Utilization of *Vigna mungo* flour as fat mimetic in biscuits: Its impact on antioxidant profile, polyphenolic content, storage stability, and quality attributes

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**Abstract**

In the present study, an effort was made to incorporate *Vigna mungo* flour (VMF) as a fat mimetic for the development of functional biscuits. Rheological properties of dough, pasting profile, total phenolic content (TPC) and total flavonoid content (TFC), antioxidant activities (1, 1-diphenyl-2-picrylhydrazyl [DPPH] inhibition and ferric reducing antioxidant power [FRAP]), shelf life, and nutritional and sensory properties of the samples were analyzed. An increase in DPPH inhibition, FRAP, TPC, and TFC was observed with increase in the concentration of VMF in wheat flour (WF). The addition of VMF resulted in increased water absorption (58.71–72.40%) and dough stability time (6.30–13.11 min). Set back viscosities (479–81 torque) decreased significantly (*P* ≤ 0.05), whereas break down viscosities (378–447 torque) increased. The baking process decreased the IC₅₀ values of DPPH inhibition (255.40–169.21 mg/ml) and FRAP (114.45–34.12 mg/ml), although TPC and TFC decreased. The study of shelf life for 2 months revealed no significant difference (*P* ≤ 0.05) in antioxidant, phenolics, and flavonoids contents of VMF-WF biscuits. Furthermore, the antioxidants, TFC, and TPC compounds were undetected in control throughout the storage period. The increase in both peroxide value (3.9 mEqO₂/kg of fat) and free fatty acid (0.87%) was significantly lower (*P* ≤ 0.05) than that of the control biscuits during storage but were in an acceptable range. It correlates to the enhanced activity of natural antioxidants as TPC and TFC in VMF-WF biscuits, which are responsible for the decrease in the rate of oxidation. Nutritional data of VMF-WF biscuits revealed that protein (11.20–15.85%), crude fiber (0.21–2.01%), and ash content (0.53–4.01%) significantly increased (*P* ≤ 0.05) and calories (497–430 kcal/100 g) reduced because of the addition of fat mimetic. Biscuits prepared with VMF 15% demonstrated superior quality as compared with control. In conclusion, it could be suggested that the addition of VMF as a fat mimetic in biscuits may increase antioxidant and polyphenolic contents with enhanced storage stability. The reduced calories, increased protein, fiber, mineral contents, and enhanced antioxidant activity considerably increase the nutritional status of the biscuits.
1 | INTRODUCTION

It is generally known that high oxidative stress is the leading cause of nutrient-deficiency diseases. Hence, antioxidant-fortified and enriched foods with multiple healing properties will be highly beneficial to prevent and may cure such disorders. Food items modified along these lines can be manufactured on the business scale and offer not only satisfaction but also provide the therapeutic potential to health-conscious consumers (Lobo, Patil, Phatak, & Chandra, 2010). *Vigna mungo* (VM), also known as black gram, which belongs to family Leguminosae, is a highly consumed legume in Pakistan due to its therapeutic importance (Haq et al., 2014). The high carbohydrate contents present in VM seeds indicate its potential use as a significant source of energy to prevent marasmus in infants. Like other legumes, its seeds are also rich in protein content and a significant amount of dietary fiber (Haq et al., 2014). It is just because of the diverse functional properties of VM it can be considered as a great source of fat replacement. For instance, the presence of the nonpolar compounds in VM, which provides functional characteristics of fats by facilitating solubilization of flavor compounds, which are only soluble in lipids. However, the polar nature of VM facilitates water binding, which helps to generate a sense of creaminess and lubricity in foods similar to that found in full-fat products. (O’Connor & O’Brien, 2016).

Polyphenols are the phytochemicals found in plants and are well known for their antioxidant activity. In a study conducted by Girish, Pratape, and Rao (2012), the acetone extract of VM and its milled fraction showed a higher amount of total phenols as compared with ethyl alcohol extract (Girish et al., 2012). Anthocyanins are another phytochemicals that belong to the polyphenolic family. It is present in various colors, mainly red and blue, particularly present in fruits and vegetables (Khoo, Azlan, Tang, & Lim, 2017). The polyphenol contents were also high in the seed coat of VM (Girish et al., 2012). In cooked black bean and chickpea seeds, anthocyanin content ranged from 1.5 to 4.8 mg/100 g (Silva-Cristobal, Osorio-Díaz, Tovar, & Bello-Pérez, 2010); on the other hand, Girish et al. (2012) showed 9.79 mg/100 g in the whole VM seeds. Dietary fibers often play a significant role in preventing diseases. Insoluble dietary fiber helps in water absorption (WA), which is beneficial for healing constipation, as it makes the fecal material bulkier. Hence, the elimination of fecal material becomes easier from the digestive tract. On the other hand, soluble dietary fiber surrounds very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) and inhibits their absorption in the blood (Soliman, 2019). The percentage of soluble dietary is about 2.6% to 9.3% in VM milled fractions (Girish et al., 2012).

The literature revealed that unleavened flatbread prepared by replacing wheat flour (WF) with kidney bean and VMF at a level of 5% to 20% showed a good quality similar to that of control. However, the higher rate of substitution of kidney beans and VMF affected color, flavor, texture, taste, and overall acceptance of flatbread (Wani, Sogi, Sharma, & Gill, 2016). In another study, the biscuit prepared from multicereal and legume flour including VM showed that protein content increased as the amount of pulses increased (Vasanthakumari & Jaganmohan, 2018). While in search of fat replacement, Román, Santos, Martínez, and Gómez (2015) reported that WF paste with added emulsifier could be used as a fat replacer in a cake formulation. Peterkuchukwu et al. (2018) utilized toasted watermelon seed meal as a fat substitute in WF biscuits. Furthermore, numerous studies based on the utilization of dietary fibers or inulin as a fat replacer in baked products have successfully been carried out (Rodríguez-García, Puig, Salvador, & Hernando, 2012).

Biscuits have always been recognized as one of the most traditional bakery product rich in fat content, and because there is no information available in the literature for the utilization of VMF as fat mimetic in biscuits, the present study was an attempt to investigate the effect of VMF addition on the physicochemical, nutritional, nutraceutical, shelf life, and sensory properties of the dough and of the biscuits.

2 | MATERIALS AND METHODS

2.1 | Raw materials

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

2.2 | Preparation of VMF

The pretreatment was given to whole VM seeds prior to milling in order to deactivate the polyphenol oxidase (PPO). Whole VM seeds were heated to 88°C for 15 s in a hot air oven (Taranto et al., 2017). Whole VM seeds (about 5 kg) were milled by using laboratory miller (TAISITE, FW100) and sieved from screen having a mesh size of 60 μm.

VM flour (VMF) was added to WF in varying concentrations (10, 15, 20, 25, and 50% W/W corresponding to the amount of fat used in biscuits).
2.3 | Physicochemical properties of flour

2.3.1 | Water absorption and oil binding capacities of flour

Water absorption capacity (WAC) and oil binding capacity (OBC) were determined by the method of Ahmed, Mulla, and Arfat (2017). Briefly describing the WAC was measured by taking 10 ml of distilled water was taken in a 20 ml centrifuge tube than 1 g of the flour sample was added to it, and the mixture was vortexed for 2 min. Then, the tube was centrifuged at 2,200×g for about 20 min. The supernatant was decanted, and the residual pellets were weighed and analyzed for WAC. The same procedure was used to determine OBC using soya bean oil.

2.3.2 | Farinograph properties of flour

The effect of the incorporation of VMF in WF in various concentrations on rheological properties was studied by using farinograph (mixer bowl 300 g, Brabender OHG, Duisburg, Germany) following the AACC Method 54-21 (AACC, 2010). The parameter measured included water absorption (WA), dough development time (DDT), the degree of softening (DoS), dough stability time (DST), and farinograph quality number (FQN).

2.3.3 | Pasting properties of flour samples

The pasting properties were also investigated by using Microvisco-amylo-graph (Brabender, Duisburg, Germany) according to the AACC Method 22-10(AACC, 2010). The parameters measured for each sample include the average values for gelatinization (G), peak viscosity (PV), final viscosity (FV), breakdown (BD), and setback (SB) viscosities.

2.4 | Preparation of biscuits

Control and VMF-WF biscuits were prepared by incorporating different levels of fat. For the preparation of biscuits samples, fat was replaced from the recipe by VMF at concentrations of 10%, 15%, 20%, 25%, and 50%. The standard biscuits recipe was followed, which consists of flour 100 g (VMF and WF 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, baking powder 1 g, soya lecithin 0.25 g, and water 20 ± 5 ml. First, icing sugar and fat were blended in a dough mixer (model KMX 51, Kenwood, Havant, UK) for 3 min, then whole egg and lecithin were added to the blend of fat and sugar and mixed again for 5 min. More ingredients: WF, baking powder, salt, glucose, and water were added later and kneaded for 3 min. The dough was sheeted and sliced into circular shapes using a biscuits cutter. Biscuits were then baked in a preheated oven (model R-892 P, SHARP, Thailand) at 180°C for 20 min. Biscuits were cooled at ambient temperature and stored in airtight bottles. Textural and sensorial properties were determined on the day of biscuits manufacturing.

2.5 | Antioxidant analysis

Antioxidant analysis of VMF-WF (10–50%), VMF biscuits, and control biscuits was carried out by free radical scavenging activity and ferric reducing antioxidant power.

2.5.1 | Preparation of extract from VMF-WF and biscuits

The extracts of VMF-WF and biscuits were prepared based on the technique of Sai, Thapa, Devkota, and Joshi (2019). The VMF-WF, control, and VMF biscuits powder were added to 10 ml of 80% acetone at the concentration of 60, 125, 185, and 250 mg/ml, and then, samples were prepared and analyzed as described by Sai et al. (2019).

2.5.2 | Free radical scavenging activity

The technique mentioned by Mulla and Ahmed (2019) with slight modification was utilized for determining the antioxidant activity of VMF-WF, VMF-WF biscuits, and control biscuits. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution was prepared by dissolving 33.9 mg in 100 ml of methanol. One milliliter each of the concentrations of the prepared samples of 60, 125, 185, and 250 mg/ml was mixed with 1 ml of DPPH solution in test tubes 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 % scavenging activity determined as follows.

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

2.5.3 | Ferric/ferricyanide (Fe}^{3+}\text{) reducing antioxidant power}

Ferric/ferricyanide (Fe}^{3+}\text{) reducing antioxidant power (FRAP) was analyzed by the method of Mulla and Ahmed (2019). Perl's Prussian color formation was determined by measuring absorbance at 700 nm. An increase in absorbance values indicated more antioxidant activity (Mulla & Ahmed, 2019).

2.6 | Total phenolic content

Total phenolic content (TPC) of VMF-WF and biscuits samples were determined using Folin–Ciocalteu reagent, as per the method of Salar...
and Purewal (2017). The absorbance of the extracts was recorded at 765 nm against the blank, and results were expressed as gallic acid equivalent (GAE), mg GAE/100 g extract on dry weight (dw) basis.

2.7 | Total flavonoid content

Total flavonoid content (TFC) of VMF-WF and biscuits samples was determined by the method of Gbenga-Fabusiwa, Oladele, Oboh, Adefegha, and Oshodi (2018). The absorbance of the extracts was measured at 510 nm using catechin as standard, and results were expressed as mg CAE/100 g of the extract on dw basis.

2.8 | Evaluation of changes in biscuits during storage

VMF-WF biscuits and control biscuits were stored at room temperature (27 ± 2°C) in an airtight container at a relative humidity of 67%. After 15 days, the shelf life study was performed for 2 months. Biscuits samples were analyzed for free fatty acid (FFA) value, peroxide value (PV), antioxidant activities, TPC, TFC, and water activity ($a_w$).

2.8.1 | Determination of FFA and PV

FFA content of biscuits samples was analyzed by the AOAC Method Cc 5a-40 (AOAC, 2010). The FFA content calculated as the percentage of oleic acid according to the following equation:

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

PV of biscuits samples was analyzed by the AOAC Method Cd 8–53, (AOAC, 2010).

2.8.2 | Evaluating the effect of storage changes on antioxidants and phenolic compounds

The impact of storage conditions on the antioxidant activity was determined by FRAP and DPPH tests, whereas TPC and TFC were determined as described earlier in the above sections (2.6 to 2.8).

2.8.3 | 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 ($a_w = 0.22$; Suriya, Rajput, Reddy, Haripriya, & Bashir, 2017). A sufficient amount of sample was taken in the sample holder, and precaution was made so that sample does not touch the sensor.

2.9 | Evaluation of biscuits quality

2.9.1 | Textural analysis

The effect of replacement of fat on breaking strength (hardness) was determined using a texture analyzer (UTM, Zwick/Roell, Germany) according to the method of Ali et al. (2018). Assembly of three bends flexure was used for determining the biscuit texture measuring force in the N unit (load cell: 5 kg, pretest speed: 1.0 mm/s, test speed: 5.0 mm/s, posttest speed: 10.0 mm/s, distance: 10 mm, trigger force: 50 N).

2.9.2 | Color analysis

The color was measured using an NH 300 Calorimeter (China) according to the method of Ohizua et al. (2017). The color values L’, a’, and b’ were recorded at different points of the biscuits. L’ value is the lightness variable from 100 for perfect white to zero for black, whereas a’ and b’ values are the chromaticity values that indicate (+) redness/(-) greenness and (+) yellowness/(-) blueness, respectively, each value being the average of four measurements.

2.9.3 | Nutritional analysis

Nutritional analysis of control and VMF fortified biscuits included the estimation 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, 2010). Fat content was determined by the AACC Method 30-25 (AACC, 2010) by utilizing the Soxhlet Apparatus (Thermo Fisher Scientific). The crude fiber was evaluated on a fiber digester (Marconi, MA-444, Brazil) as per the AACC Method 32-10 (AACC, 2010), and moisture content was determined by using moisture analyzer (Brabender 51–55, CW Brabender, Duisbury, NJ, USA). The carbohydrate contents were determined by difference from the protein, fat, moisture, ash, and crude fiber contents. Calories were measured by applying the Atwater general factor system: carbohydrate (4 kcal/g), lipid (9 kcal/g), and protein (4 kcal/g).

2.9.4 | Sensory evaluation

Sensory examination was carried out in the baking laboratory following the method of Ali et al. (2018). Biscuits samples were evaluated by 40 panelists male and female (ages 24–45) comprised mainly of
students and staff members of the Department of Food Science and Technology, University of Karachi (Karachi, Pakistan). Panelists used 9-point hedonic scale (1, extremely dislike to 9, extremely like for analyzing the biscuit samples for taste, color, appearance, texture, and overall acceptability). Samples of biscuits were randomly selected and then labeled with three-digit numbers and presented to the panelists on white plastic plates in random order.

2.10 | Statistical analysis

All the analyses were performed in triplicate. The data were analyzed by ANOVA using SPSS (Version 17.0. Inc, Chicago, USA) statistical program. Duncan's multiple range tests were applied to identify any significant differences among the treatments. Significant levels were defined at $P \leq 0.05$.

3 | RESULTS AND DISCUSSION

3.1 | Physicochemical properties of flour

3.1.1 | Water absorption and oil absorption capacities of flour

WAC and OBC are the critical functional properties of the flour blends because they determine the texture, mouthfeel, and yield of the end product (Cheng & Bhat, 2016). Table 1 represented WAC of flour samples ranged between 145.60% and 162.24%. The highest WAC observed for VMF 50% (162.24%) followed by VM 25% (155.40%) and VM 10% (146.96%), whereas the WF (145.60%) showed the lowest WAC. Results suggest that the addition of VMF to WF influenced the amount of WA. The increase in WA due to the addition of rice, green gram, and potato has been reported in the previous study (Chandra, Singh, & Kumari, 2015). Higher WAC might be due to the difference in fiber contents of VMF (Mudgil, Barak, & Carcea, 2017). The addition of VMF to WF increased the gluten network to an optimum extent and hence improved the mixing of lentil and WF blends (Turfani, Narducci, Durazzo, Galli, & Carcea, 2017). The reason for enhanced DDT could be the higher protein content of VMF-WF blends. Ali et al. (2018) reported a similar finding that WA for wheat increases with the addition of black gram flour. However, the amount of VMF added in this study was much higher (10–50%), and the values of WA were much lower. The impact of soluble and insoluble dietary fiber on the rheological quality of dough has already been demonstrated by Issarny, Cao, Falk, Seetharaman, and Bock (2017). The addition of VMF to WF is responsible for weakening the gluten network to an optimum extent and hence improved the baking quality of dough for biscuits, which require weak gluten strength. The WA, DDT, and FQN for VMF 10%, and VMF 15% did not differ significantly ($P \leq 0.05$; Table 1). In this study, DDT was increased significantly ($P \leq 0.05$) as VMF-WF concentration increased up to 25%. Similarly, literature revealed that DDT increased due to the mixing of lentil and WF blends (Turfani, Narducci, Durazzo, Galli, & Carcea, 2017). The reason for enhanced DDT could be the higher affinity of VMF for higher WA by other hydrophilic biopolymers of

### TABLE 1  Effect of different ratios Vigna mungo flour (VMF) addition on farinograph properties, water absorption capacity (WAC), and oil binding capacity (OBC) of wheat flour

| Samples  | WA%     | DDT (min) | DST (min) | FQN | WAC%     | OBC%     |
|----------|---------|-----------|-----------|-----|----------|----------|
| Control  | 58.71 ± 0.2,  | 1.67 ± 0.01, | 8.58 ± 0.18, | 88 ± 1.12, | 145.60 ± 1.01, | 153.17 ± 2.10, |
| VMF 10%  | 63.60 ± 0.20,  | 5.90 ± 0.12, | 6.30 ± 0.10, | 77 ± 1.09, | 146.96 ± 1.01, | 150.21 ± 2.11, |
| VMF 15%  | 64.52 ± 0.33,  | 7.81 ± 0.24, | 8.81 ± 0.40, | 120 ± 2.24, | 148.60 ± 1.05, | 149.71 ± 1.07, |
| VMF 20%  | 64.92 ± 0.51,  | 7.80 ± 0.22, | 9.62 ± 0.20, | 120 ± 2.01, | 150.26 ± 2.03, | 146.82 ± 2.01, |
| VMF 25%  | 65.81 ± 0.58,  | 8.10 ± 0.16, | 10.21 ± 0.33, | 127 ± 1.04, | 155.40 ± 1.04, | 140.30 ± 1.00, |
| VMF 50%  | 72.40 ± 0.81,  | 11.50 ± 0.14, | 13.11 ± 0.40, | 139 ± 2.02, | 162.24 ± 1.08, | 128.46 ± 1.11, |
| VMF      | -       | -         | -         | -   | 200.31 ± 1.02, | 75.22 ± 1.03, |

Note: Means with different subscript letters within a column differ significantly, and they were calculated by Duncan method ($P \leq 0.05$); each value is expressed as mean ± standard deviation ($n = 3$).

Abbreviations: DDT, dough development time; DST, dough stability time; FQN, farinograph quality number; WA, water absorption.

3.1.2 | Farinograph properties of dough

Farinograph properties of dough were studied on the basis of DDT, DST, DoS, and FQN. A significant increase ($P \leq 0.05$) in WA was observed for VMF-WF blends. Ali et al. (2018) reported a similar finding that WA for wheat increases with the addition of black gram flour. However, the amount of VMF added in this study was much higher (10–50%), and the values of WA were much lower. The impact of soluble and insoluble dietary fiber on the rheological quality of dough has already been demonstrated by Issarny, Cao, Falk, Seetharaman, and Bock (2017). The addition of VMF to WF is responsible for weakening the gluten network to an optimum extent and hence improved the baking quality of dough for biscuits, which require weak gluten strength. The WA, DDT, and FQN for VMF 10%, and VMF 15% did not differ significantly ($P \leq 0.05$; Table 1). In this study, DDT was increased significantly ($P \leq 0.05$) as VMF-WF concentration increased up to 25%. Similarly, literature revealed that DDT increased due to the mixing of lentil and WF blends (Turfani, Narducci, Durazzo, Galli, & Carcea, 2017). The reason for enhanced DDT could be the higher affinity of VMF for higher WA by other hydrophilic biopolymers of
flour that swell and delay the mixing time, that is, the DDT for gluten to form the network. WF showed lower DDT, the lesser DoS, and less time for dough stability than the different ratios of VMF-WF blends (Ali et al., 2018). An increase in fiber content with an increased amount of VMF in WF might be responsible for the differences in the mixing profile of the flour blends (Bae, Lee, Hou, & Lee, 2014). Another reason could be the increased FQN for VMF-WF blends, which gave a hardening effect to the dough and ultimately delayed the DDT, DST, and DoS (Ali et al., 2018).

### 3.1.3 Pasting properties of flour

The pasting properties of WF and different blends of VMF-WF were measured under the controlled heating, holding, and cooling conditions. The results obtained (Table 2) revealed the significant differences ($P \leq 0.05$) in the pasting profiles of the flour samples. Pasting functionalities of flour are usually influenced by their particle size, amylose content, the ratio of amylose to amylopectin, and chemical structure of amylopectin of starch granules and also by lipid components present in flour. Amylopectin content in starch mainly contributes to the swelling strength of starch molecules. On the other hand, amylose and lipid contents retard the process of swelling (Vamadevan & Bertof, 2020). PV varied from 1.037 to 1.455 (torque) for all the flour samples. The highest PV was observed for VMF 20%, followed by VMF 15%, which may be associated with swelling power or water absorption ability of starch. The low PV was observed for VMF 50%, and VMF 25% might be due to increased protein to protein, starch to starch, and protein to starch interaction upon addition of a higher percentage of VMF. Another reason for decreased viscosity was weaker resistance of wheat with a higher amount of VMF under heating and shear mixing due to an increased level of nonstarch polymers (NSPs; Bae et al., 2014). The BD values of all the samples ranged from 378 to 571 (torque). The lowest BD among different levels of VMF-WF was observed for VMF 50%, probably due to the dilution of wheat starch with numerous components present in VMF. SB value decreased with the increase in the level of VMF. Julianti, Rusmarilin, & Yusraini (2017) also reported earlier for a decrease in the BD and SB values when sweet potato, maize, soybean, and xanthan gum were added in WF. The lesser value of SB demonstrated a lesser degree of retrogradation of a starch paste after baking (Devi, Sindhu, & Khatkar, 2019). Hence, lower SB viscosity indicates that the less amylose molecules will be the part of retrogradation of the VMF-WF blends during cooling. The substitution of VMF will lower the staling rate of the biscuits made from these blends (Wang, Li, Copeland, Niu, & Wang, 2015). Results suggested that adding VMF could significantly inhibit the retrogradation of the gelatinized flour samples. The decrease in retrogradation may be due to the presence of phenolic components which do not allow amylose molecules to arrange themselves together (Fu, Chen, Luo, Liu, & Liu, 2015).

### 3.2 Antioxidant analysis

#### 3.2.1 Determination of free radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl

In the current analysis, the antioxidant activity of the samples was determined by the DPPH assay. The DPPH radical scavenging activity, IC$_{50}$ values of VMF-WF blends, and biscuits samples during storage are given in Table 3a and Table 3b, respectively. Increasing the level of VMF in the WF increased the DPPH radical scavenging activity, that is, decreased IC$_{50}$ values (Table 3a). Control biscuits showed the highest IC$_{50}$ values hence the lowest DPPH radical scavenging activity during the day of baking, which further became undetectable, whereas no significant difference ($P \leq 0.05$) was observed in IC$_{50}$ values of VMF-WF biscuits till second week of storage. However, IC$_{50}$ values of VMF-WF biscuits samples became stable after the sixth week of storage, as shown in Table 3b. This increased scavenging activity contributes to the presence of radical scavengers or inhibitors in VMF and hence has a probability of acting as a primary antioxidant. Baking furthermore improved the radical scavenging capacity; the increment in antioxidant action might be attributed to the development of melanoids during baking and formation of more reducing low molecular weight compound due to hydrolysis (Sharma & Gujral, 2014). Similar results were reported by Jan et al. (2015) when the varying concentration of buckwheat flour was incorporated in wheat-based biscuits samples. These brown color pigments

#### Table 2 Microvisco-amylograph properties of wheat flour and different ratios of Vigna mungo flour (VMF) incorporated

| Samples     | G (torque) | Peak.V (torque) | HPs (torque) | CPs (torque) | CPe (torque) | HPe (torque) | BD (torque) | SB (torque) |
|-------------|------------|-----------------|--------------|--------------|--------------|--------------|-------------|-------------|
| Control     | 24 ± 0.1   | 1.037 ± 10a     | 717 ± 4a     | 717 ± 5a     | 1.486 ± 10b  | 1.542 ± 10c  | 378 ± 10a   | 479 ± 10a   |
| VMF 10%     | 32 ± 0.3d  | 1.436 ± 10a     | 1.112 ± 11b  | 864 ± 7c     | 1.399 ± 15a  | 1.394 ± 16a  | 571 ± 8a    | 526 ± 7a    |
| VMF 15%     | 34 ± 0.4a  | 1.442 ± 15a     | 1.312 ± 12c  | 842 ± 6a     | 1.325 ± 13a  | 1.382 ± 16a  | 462 ± 7a    | 466 ± 5a    |
| VMF 20%     | 35 ± 0.6l  | 1.455 ± 10b     | 1.350 ± 13e  | 860 ± 7e     | 1.250 ± 11e  | 1.250 ± 13c  | 455 ± 7a    | 321 ± 5a    |
| VMF 25%     | 30 ± 0.2a  | 1.430 ± 12c     | 1.380 ± 14f  | 850 ± 9b     | 1.090 ± 9b   | 1.130 ± 12a  | 451 ± 6a    | 157 ± 3b    |
| VMF 50%     | 31 ± 0.3d  | 1.418 ± 12c     | 1.181 ± 10c  | 829 ± 8b     | 921 ± 6b     | 1.059 ± 11a  | 447 ± 5.1b  | 81 ± 2.2a   |

Note: Means with different subscript letters within a column differ significantly, and they were calculated by Duncan method ($P \leq 0.05$); each value is expressed as mean ± standard deviation ($n = 3$).

Abbreviations: BD, break down; SB, setback; CPe, cooling period end; CPs, cooling period start; G, gelatinization; HPe, heating period end; HPs, heating period start; Peak.V, peak viscosity.
(melanoids) were found to be responsible for higher antioxidant activity (Sharma & Gujral, 2014). These compounds are beneficial in the sense that they can extend the shelf life of food products as that of biscuits, which contains high amount of fat that is responsible for the development of rancidity during storage (Sharma & Gujral, 2014).

### 3.2.2 Ferric/ferricyanide (Fe³⁺) reducing antioxidant power

The results of the reducing power of VMF-WF blends and changes in storage of biscuits samples are shown in Table 3a and Table 3b, respectively. As predicted, the control biscuits sample demonstrated the lesser FRAP, whereas the IC⁵₀ value decreased with the increase of the quantity of the VMF-WF in biscuits samples. The higher level of fat replacement in biscuits revealed stronger reducing power (i.e., lower IC⁵₀ values). The antioxidant activity declined after fourth week and then was stable till the eighth week of the storage as there was no notable difference between IC⁵₀ values of the sixth and eighth weeks. Furthermore, during storage, Caleja, Barros, Antonio, Oliveira, and Ferreira (2017) had reported no change in FRAP when fennel and chamomile extracts were added in biscuits. Similar to DPPH activity, the FRAP improved as lower IC⁵₀ values were observed in biscuits after baking (Table 3b) as compared with VMF-WF blends (Table 3a). The increment in reducing power upon baking has been previously reported in biscuits prepared by incorporation of buckwheat flour in WF (Jan et al., 2015).

### 3.3 Total phenolic content

The phenolics have always demonstrated the potential of protecting the oxidative stress in human health through their antioxidant, anti-radical, and anti-inflammatory attributes (Šibul et al., 2016). The TPC
of different levels of VMF added in biscuits samples during storage are presented in Table 4. Despite a greater decrease in TPC of VMF-WF biscuits during baking as compared with VMF-WF blends (Table 3a), the TPC values in VMF-WF biscuits were still higher than that of control biscuits. A similar decrease in TPC of buckwheat flour was observed when heated to a temperature of 180°C (Jan et al., 2015). The reduction in TPC may be due to alteration in the chemical structure of the phenolic compounds (Sharma & Gujral, 2011). Biscuits prepared with VMF 50% showed the highest TPC throughout the storage period till eighth week, followed by VMF 25% and VMF 20%, between the initial days and second week of storage. There was no notable difference in TPC between the initial days and second week of storage, although TPC reduced from the fourth week of storage period. Even then, all VMF-WF biscuits samples had high TPC when compared with control biscuits. Because pulses are known to be a valuable source of fiber, mineral, and protein, they are reported to carry a wide range of polyphenolic compounds (Carbonaro, 2011). Mainly phenolic acids, flavonoids, and proanthocyanidins were found to be present (Carbonaro, 2011). Earlier studies on various legumes extract reported TPC as 14.3, 12.1, and 10.8 mg GAE/g in horse gram, cowpea, and chickpea flour, respectively (Sreerama, Sashikala, & Pratape, 2012). The TPC differ from cultivar to cultivar in lentils, due to the diverse geographical location and conditions of the cultivars (Alishik, de Camargo, & Shahidi, 2015).

### 3.4 | Total flavonoid content

Phenol and flavonoids neutralized the pace of oxidation and reduction due to the formation of the reactive oxidation species (ROS) precursors of free radicals (Kaurinovic & Vastag, 2019). The TFC in biscuits samples increased with an increased amount of VMF, as shown in Table 4. Biscuits prepared with VMF 50% showed the highest TFC, like in the case of TPC throughout the storage period of 8 weeks (2 months) followed by VMF 25% and VMF 20%. Although the formulation of TFC reduced from the sixth week of the storage period, still, the VMF-WF biscuits had had high TFC as compared with control biscuits samples, which revealed that WF is deficient in flavonoids that are the abundant phenolics in the biscuits. These results also proved the positive correlations between TPC and TFC in plant food (Kheirkhah, Baroutian, & Quek, 2019).

### 3.5 | Evaluation of changes in edible quality of biscuits during storage

#### 3.5.1 | PV and FFA content of biscuits samples

It was clear, as seen from Figure 1a, that initially, FFA was not detectable in VMF-WF biscuit samples. However, a gradual increase (up to 0.87%) was observed during storage, indicating the capability of antioxidants present in VMF-WF biscuits in reducing the formation of % FFA. The FFA in the first 2 weeks got visible from second to fourth week % FFA increased gradually, but from the fourth week onwards, the increase in % FFA was relatively rapid. Moreover, the rate of FFA formation was relatively high in control biscuits compared to the VMF-WF biscuits. The results turned out to be very similar to the earlier research work carried out by Shukla, Jain, Genitha, and Tirkey (2013). The biscuits samples were also analyzed for the formation of PV, and the results are presented in Figure 1b. The results from PV also showed a similar pattern, that is, like FFA, a gradual increase.

### TABLE 4

| Samples | TPC (mg GAE/100 g dw) | 2nd week | 4th week | 6th week | 8th week |
|---------|----------------------|---------|---------|---------|---------|
| Control | 14.430 ± 0.12a       | Nd      | Nd      | Nd      | Nd      |
| VMF 10% | 19.74 ± 2.18d        | 19.64 ± 1.31d | 17.50 ± 1.14c | 15.21 ± 1.12b | 15.19 ± 1.1b |
| VMF 15% | 39.21 ± 2.21f        | 38.81 ± 1.43f | 34.44 ± 1.25f | 21.12 ± 1.21a | 19.87 ± 1.08f |
| VMF 20% | 85.49 ± 3.12g        | 85.21 ± 1.97g | 80.12 ± 1.45g | 69.13 ± 1.32g | 67.93 ± 1.11g |
| VMF 25% | 89.34 ± 3.15h        | 87.01 ± 2.48h | 83.90 ± 2.35h | 70.16 ± 3.00h | 69.13 ± 2.41h |
| VMF 50% | 105.13 ± 3.21i       | 104.65 ± 3.11i | 98.68 ± 3.07i | 90.10 ± 3.17i | 88.21 ± 3.04i |
| TFC (mg CE/100 g dw) | 18.56 ± 1.01a | Nd | Nd | Nd | Nd |
| Control | 104.88 ± 2.21c       | 104.48 ± 1.44c | 100.12 ± 1.25d | 97.88 ± 1.23b | 95.81 ± 1.22a |
| VMF 10% | 359.70 ± 2.74h       | 357.54 ± 2.46h | 331.05 ± 2.42e | 314.14 ± 1.14d | 312.54 ± 1.12d |
| VMF 15% | 395.80 ± 3.45h       | 393.12 ± 3.21f | 352.11 ± 3.15g | 336.86 ± 3.14f | 332.76 ± 3.11ef |
| VMF 20% | 433.97 ± 4.51m       | 430.13 ± 4.43g | 385.01 ± 3.32f | 378.98 ± 3.32g | 375.99 ± 3.25f |
| VMF 25% | 473.60 ± 4.82n       | 472.11 ± 4.89n | 441.04 ± 4.61m | 428.78 ± 3.41kl | 426.21 ± 3.33kl |

Note: Means followed by different subscript letters in the row differ significantly (P ≤ 0.05). Calculations were made using the Duncan method. Each value is expressed as mean ± SD (n = 3). Abbreviations: CE, catechin equivalent; dw, dry weight of the sample; GAE, gallic acid equivalent; Nd, not detected.

Control represents biscuits without fat replacement.
in % PV was observed when biscuits were stored for 8 weeks. The % PV for control biscuits increased from 0.9 to 0.53 mEqO₂/kg of fat for 2 months, whereas the % PV value of all VMF-WF biscuits samples increased from 0.2 to 3.9 mEqO₂/kg of fat, which was in the acceptable range of PV (<5%; Kumar, Vijaykrishnaraj, & Prabhasankar, 2018). Least change in % PV was observed for 50% VMF (0.2–2.1) and highest in 10% VMF (0.67–3.9), that is, PV contents increased with the decrease in VMF ratio. Similarly, an increase in PV was observed in snacks prepared from WF, broken rice, and legumes by-product (Tiwari, Gunasekaran, Jaganmohan, Alagusundaram, & Tiwari, 2011).

3.5.2 | Water activity to evaluate the possibility of microbial growth

The effect of storage on water activity of control and VMF-WF biscuits are shown in Table 5. An increase in water activity of control and VMF-WF biscuits was observed with increases in storage time. The water requirement for the growth of microorganisms was expressed in terms of moisture available or water activity (Tapia, Alzamora, & Chirife, 2020). The water activity of control biscuits increased from 0.62 at 0 day to 0.71 after 2 months of storage. A similar trend was observed earlier for the storage of bread (Bhise & Kaur, 2014). In our study, even at 10% VMF, the water activity was found to be 0.58 at the eighth week of storage, which assured the safety of the product with respect to microbial profile. Rodríguez-García, Laguna, Puig, Salvador, and Hernando (2013) also reported a similar trend of decreased water activity when fat was replaced by inulin in biscuits samples. The lower water activity of VMF-WF biscuits might be due to higher...
water-binding capacity of VMF, as less water evaporated during baking, and less free water remains in the biscuits, thus contributed longer shelf life (Rodríguez-García et al., 2013). No significant ($P \leq 0.05$) change was observed in water activity of control and VMF-WF biscuits from 1st day till second week of storage. Although water activity increased after fourth week of storage and this remained constant up to 8 weeks. Morais et al. (2018) also reported a similar behavior of storage changes in biscuits samples. The increased water activity can be attributed to the increased moisture of biscuits with respect to storage. Therefore, it can be considered that limiting the water activity at 0.6 will prevent microbial proliferation. The VMF-WF biscuits were below that range for developing microorganisms and hence maintain the shelf life of the product after 2 months, as evidenced by the results of water activity (Table 6).

3.6 | Evaluation of quality attributes

3.6.1 | Textural analysis of biscuits

The textural profile of biscuits was determined by using the textural analyzer, as shown in Figure 2. There was a significant difference in hardness (N) that varied from 34.39 in 50% substituted biscuits to 25.98 in 25% and 23.37 in 20% substituted biscuits. The hardness further decreased by decreasing the amount of VMF to 10%. Hardness, which is related to the force necessary to break the biscuits, increased with the increased concentration of VMF in biscuits. The increase in hardness is related to the increased quantity of carbohydrate contents like starch molecules and the reduced amount of fat. However, biscuits prepared with VMF 10% and VMF 15% showed a remarkable acceptable texture profile similar to control biscuits as earlier reported by Ali et al. (2018) when the amount of black gram flour increased in the biscuits.

3.6.2 | Color analysis of biscuits

The surface color of the biscuits decreased with respect to lightness ($L^*$) and yellowness ($b^*$) as the amount of VMF increased in the biscuits (Table 6), whereas an increase in $a^*$ (redness) was observed with an increase in the concentration of VMF in the biscuits. Similarly, Jan et al. (2015) observed decreased $L^*$ and $b^*$ values and increased in $a^*$ value in cookies prepared with blends of buckwheat and WFs. The browning reactions may be due to Maillard browning and caramelization of sugars that are considered to produce brown pigments during baking (Laguna, Salvador, Sanz, & Fiszman, 2011). The browning reactions in baking are influenced by many factors such as water activity, pH, temperature, sugars, and the ratio of amino compounds (Sharma & Gujral, 2013).

### Table 5

| Samples | Initial  | 2nd week | 4th week | 6th week | 8th week |
|---------|---------|----------|----------|----------|----------|
| Control | 0.62 ± 0.02h | 0.61 ± 0.02h | 0.70 ± 0.04i | 0.71 ± 0.02i | 0.71 ± 0.02i |
| VMF 10% | 0.55 ± 0.02a | 0.55 ± 0.01a | 0.57 ± 0.01ef | 0.57 ± 0.02ef | 0.58 ± 0.01f |
| VMF 15% | 0.52 ± 0.01d | 0.52 ± 0.03d | 0.56 ± 0.02ef | 0.56 ± 0.01ef | 0.56 ± 0.01ef |
| VMF 20% | 0.51 ± 0.02d | 0.52 ± 0.02d | 0.55 ± 0.02ef | 0.56 ± 0.02ef | 0.57 ± 0.01ef |
| VMF 25% | 0.47 ± 0.03s | 0.47 ± 0.02s | 0.50 ± 0.03d | 0.51 ± 0.02d | 0.51 ± 0.01d |
| VMF 50% | 0.35 ± 0.01a | 0.34 ± 0.01a | 0.38 ± 0.01b | 0.38 ± 0.02b | 0.38 ± 0.01b |

Note: Means followed by different subscript letters in the row differ significantly ($P \leq 0.05$). Calculations were made using the Duncan method. Each value is expressed as mean ± SD ($n = 3$).

*Control represents biscuits without fat replacement.

### Table 6

| Samples | Color values | $L^*$ | $a^*$ | $b^*$ |
|---------|--------------|------|------|------|
| Control | 73.12 ± 0.82i | 6.13 ± 0.01a | 43.12 ± 0.21i |
| VMF 10% | 67.41 ± 0.67e | 6.91 ± 0.03a | 38.11 ± 0.15a |
| VMF 15% | 54.33 ± 0.34d | 7.30 ± 0.02a | 33.14 ± 0.13a |
| VMF 20% | 48.29 ± 0.13c | 7.81 ± 0.01a | 26.31 ± 0.10a |
| VMF 25% | 42.14 ± 0.11b | 8.11 ± 0.02a | 18.35 ± 0.10a |
| VMF 50% | 36.21 ± 0.10a | 10.32 ± 0.05f | 8.21 ± 0.06a |

Note: Means followed by different subscript letters in the same column differ significantly ($P \leq 0.05$). Calculations were made using the Duncan method. Each value is expressed as mean ± SD ($n = 4$).

*Control represents biscuits without fat replacement.
3.6.3 Nutritional analysis of biscuits

Changes in the nutritional composition of biscuits produced were compared with the control and are summarized in Table 7. The VMF-WF biscuits resulted in significantly (P ≤ 0.05) higher in ash content than the control samples. Moisture content decreased with the increasing concentration of VMF and ranged from 4.80% (10% VMF) to 11.20% (control). The VMF-WF biscuits showed enhanced level of protein, as legumes have more protein than cereals (Stone, Nosworthy, Chiremba, House, & Nickerson, 2019). The protein in 10% VMF was higher in quantity as compared to the control biscuits. It was observed that protein increased significantly (P ≤ 0.05) in all the VMF. In this study, amino acids were not examined. The literature revealed that increasing the amount of legume flour in baked products enhanced the fundamental amino acid profile (Stone et al., 2019). Similar observations regarding protein and ash contents were reported in the previous study when legumes flour was utilized in the preparation of WF-based cookies (Cheng & Bhat, 2016). All the VMF-WF biscuits contain significantly more dietary fiber than the traditional WF biscuits as expected. Meanwhile, the calorific value of all the VMF-WF biscuits also considerably reduced in comparison with the control (497 kcal/100 g). The decreased calorific value of VMF-WF biscuits was confirmed by the previous study when biscuits samples were substituted with olive pomace (Lin et al., 2017).

### Table 7

| Samples     | Moisture content % | Ash %          | Protein %       | Fat %          | Crude fiber % | Carbohydrate % | kcal/100 g |
|-------------|--------------------|----------------|----------------|----------------|---------------|----------------|-------------|
| Controla    | 5.02 ± 0.12a       | 0.53 ± 0.01a   | 11.20 ± 0.10a   | 24 ± 0.20g     | 0.21 ± 0.26a  | 59.04 ± 0.80b  | 497         |
| VMF         | 11.20 ± 0.30d      | 5.20 ± 0.10f   | 26.10 ± 0.50g   | 5.1 ± 0.01i    | 5.02 ± 0.42h  | 52.4 ± 0.30a   | 360         |
| VMF 10%     | 5.20 ± 0.14c       | 1.45 ± 0.03b   | 14.01 ± 0.12b   | 20.01 ± 0.14f  | 1.10 ± 0.20b  | 59.33 ± 0.42c  | 473         |
| VMF 15%     | 5.31 ± 0.10c       | 1.83 ± 0.01c   | 14.21 ± 0.11bc  | 18.21 ± 0.10e  | 1.33 ± 0.11b  | 60.44 ± 0.57d  | 462         |
| VMF 20%     | 5.01 ± 0.11c       | 3.56 ± 0.02d   | 14.75 ± 0.15d   | 16.05 ± 0.21d  | 1.78 ± 0.21i  | 60.63 ± 0.64e  | 446         |
| VMF 25%     | 4.92 ± 0.12a       | 3.70 ± 0.06de  | 15.09 ± 0.20de  | 14.21 ± 0.10c  | 1.88 ± 0.13cd | 62.08 ± 0.60a  | 435         |
| VMF 50%     | 4.82 ± 0.13a       | 4.01 ± 0.09e   | 15.85 ± 0.22l   | 13.01 ± 0.11b  | 2.01 ± 0.17e  | 62.31 ± 0.54f  | 430         |

Note: Means followed by different letters in the same column differs significantly (P ≤ 0.05). Calculations were made using the Duncan method. Each value is expressed as mean ± SD (n = 3).

*Control represents biscuits without fat replacement.

3.6.4 Sensory evaluation of biscuits

The sensory scores for the appearance, texture, taste, color, and overall acceptability of control and VMF-WF biscuits are presented in Figure 3. Biscuits with VMF 10% demonstrated similar results as that of the control sample in terms of overall acceptability. On the other hand, fat replacement by 15% VMF showed good sensory profile for color, which scored higher when we compared with control biscuits, and it was not significantly (P ≤ 0.05) different from control biscuits in terms of appearance, taste, texture, and overall acceptability. As the replacement of fat increased beyond 15%, the taste, which is one of the essential attributes, declined due to unpleasant mouthfeel, and texture became harder as confirmed from the observations of the texture analyzer (Figure 2). The texture was strong and firm in biscuit samples with an increase in substitution of fat (VMF 20% and VMF 25%), as fat helps to moisturize, makes the texture softer, and structure of baked food product acceptable to consumer (Mamat et al., 2014). In view of the reduced fat content of dough in the dough formulation, the flour protein particles were better hydrated during mixing because of the available excess water for wetting the flour components, consequently higher hydration of gluten and harder dough were obtained that produce harder biscuits on baking (Rodriguez-Garcia et al., 2013).
4 | CONCLUSION

In view of the tremendous advancement in the application of many emerging technologies in the bakery industry, new varieties of biscuits have been developed rapidly worldwide, which are being accepted and mainly consumed. Interestingly, the development of baked products having the nutraceutical properties might be attractive for customers who are conscious about their health. The VMF proved it's self an excellent source of phenolics and flavonoids, and they are responsible for the antioxidant potential. Moreover, the antioxidant activity may be retained for 2 months. Fat mimetic by 15% VMF in biscuits showed overall excellent quality and sensory attributes. The present study opens the doors for commercial production of nutraceutical biscuits providing low calories, reduced fat, but rich in proteins, minerals, and dietary fibers. In addition, legumes (Dal) as fat replacer is not only economical but is a new approach of consuming legumes in the diet which are regarded health-promoting from multiple angles.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHOR CONTRIBUTIONS

Syed Muhammad Ghufran Saeed and Syed Arsalan Ali conceptualized this research. Syed Arsalan Ali and Lubna Mobin conducted the experiments. Syed Muhammad Ghufran Saeed, Rashida Ali, and Syed Asad Sayeed supervised the work. Syed Arsalan Ali prepared the original manuscript draft, and Rashida Ali, Syed Muhammad Ghufran Saeed and Shahina Naz contributed to the manuscript revision.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This article does not contain any In-Vivo studies with human participants (except biscuits sensory test) or animals performed by any of the authors.

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