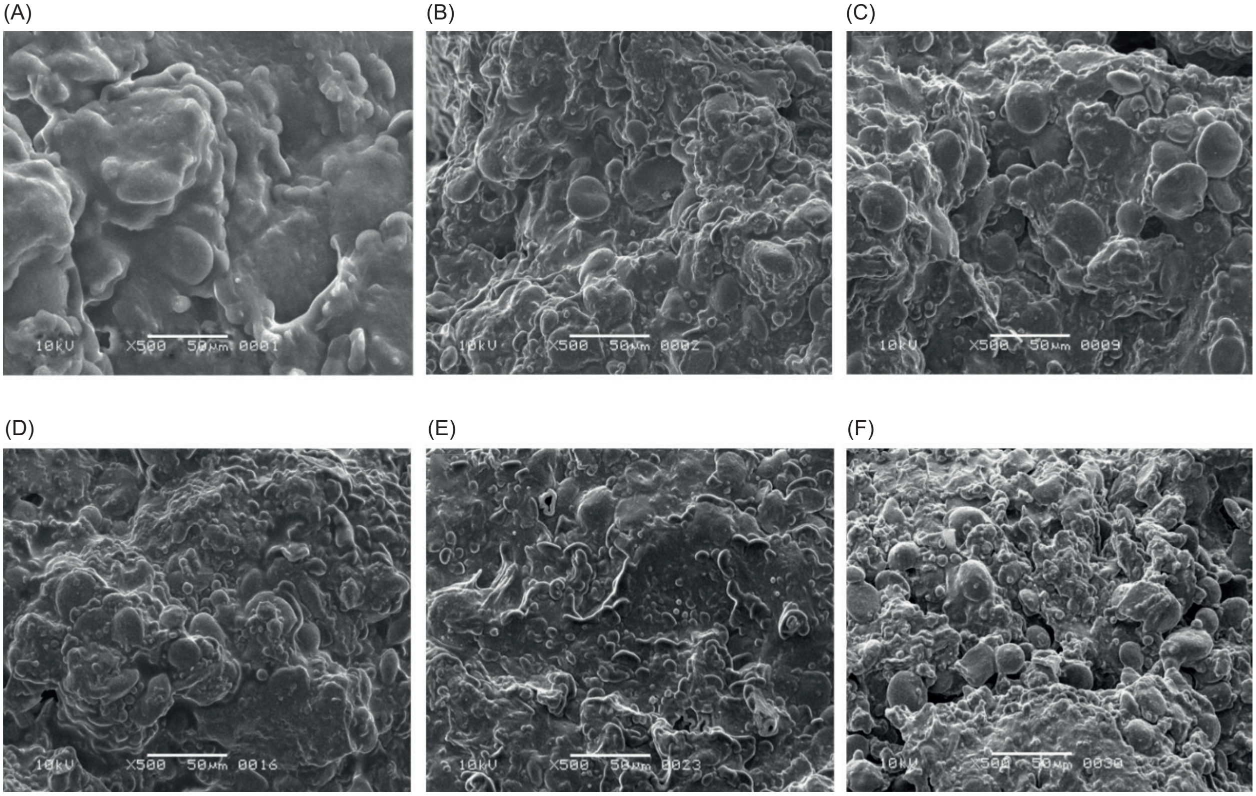

Figure 1. Scanning electron micrograph of biscuits dough (magnification, 500×): (A) control, (B) 10% bottle gourd flour, (C) 15% bottle gourd flour, (D) 20% bottle gourd flour, (E) 25% bottle gourd flour, and (F) 50% bottle gourd flour.

(Figure 1C), the BGF proteins were concentrated, and more and more large and small wheat starch granules were seen trapped in protein fibrils of BGF (Figure 1D and E). Similar observations were also depicted by Dachana *et al*. (2010) in their study on moringa leave powder which was used for the manufacturing of biscuits dough. Higher amount of fat substitution (Figure 1F) by higher level of fiber in BGF resulted in disrupted gluten matrix. Similarly, a reduction in gluten matrix was observed by Indrani *et al*. (2010) upon addition of 20% multi-grains. The literature revealed that even though microstructural properties of fat mimetics are quite different from fat when they are used to replace fat in food products, the end products show comparable qualities to the standard that is in agreement with our observations (Patel *et al*., 2020).

Free radical scavenging activity

The IC_{50} value of flour blends and biscuits samples are given in Tables 6 and 7, respectively. As predicted, the control biscuits demonstrated the lowest antioxidant activity and the IC_{50} value of BGF-WF biscuits reduced with the increment in the quantity of the BGF in biscuit samples. The increase in antioxidant activity of BGF-WF biscuits was probably due to the presence of bioactive compounds with hydrogen-donating ability such as vitamins, flavonoids, and phenols (Wojtunik-Kulesza *et al*., 2020). Results showed that storage time had no impact on antioxidant activity till the second week; however, it became obvious after the fourth week. Caleja *et al*. (2017) reported similar findings when fennel and chamomile extracts were used in biscuit samples; however, storage time did not influence antioxidant activity until the eighth week of storage. The development of antioxidative melanoidin pigments during baking further improved the free radical scavenging capacity (Sharma and Gujral, 2014). Similar observations were reported by Mabogo *et al.* (2021) when varying concentrations of unripe banana flour was incorporated in wheat-based biscuit samples.

Ferric/Ferricyanide (Fe^{3+}) reducing antioxidant power

The results of reducing the power of BGF-WF blends and biscuit samples are shown in Tables 6 and 7, respectively. FRAP value of biscuit samples increased with the incorporation of BGF. BGF-WF biscuits showed maximum reducing capacity, on the other hand, control biscuit samples demonstrated the highest IC_{50} value, hence the lowest reducing ability, which further became undetectable

Table 6. Antioxidant activity (DPPH and Ferric-reducing antioxidant power) and bioactive compounds (Total phenol content (TPC) and Total flavonoid content (TFC) of different levels of Bottle gourd flour (BGF)) incorporated in wheat flour.

Antioxidant activity			Bioactive compounds	
Samples	**DPPH (IC_{50})**	**FRAP (IC_{50})**	**TPC (mg GAE/100 g DW)**	**TFC (mg CE/100 g DW)**
Wheat flour	488.12 ± 3.15^{f}	327.21 ± 4.52f	16.73 ± 0.11^{a}	31.16 ± 1.01^{a}
BGF 10%	301.40 ± 1.29^{e}	127.35 ± 0.51^{e}	334.64 ± 1.18^{b}	174.82 ± 2.81^{b}
BGF 15%	245.62 ± 1.21^{d}	121.34 ± 0.21^{d}	437.81 ± 2.31^{c}	385.30 ± 2.14^{c}
BGF 20%	221.04 ± 1.01^{c}	89.32 ± 0.45^{c}	506.69 ± 3.42^{d}	456.83 ± 4.45^{d}
BGF 25%	200.60 ± 0.92^{b}	78.81 ± 0.13^{b}	543.37 ± 3.55^{e}	484.67 ± 4.61^{e}
BGF 50%	187.21 ± 0.43^{a}	57.62 ± 0.10^{a}	667.16 ± 3.81^{f}	583.65 ± 4.72^{f}

Means with different letters in the column differ significantly. They were calculated by the Duncan method (P ≤ 0.05). Each value was expressed as mean ± standard deviation (n = 3), Nd, not detected.
Where, GAE, gallic acid equivalent. CE, catechin equivalent. dw, dry weight of the sample. Nd, not detected.

Table 7. DPPH radical scavenging activity, Ferric/Ferricyanide (Fe^{3+}) reducing antioxidant power (FRAP), and Alpha amylase inhibition about storage period at different levels of Bottle gourd flour (BGF) incorporated in biscuit samples.

Samples	Initial	Second week	Fourth week	Sixth week	Eighth week
DPPH-Scavenging activity- IC_{50} (mg/mL)					
Control	500.02 ± 3.15^{p}	Nd	Nd	Nd	Nd
BGF 10%	151.63 ± 1.29^{j}	152.12 ± 1.37^{jk}	166.44 ± 1.55^{l}	183.82 ± 1.82^{o}	185.02 ± 1.87^{o}
BGF 15%	139.91 ± 1.21^{i}	140.03 ± 1.23^{i}	154.24 ±1.28^{k}	178.87 ± 1.33^{m}	180.51 ± 1.41^{m}
BGF 20%	93.95 ± 1.01^{c}	93.98 ± 1.09^{c}	131.69 ±1.17^{gh}	167.87 ± 1.36^{l}	168.39 ± 1.21^{l}
BGF 25%	88.14 ± 0.92^{b}	88.94 ± 1.11^{b}	122.75 ±1.16^{f}	133.89 ± 1.25^{gh}	134.89 ± 1.28^{hg}
BGF 50%	68.36 ± 0.43^{a}	69.00 ± 0.81^{a}	96.56 ± 0.94^{d}	105.57 ± 1.11^{e}	108.55 ± 1.12^{e}
FRAP- IC_{50} (mg/mL)					
Control	350.14 ± 4.52^{o}	Nd	Nd	Nd	Nd
BGF 10%	52.44 ± 0.4^{i}	53.14 ± 0.42^{hij}	56.60 ± 0.44^{l}	59.74 ± 0.48^{m}	60.82 ± 0.52^{m}
BGF 15%	50.88 ± 0.3^{h}	52.88 ± 0.33^{h}	55.24 ± 0.36^{k}	57.45 ± 0.37^{l}	58.71 ± 0.44^{l}
BGF 20%	42.53 ± 0.15^{e}	43.24 ± 0.17^{e}	54.06 ± 0.25^{j}	55.40 ± 0.28^{k}	57.61 ± 0.31^{kl}
BGF 25%	33.87 ± 0.13^{d}	35.30 ± 0.1^{d}	45.08 ± 0.14^{f}	49.80 ± 0.17^{g}	50.13 ± 0.24^{g}
BGF 50%	12.69 ± 0.12^{a}	13.11 ± 0.11^{a}	22.51 ± 0.12^{b}	28.89 ± 0.14^{c}	29.14 ± 0.15^{c}
Alpha amylase inhibition -IC_{50} (mg/mL)					
Control	Nd	Nd	Nd	Nd	Nd
BGF 10%	105.78 ± 4.04^{i}	109.88 ± 4.21^{i}	121.56 ± 4.32^{l}	128.59 ± 4.41^{m}	132.02 ± 4.60^{n}
BGF 15%	96.50 ± 2.12^{g}	100.67 ± 3.11^{gh}	119.51 ± 3.20^{kl}	124.38 ± 3.28^{m}	128.51 ± 3.35^{m}
BGF 20%	62.42 ± 1.46^{c}	68.06 ± 1.52^{c}	92.22 ± 1.73^{f}	112.80 ± 3.00^{j}	120.62 ± 3.23^{l}
BGF 25%	50.04^{b} ± 1.57^{a}	54.94^{b} ± 1.40^{a}	89.15 ± 1.56^{e}	90.79 ± 2.12^{f}	100.50 ± 3.17^{h}
BGF 50%	47.94 ± 1.11^{a}	50.41 ± 1.70^{a}	80.81 ± 2.04^{d}	89.62 ± 2.09^{e}	93.02 ± 3.15^{fg}

Means with different letters in the row differ significantly. They are calculated by Duncan method (P ≤ 0.05).
Each value expressed as mean ± SD (n = 3).
Nd, not detected.
Control represents biscuits without fat mimetic.

during storage. Results of storage stability also showed a similar pattern as that of DPPH for BGF-WF biscuits, there was no significant (P ≤ 0.05) difference between the IC_{50} value of initial and second week of storage, although reducing power decreased from the fourth week of storage and further decreased to sixth week. However, there was no significant (P ≤ 0.05) difference in IC_{50} values between sixth to eighth weeks of storage. Similar findings were reported by Caleja *et al.* (2017) when fennel and chamomile extracts were used in biscuit samples.

Like the DPPH test, baking further improves the FRAP as lower IC_{50} values were witnessed in biscuits (Table 7) when compared to flour samples (Table 6). An increase in reducing power upon baking has been reported by Mabogo *et al.* (2021) for biscuits prepared by incorporation of unripe banana flour in wheat flour.

Total phenolic content

Table 8 represents the TPC of BGF-WF biscuit samples during storage. Biscuits prepared with BGF 50% showed the highest TPC throughout the storage period of 8 weeks followed by BGF 25% and BGF 20%. It was observed that there is no significant ($P \leq 0.05$) difference between the first day and second week of storage. Although TPC reduced from the fourth week of storage, all BGF-WF biscuit samples have higher TPC as compared to control biscuit samples which exhibited the least TPC (18.56 mg GAE/100g dw). Despite greater decrease in TPC of BGF-WF biscuits during baking was found compared to BGF-WF blends (Table 6), the TPC value in BGF-WF biscuits was still higher than that of control biscuits. Jan *et al.* (2015) also found a decrease in TPC of buckwheat flour when heated to a temperature of 180°C. The decrease in TPC was may be due to alteration in the chemical structure of the phenolic compounds (Pintado *et al.*, 2021). In previous research, various natural sources of phenolic compounds were added to bakery products to increase the nutraceutical value (Pattnaik *et al.*, 2021).

Total flavonoid content

From Table 8, it can be observed that TFC in biscuit samples increased with the level of addition of BGF. Biscuits prepared with BGF 50% showed the highest TFC throughout the storage period of 8 weeks, followed by BGF 25% and BGF 20%. Natural flavonoid-containing ingredients were also added in bakery products to increase the bioactive compounds, as reported by many researchers; for instance, a study reported that biscuits prepared with water chestnut flour demonstrated enhanced TFC (Shafi *et al.*, 2016).

Inhibition of alpha-amylase activity In-vitro

The methanolic extract of BGF-WF biscuits samples showed significant ($P \leq 0.05$) inhibitory activity against alpha-amylase. From Table 7, it can be observed that IC_{50} values decreased with increasing ratio of BGF. The follow-up of storage changes in biscuits samples revealed that there was no significant ($P \leq 0.05$) change in enzyme inhibition up to 2 weeks of storage; however, IC_{50} of all BGF biscuits gradually increased after the second week of storage, and the inhibition activity was almost undetectable in control biscuits. Petrus *et al.* (2012) reported that antioxidant compounds such as catechin, phenolics, flavonoids, alkaloids, and triterpenoids possess anti-diabetic activity. Epicatechin, a flavonoid is known to possess insulin-like properties, while epigallocatechin gallate is

Table 8. Total phenol content (TPC) and total flavonoid content (TFC) concerning storage period at different levels of Bottle gourd flour incorporated in biscuit samples.

Samples	Initial	Second week	Fourth week	Sixth week	Eighth week
TPC-(mg GAE/100 g dw)					
Control	27.430^{a} ± 0.12	Nd	Nd	Nd	Nd
BGF 10%	326.66^{f} ± 2.18	323.33^{f} ± 1.31	255.5^{c} ± 1.14	230.00^{b} ± 1.12	225.50^{b} ± 1.1
BGF 15%	383.33^{h} ± 2.21	380.66^{h} ± 1.43	319.33^{e} ± 1.25	280.00^{d} ± 1.21	278.00^{d} ± 1.08
BGF 20%	413.83^{j} ± 3.12	410.88^{j} ± 1.97	391.66^{i} ± 1.45	371.66^{g} ± 1.32	368.50^{g} ± 1.11
BGF 25%	525.00^{p} ± 3.15	520.00^{p} ± 2.48	469.33^{n} ± 2.35	421.66^{k} ± 3.00	418.50^{k} ± 2.41
BGF 50%	638.33^{q} ± 3.21	634.00^{q} ± 3.11	486.00^{o} ± 3.07	460.00^{m} ± 3.17	454.16^{l} ± 3.04
TFC-(mg CE/100 g dw)					
Control	18.56^{a} ± 1.01	Nd	Nd	Nd	Nd
BGF 10%	104.32^{e} ± 2.21	100.84^{e} ± 1.44	90.24^{c} ± 1.25	76.16^{b} ± 1.23	74.24^{b} ± 1.22
BGF 15%	121.60^{f} ± 2.74	119.84^{f} ± 2.46	104.32^{e} ± 2.42	88.96^{d} ± 1.14	87.04^{d} ± 1.12
BGF 20%	380.51^{i} ± 3.45	376.66^{i} ± 3.21	338.83^{h} ± 3.19	330.5^{g} ± 3.14	326.66^{g} ± 3.11
BGF 25%	451.66^{l} ± 4.51	447.16^{l} ± 4.43	397.18^{k} ± 3.32	371.66^{j} ± 3.32	366.00^{j} ± 3.25
BGF 50%	558.33^{n} ± 4.82	552.00^{n} ± 4.89	460.00^{m} ± 4.61	397.16^{k} ± 3.41	394.00^{k} ± 3.33

Means followed by different letters in the raw differs significantly ($P \leq 0.05$). Calculations were made using the Duncan method. Each value is expressed as mean ± SD (n = 3), where GAE, gallic acid equivalent. CE, catechin equivalent. dw, dry weight of the sample. Nd, not detected.
*Control represents biscuits without fat replacement.

considered to be an effective hypoglycemic agent (Sun *et al.*, 2020). Data reported in Table 8confirmed that BGF-WF biscuits have a significant ($P \leq 0.05$) amount of bioactive phenolics and flavonoids. Being inhibitors of alpha-amylase activity, these antioxidant compounds in the BGF biscuits could induce anti-diabetic potential in the biscuits and hence are helpful in controlling diabetes.

Anti-hyperglycemic of BGF biscuits In-vivo

Table 9 shows the mean blood glucose responses after the consumption of glucose and the test foods, that is, biscuits prepared with BGF 15% (selected due to good sensorial profile) and the control biscuits in healthy human subjects. The IAUC for glucose was found to be 143 mmol/L, and for BGF 15% and control were 31.05 mmol/L and 59.25 mmol/L, respectively. GI of control and BGF biscuits were 41.18 and 21.58, respectively. Although both Control and BGF biscuits contained an equal quantity of sugar, biscuits with BGF 15% significantly ($P \leq 0.05$) resulted in a lower glycemic response at the 120th minute of intake. Apart from phytochemicals, BG is a blend of soluble and insoluble fiber; however, impact on a glycemic level could be credited more for the presence of soluble fiber (Luan and Hong, 2016). The mechanism of action may be achieved through a reduction in both fasting blood glucose and insulin concentrations. This occurs because of water-soluble gel-forming fibers. These dietary fibers form a viscous solution in the small intestine, which reduces the contact and mixing of macronutrients with digestive enzymes, and this delays the absorption of glucose, which consequently reduces the postprandial plasma glucose and insulin levels (Mcrae, 2018). Another reason for decrease in blood glucose profile was credited to phenolics present in BGF-WF biscuits samples as these phenolic compounds are bioactive compounds with antioxidant potential, and hypoglycemic, hypolipidemic, and anti-tumor properties (Fombang and Saa, 2016). A similar trend of blood glucose modulation was observed in a study in which moringa tea was ingested by healthy human subjects for determination of glycemic response (Fombang and Saa, 2016). In previous research, various solvent extracts of BG have also been shown to exhibit antihyperglycemic activity in animal subjects, because of the presence of secondary metabolites; for instance, phenolic and flavonoids (Luan and Hong, 2016).

Peroxide value of biscuit samples

Figure 2A shows that PV was less than three mEq O_2/kg of fat for all the BGF biscuit samples during storage which is considered safe value according to Poilish Standard, PN-A-86908:1966. Less PVs of the BGF-WF biscuit samples were because of the two main reasons: first, the presence of antioxidants that contributed by controlling oxidation and hence peroxide formation. Second, fat replacement, the greater the amount of fat replaced by BGF, lesser the amount of fat that remained for the process of oxidation. In a previous study, addition of chokeberry polyphenols extract to butter cookies resulted in a higher PV than the recommended value after ninth week of storage (Bialek *et al.*, 2016) which is contradictory to our findings. However, PV range after 6 weeks of preparing the biscuits with the addition of grape pomace extract was comparable to our findings (Zaky *et al.*, 2020). There was a gradual increase of PV in all the BGF-WF biscuits samples during storage; on the other hand, control biscuits had the highest PV of 5.53 at the eight week of storage.

Free fatty acid in biscuit samples

Data reported in Tables 6, 7, and 8 demonstrated that BGF-WF blends and BGF-WF biscuits possessed significant ($P \leq 0.05$) antioxidant activity and hence were helpful for the inhibition of oxidation of fats in biscuits. During storage, increased FFA value was observed in all the biscuit samples. The increase was remarkably greater

Table 9. Blood glucose responses (mmol/L), incremental area under the curve (IUAC), and glycemic index (GI) in normal healthy volunteers following glucose, tested, and control biscuits.

Samples	Time Intervals (min)					IAUC	GI
	0	30	60	90	120		
Glucose	5.90 ± 0.13^b	7.83 ± 0.24^c	8.43 ± 0.25^c	5.74 ± 0.12^b	5.23 ± 0.1^b	143.00	–
BGF 15%	5.30 ± 0.11^a	5.45 ± 0.13^a	5.80 ± 0.16^a	5.63 ± 0.14^a	5.19 ± 0.1^a	31.05	21.58
Control*	5.96 ± 0.17^b	6.75 ± 0.22^b	6.83 ± 0.25^b	6.25 ± 0.18^c	5.91 ± 0.14^c	59.25	41.18

Means with different letters in the column differ significantly. They are calculated by the Duncan method ($P \leq 0.05$). Each value expressed as mean ± SD (n = 15 healthy female volunteers).
BGF, bottle gourd flour. IAUC, incremental area under the curve. GI, glycemic index.
*Control represents biscuits without fat mimetic.

in control (1.1%) as compared to BGF-WF biscuit samples. From Figure 2B, it was clear that initially the FFA was not detected in BGF-WF biscuit samples; however, a gradual increase (up to 0.9 %) was observed during storage indicating the capability of antioxidants present in BGF in reducing the formation of FFA. Therefore, the increment of FFA level in BGF biscuits was relatively slow. Similar observations were reported when a flavonoid-rich extract from green tea was incorporated in biscuit samples (Navaratne and Senaratne, 2014). Another study in which pomegranate peel was added in different ratios in biscuit samples was also in agreement with our findings (Ismail *et al.*, 2014). Hence, it can be concluded that BGF was capable of controlling the formation of FFA in biscuit samples when used as a fat mimetic.

Water activity to evaluate the possibility of microbial growth

Table 10 shows that an increase in the storage period increases the water activity of control and BGF-WF biscuits. The water requirement for the growth of microorganisms is expressed in terms of moisture available or water activity (Macedo *et al.*, 2020). In this study, even at 10% BGF, the water activity was found to be 0.58 at the eighth week of storage which assured the safety of product with respect to microbial profile (Morais *et al.*, 2018). Rodríguez *et al.* (2013) also reported a similar trend of decreased water activity when fat was replaced by inulin in biscuit samples. Lower water activity of BGF biscuits might be due to higher water-binding capacity of BGF, as less water evaporated during baking, and less free water remained in the biscuits, thus contributing to longer shelf life (Rodríguez *et al.*, 2013). No significant ($P \leq 0.05$) change was observed in water activity of control and BGF biscuits from first day till the second week of storage. Although water activity increased after the fourth week of storage and this remained constant up to 8 weeks. Morais *et al.* (2018) also reported a similar trend of water activity in biscuit samples. The increased water activity can be attributed to the increased moisture of biscuits with respect to storage, but it remained limited under the value of 0.6 for 2 months to inhibit microbial growth

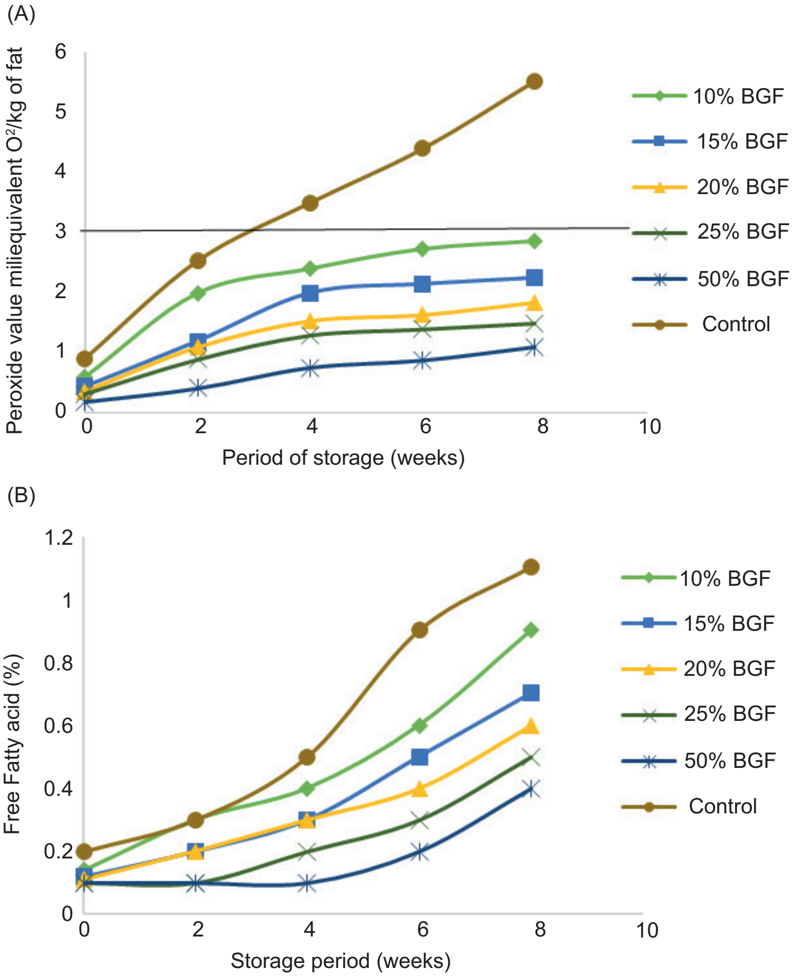

Figure 2. (A) Peroxide value levels in control and Bottle gourd flour incorporated biscuits stored for 2 months. (B) Free fatty acid (%) levels in control and bottle gourd flour incorporated biscuits stored for 2 months.

Table 10. **Water activity concerning storage period at different levels of bottle gourd flour (BGF) incorporated in biscuit samples.**

Samples	Initial	Second week	Fourth week	Sixth week	Eighth week
Control*	0.62 ± 0.02^{h}	0.61 ± 0.02^{h}	0.70 ± 0.02^{i}	0.71 ± 0.02^{i}	0.71 ± 0.02^{i}
BGF 10%	0.55 ± 0.08^{e}	0.55 ± 0.01^{e}	0.57 ± 0.04^{ef}	0.57 ± 0.02^{ef}	0.58 ± 0.01^{f}
BGF15%	0.52 ± 0.01^{d}	0.52 ± 0.03^{d}	0.56 ± 0.02^{ef}	0.56 ± 0.01^{ef}	0.56 ± 0.01^{ef}
BGF 20%	0.51 ± 0.02^{d}	0.52 ± 0.02^{d}	0.55 ± 0.05^{ef}	0.56 ± 0.02^{ef}	0.57 ± 0.01^{ef}
BGF 25%	0.47 ± 0.05^{c}	0.47 ± 0.08^{c}	0.50 ± 0.03^{d}	0.51 ± 0.02^{d}	0.51 ± 0.01^{d}
BGF 50%	0.35 ± 0.01^{a}	0.34 ± 0.01^{a}	0.38 ± 0.07^{b}	0.38 ± 0.07^{b}	0.38 ± 0.01^{b}

Means with different letters in the row differ significantly ($P \leq 0.05$). They are calculated by the Duncan method.
*Control represents biscuits without fat mimetic.

(Morais *et al.*, 2018) and ensure product stability. On the other hand, water activity of control biscuits increased from 0.6 to 0.71 during 2 months of storage.

Physical properties of biscuit samples

Table 11 summarized the effects of the BGF as a fat mimetic on the physical properties of biscuits such as diameter, thickness, spread ratio, hardness, and fracturability. A standard decrease was observed in the diameter of fat mimetic biscuit samples, although biscuits prepared with BGF 10% and BGF 15% showed a maximum increase in diameter when compared with the control sample. In addition to this, biscuits containing BGF 10%, BGF 15%, and BGF 20% showed less thickness than control. BGF significantly ($P \leq 0.05$) increased the spread ratio at the level of 10 and 15%, which was more than the control. Similarly, Kuchtová *et al.* (2018) and Mildner *et al.* (2013) reported an increase in the spread ratio of biscuit samples produced by incorporation of grape skin and white grape pomace, respectively. An increased amount of dietary fiber in BGF biscuits could be the reason for increased thickness, reduced diameter, and decreased spread ratio as a higher amount of fat was replaced with BGF. It has already been reported that the addition of dietary fiber from various sources and substitutes has a negative effect on the diameter, thickness, and spread ratio of biscuits/cookies (Saka *et al.*, 2020). Another reason for the variation in these physical parameters could be the dilution of gluten network in biscuit dough (Kohajdová *et al.*, 2014). Hardness and fracturability are textural properties that attract significant attention in the evaluation of baked goods; these parameters should be as low as possible (Saka *et al.*, 2020). The increased amount of BGF from 10 to 15% resulted in considerable less hardness and fracturability; however, hardness increased when fat was replaced beyond 20%. This is possibly related to the high WAC of BGF, as it has been reported by Saeed *et al.* (2021) that components that enhance the water absorption of dough results in the development of the complex gluten network and ultimately hardens the texture of the biscuit samples.

Color characteristics of biscuits

Color plays an essential role in determining the quality of products in food processing industries and food engineering research (Palamthodi *et al.*, 2019). Results of color measurements of biscuits made with different levels of BGF are given in Table 12. It was found that the lightness L* of the biscuits exhibited a decreasing trend with an increasing level of BGF. The reducing values of

Table 11. **Effect of bottle gourd flour (BGF) on physical properties (dimension and texture) of biscuits.**

Sample	Diameter (mm)	Thickness (mm)	Spread ratio (mm)	Breaking force (N)	Fracturability (mm)
Control*	42.67 ± 0.56^{d}	9.07 ± 0.52^{d}	4.71 ± 0.10^{d}	22.90 ± 0.39^{d}	2.10 ± 0.12^{b}
BGF 10%	42.74 ± 0.40^{e}	8.06 ± 0.2^{a}	5.30 ± 0.14^{f}	17.42 ± 0.2^{a}	0.87 ± 0.11^{a}
BGF 15%	42.84 ± 0.88^{f}	8.26 ± 0.20^{b}	5.18 ± 0.12^{e}	20.87 ± 0.23^{b}	0.90 ± 0.12^{a}
BGF 20%	41.74 ± 0.51^{c}	8.68 ± 0.21^{c}	4.67 ± 0.13^{c}	24.77 ± 0.30^{c}	2.45 ± 0.14^{c}
BGF 25%	41.59 ± 0.42^{b}	9.32 ± 0.24^{e}	4.37 ± 0.10^{b}	27.42 ± 0.41^{e}	2.74 ± 0.17^{d}
BGF 50%	40.78 ± 0.33^{a}	10.68 ± 1.02^{f}	3.81 ± 0.10^{a}	31.51 ± 0.44^{f}	2.81 ± 0.19^{e}

Values expressed are the mean ± standard deviation ($n = 3$). Means in the column with different superscripts are significantly ($P \leq 0.05$) different.
*Control represents biscuits without fat mimetic.

Table 12. Effect of bottle gourd flour (BGF) on the color of biscuit samples.

Samples	L*	a*	b*
Control*	73.12 ± 0.82^{f}	6.13 ± 0.01^{a}	43.12 ± 0.21^{f}
BGF 10%	70.41 ± 0.67^{e}	6.91 ± 0.03^{b}	38.11 ± 0.15^{e}
BGF 15%	64.33 ± 0.34^{d}	7.30 ± 0.02^{c}	33.14 ± 0.13^{d}
BGF 20%	58.29 ± 0.13^{c}	7.81 ± 0.01^{d}	26.31 ± 0.10^{c}
BGF 25%	52.14 ± 0.11^{b}	8.11 ± 0.02^{e}	18.35 ± 0.10^{b}
BGF 50%	46.21 ± 0.10^{a}	10.32 ± 0.05^{f}	8.21 ± 0.06^{a}

Values expressed are the mean ± standard deviation (n = 4).
Means in the column with different superscripts are significantly (P ≤ 0.05) different.
*Control represents biscuits without fat mimetic.

L* indicated that the biscuits were darker at higher levels of BGF compared to the control sample. The effect might be due to the presence of natural pigments or the Maillard reaction products that were formed from amino acids and reducing sugars during baking (Mildner *et al.*, 2013). Furthermore, it was shown that the higher levels of BGF in biscuits increased redness (higher a* value) and decreased yellowness (lower b* value). This may be because BG contains a good amount of anthocyanin, a red-blue natural pigment (Palamthodi *et al.*, 2019). Kuchtová *et al.* (2018) and Sharma and Gujral (2014) reported a decrease in the color values of L*, an increase in redness a*, and decreased yellowness b* when grape seeds and buckwheat flour were incorporated in biscuits, respectively, and it is in agreement with our findings.

Nutritional analysis of biscuits

The nutrient composition of BGF, control, and fat mimetic biscuits are summarized in Table 13. BGF was found to be an excellent source of crude fiber (26.30%), protein (20.21%), and minerals (ash content 4.23%). All the fat mimetic biscuits showed higher protein, ash, and crude fiber contents than the control. As expected, fat content reduced with the level of addition of BGF than the control sample. As the concentration of BGF increased, the moisture content of biscuit samples reduced. According to Camelo-Méndez *et al.* (2018), moisture reduction was due to the decrease in the gluten network with the increase in the amount of BGF. The crude fiber, protein, and ash content increased with the increased amount of BGF in biscuit samples. The control sample showed a low value of crude fiber (6.0%) while the biscuits containing BGF 50% and BGF 25% possessed the highest crude fiber. The carbohydrate content and energy (Kcal) values were the highest in control (59.04% and 255.92 Kcal) and lowest in biscuits with BGF 50% (43.25% and 219.68 Kcal). Therefore, BGF biscuit samples showed less energy value and the reason was fat substitution.

Sensory characteristics of biscuits

Figure 3 presents the sensory scores of the biscuit samples (50% replacement of fat in biscuits excluded from the data reported because of the undesirable sensory profile). Biscuits with BGF10% demonstrated similar results as that of control sample in terms of taste, texture, color, and overall acceptability. On the other hand, at the level of BGF15%, score for color was higher than the control biscuits, although appearance, taste, texture, and overall acceptability were not significantly (P ≤ 0.05) different from control biscuits. As the level of BGF increased beyond 15%, taste which is one of the essential attributes, declined due to unpleasant mouthfeel, bitterness, and the texture also became harder. The bitterness was may be due to the interaction between a high amount of phenolic compounds in BGF and saliva present in the mouth (Kuchtová *et al.*, 2018) while the hard texture could be due to the low amount of fat in the biscuit recipe as fat

Table 13. Nutritional analysis of bottle gourd flour (BGF), control, and fat mimetic biscuits.

Samples	Moisture content %	Ash %	Protein %	Fat %	Dietary fiber %	Carbohydrate %	Kcal/100 g
Control*	5.02 ± 0.12^{a}	0.53 ± 0.01^{a}	11.20 ± 0.10^{a}	24.02 ± 0.20^{g}	0.21 ± 0.26^{a}	64.04 ± 0.80^{g}	517
BGF	8.01 ± 0.30^{d}	4.23 ± 0.10^{f}	20.21 ± 0.50^{f}	0.43 ± 0.01^{a}	26.30 ± 0.42^{f}	48.83 ± 0.30^{b}	280
BGF 10%	4.68 ± 0.14^{c}	2.73 ± 0.03^{b}	13.70 ± 0.12^{b}	20.21 ± 0.14^{f}	13.80 ± 0.20^{b}	49.56 ± 0.42^{c}	435
BGF 15%	4.68 ± 0.10^{c}	2.77 ± 0.01^{b}	13.90 ± 0.11^{bc}	19.11 ± 0.10^{e}	13.60 ± 0.11^{b}	50.62 ± 0.57^{d}	430
BGF 20%	4.65 ± 0.11^{b}	3.82 ± 0.02^{c}	13.60 ± 0.15^{b}	16.32 ± 0.21^{d}	14.00 ± 0.21^{c}	52.26 ± 0.64f	410
BGF 25%	4.64 ± 0.12^{b}	3.86 ± 0.06^{cd}	14.40 ± 0.20^{d}	15.01 ± 0.10^{c}	14.60 ± 0.13^{d}	52.13 ± 0.60e	401
BGF 50%	4.65 ± 0.13^{b}	3.87 ± 0.09^{ed}	18.70 ± 0.22^{e}	12.54 ± 0.11^{b}	17.30 ± 0.17^{e}	47.59 ± 0.54^{a}	378

Values expressed are the mean ± standard deviation (n = 3). Means in the column with different superscripts are significantly (P ≤ 0.05) different.
*Control represents biscuits without fat mimetic.

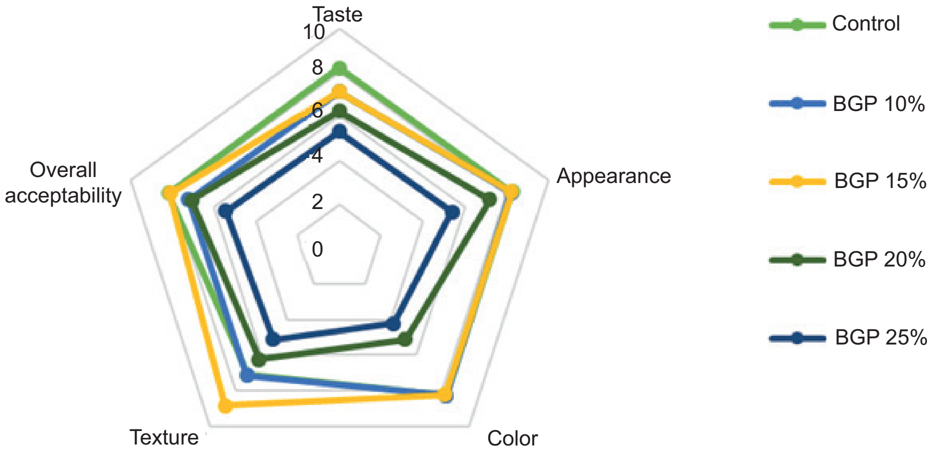

Figure 3. **Effect of fat replacement by bottle gourd flour (BGF) on the sensorial quality of biscuits. Control represents biscuits without fat mimetic.**

helps to lubricate and soften the structure of food and contributes desirable textural properties (Hasmadi *et al.*, 2014). Moreover, in the study conducted by Serin and Sayar (2017), maltodextrin and polydextrose were used as a fat replacer in biscuits which also resulted in increased hardness with the level of fat replacement.

Conclusion

The bakery industry is in constant innovation, and biscuits are products that are consumed worldwide by different classes of consumers. Therefore, the production of this type of product having nutraceutical effects may be attractive for consumers who are concerned about the choice of healthy foods. The result showed that the addition of BGF as a fat mimetic in the proportion of 10 to 15% produced desirable biscuits, and the functional properties of the wheat flour were not affected. BGF was observed to be an excellent source of phenolic and flavonoids, which makes it a valuable source of antioxidants. Incorporation of BGF raised the bioactive compounds in biscuits' enhanced antioxidant activity, and inhibited alpha-amylase. Biscuits with 15% BGF showed excellent physical properties and sensorial attributes. Therefore, administration of this formulation of biscuits in healthy volunteers for the determination of anti-hyperglycemic activity revealed a lower blood glucose response curve and glycemic index as compared to the control biscuits. Moreover, further studies are needed to improve the functional behavior and quality of biscuits.

Acknowledgment

The help of Ms. Uroosa Ejaz in proof reading of this manuscript is acknowledged. The authors are grateful to the University of Karachi for partial funding through a Dean Research Grant, and to English Biscuit Manufacturers management, particularly Dr. Zeelaf Munir and Madam Saadia Naveed, for their encouragement and support.

Ethics declarations

Conflict of interest

There is no conflict of interest.

Compliance with ethics requirements

This article contains *In-Vivo* studies with human participants (anti-hyperglycemic activity and sensory test).

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