RESEARCH ARTICLE

Food to food fortification of whole wheat flour biscuit using moringa leaves powder

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ABSTRACT

The purpose of the research was to use food to food fortification of staples, as a tool to deliver micronutrients especially minerals to the population. In this research moringa leaves powder (MLP), a local nutrient dense source, was utilized for increasing the micronutrients in whole wheat flour biscuit. Microstructural and mineral analysis of whole wheat flour (WWF) and MLP was carried out. WWF biscuit were prepared by incorporating MLP in different percentages (0, 2, 4, 6, 8 and 10%). Mineral profile, phytic acid content and microstructure of the biscuit were analyzed. The microstructural analysis showed that the gluten network was infered by inculsion of MLP, creating some cavities, but the entire protein matrix was not disrupted. The results of the mineral analysis indicated a significant increase in magnesium (42.34%), calcium(84.91%), potassium(49.82%), phosphorus (32.32%) and iron content (50.92%) of the biscuit fortified with MLP. Phytic acid of the biscuit decreased from 0.66 to 0.39%. It can be concluded that MLP can be a tool to alleviate malnutrition and solve food insecurity especially in the developing counteries.

Keywords: Biscuit; Microstructure; Mineral content; Moringa leaves powder; Phytic acid

INTRODUCTION

Food-to-food fortification is an approach that uses an interesting (contain useful amounts of micronutrients), available and accessible local resource (plant or animal) to fortify another food (Uvere et al., 2010). Food to food fortification contributes to the economic growth because it encourages the utilization of local sustainable resources thereby promoting self-sustenance and create market opportunities for locally produced foods (Burchi et al., 2011).

Moringa oleifera, commenly known as moringa in literature, is a perineal tree and belongs to family Moringacea. It is a tree with multiple uses and is cutivated in a wide variety of agroclimatic conditions around the world. This tree has great potential to play a pivotal role in improving food security and reducing malnutrition (Domenico et al., 2019). All parts (roots, leaves, flowers, seeds and pods) of the plant are considered edible in different counteries and have high nutritional value (Brilhante et al., 2017; Nouman et al., 2016). All parts of moringa have diverse therapeutic, food and feed uses (Singh and Prasad, 2013; El Sohaimy et al., 2015). The medicinal use of moringa is attributed to the presence of high content of phenolics. Moringa has a high antimicrobial, antidiabetic and antihypertensives activity (Chhikara et al., 2020). Moringa is exploited in a number of ways by the industrial sector. It can be used as food supplement, as fodder crop, compost, a plant growth enhancer, coagulant for turbid water and bio-pesticide (Nouman et al., 2016). Moringa leaves are versatile in use and can be consumed in various ways, as can be eaten freash, may be cooked or can be used as dried powder. The dried powder can be stored for a considerable length of time without greatly compromising its nutritional composition (Fahey, 2005; Fuglie, 2001). The leaves have a high content of minerals, especially iron, calcium, magnesium, potassium, copper and zinc which are essential for normal growth and development of the body (Domenico et al., 2019). Moringa leaves being nutrient dense and less expensive have the potential to be used in food to food fortification.

Minerals comprises about 4% of the total body weight and the diet must provid a substantial amount of the minerals.

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Received: 21 October 2021; Accepted: 30 January 2022

Minerals paly important role in body and if insufficient may cause certain abnormalities. Deficiency of iron may cause iron deficiency anemia, defeciency of calcium may give rise to rickets and osteoporosis, and zinc defeciency may lead to diseases of the immune system (Gaffney-Stomberg et al., 2010; Rebellato et al., 2017). Bakery products being staple are of great importance in international nutrition (Agrahar-Murugkar, 2020). Biscuit is one of the widely consumed bakery product with wheat flour as the main ingredient. Biscuit is used in many food fortification programs (Nogueira and Steel, 2018).

Previous research show that cookies prepared using moringa leaves powder resulted in increase of proteins, fibers and mineral content (Ca, Fe, P, K) of the cookies significantly (Dachana et al., 2010; Abdel-Samie and Abdulla, 2014; Mouminah, 2015). Adding moringa leaves powder to biscuit also affected the physical attributes and acceptability of the biscuit (Riaz and Wahab, 2021; Dachana et al., 2010). These authors and Hedhili et al. (2021) suggested that the limit of moringa supplemnation in cookies is upto 10%. A number of factors viz maturity, agroclimatic conditions, stage of harvest, soil type and modes of processing, affects moringa leaves composition (Murwa et al., 2017; Falowo et al., 2018). Pakistan is a developing country and food to food fortification approach by utilizing local nutrient dense resources could prove promising results in combating malnutrition. Moringa oleifera locally grown in the area was used for the research. The research was conducted with the objectives to study the microstructure of moringa supplemented biscuits. Further the mineral composition and phytic acid of moringa supplemented biscuits were determined to ivestigate its nutritional quality.

MATERIALS AND METHODS

Sample of wheat grains (Pirsabak-13 cv.) and moringa leaves was obtained from the Department of Agronomy, The University of Agriculture Peshawar. Laboratory-scale mill (Thomas-Wiley, Model 4. USA) was used for grinding the grains. The leaves were dried under shade and an electric spice grinder was used for grinding the leaves.

Preparation of biscuit

Biscuits were prepared according to the approved method of AACC (2010). The formulation composed of flour (500 g), grinded sugar (250 g), fat (250 g), egg (2 No) and baking powder (10 g). Wheat moringa biscuit (WMB) were prepared by replacing wheat flour with MLP in different proportion. Treatment WMB₀ was control with 100% whole wheat flour and no MLP. In treatment WMB₂, WMB₄, WMB₆, WMB₈ and WMB₁₀, WWF was replaced with 2, 4, 6, 8 and 10% of MLP respectively (Fig. 1).

The microstructure of WWF, MLP and biscuits was studied using Scanning Electron Microscope (JSM5910, JEOL, Japan).

Mineral analysis

WWF, MLP and biscuits were subjected to mineral analysis using atomic absorption spectrophotometer by the method of AOAC (2012).

Determination of phytates

Phytic acid of biscuit samples was determined by the method of Haug and Lantzsch (1983).

Statistical analysis

The data obtained from this study were analyzed statistically according to the standard method as described by Steel and Torrie (1960). Data for mineral analysis and phytic acid of the biscuits were subjected to complete randomized block design (CRD) with one factor and means were separated by Least Significant Difference (LSD) test using software Statistix 8.1.

RESULTS AND DISCUSSION

Microstructural analysis of whole wheat flour and moringa leaves powder

The micrographs of WWF sample are given in Fig. 2. The micrographs clearly show the starch granules (SG) distributed throughout the flour. The starch granules are of varying size that is small and large. As expected, spherical and oval starch granules are visible in the micrographs. Indentation can be identified in some granules in the micrograph of the higher magnification. These

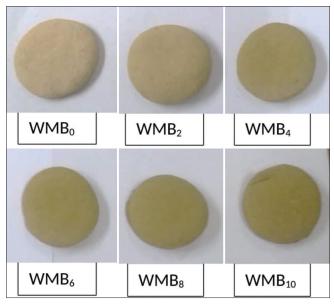


Fig 1. Images for Biscuits

indentations are considered to be the sites for attachment of smaller starch granules, the protein complexes and other cellular bodies. Protein forms a network and embeds the starch granules, this network with embedded starch granules can be seen. Aggregates of flour components can be seen in the micrographs. Close examination revealed a compact non-porous structure of the flour particles (Fig. 2a and b). Similar results were reported by Angelidis et al. (2016). They reported the arrangement of proteins as a matrix embedding starch granules when the particle size is medium. According to the author the aggregates were seen segmented into smaller segments which was attributed to the increased intensity of flour milling. Bressiani et al. (2017) also stated that in the whole wheat flour the bran and starch granules can be easily depicted because of the less interaction between starch granules and protein that can be attributed to the presence of outer layer. In a study by Srivastava et al. (2014), the microstructure of wheat flour showed both small and large granules of starch entrapped in protein matrix. The individual starch granules of circular and lentil shape, the protein matrix and the adhesion of proteins to starch granules are also observed by Roman-Gutierrez et al. (2002).

The micrographs of the MLP are given in Fig. 3. The morphology of the leaves powder can be observed from these micrographs. The leaves powder consists of a compact structure. The proteins form a complex network with other substances. The leaves powder can be seen in which different components are assembled together with irregular shapes and different sizes. The micrograph depicts various crystals which could be of components that is proteins and other substances. Dachana et al. (2010) described the micrographs of moringa leaves powder and stated that the leaves powder contained crystals which could be of various pigments, proteins and other substances. He also highlighted the presence of calcium oxalate crystals. Reddy et al. (2012) stated that the micrographs of moringa leaves powder contain an assembling of irregular shape and size particles which are confirmed by our results.

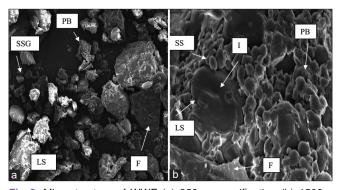


Fig 2. Microstructure of WWF (a) 250 x magnification (b) 1500 x magnification. LSG: large starch granules, SSG: Small starch granules, PB: Protein bodies, F: Fiber, I: indentation

Mineral composition of whole wheat flour and moringa leaves powder

The mineral composition of WWF and MLP is given in Table 1. All the minerals were significantly ($p \le 0.05$) higher in moringa leaves powder as compared to whole wheat flour except Na and Zn which were non significantly different.

The findings of the present study for moringa leaves powder are in line with the observed values of mineral content of different cultivars grown in Pakistan (Nouman et al., 2016). Calcium, Phosphorus and potassium which are considered very important for human bone development and maintenance are present in an appreciable amount in moringa leaves powder. Similar results of mineral composition with little variation were also recorded by Yaméogo et al. (2011), Lalas et al. (2017) and Juhaimi et al. (2017). These authors have different agroclimatic conditions, stage of harvest and drying method than that of the current study. The variation in the mineral content may be attributed to stage of harvest, soil type and modes of processing (Falowo et al., 2018). Moringa leaves contain a higher amount of iron than spinach. Due to the excessive amount of iron in moringa leaves, iron tablets can be substituted moringa leaves powder (Gopalakrishnan et al., 2016). Regular use of Moringa leaves powder can alleviate certain micronutrient deficiencies such as iron deficiency anemia (IDA). Saini et al. (2016) conducted a study on rat model and investigated the bioavailability of folate from

Table 1: Mineral composition	of WWF and MLP
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Mineral (mg/100g)	WWF	MLP		
Mg	98.50 ^b ±7.95	238.06 ^a ±3.81		
Ca	23.01 ^b ±6.75	4043.16 ^a ±89.97		
Zn	2.91±0.65	3.89±1.74		
Na	2.41±0.97	3.96±2.98		
К	474.37 ^b ±38.56	1575.91ª±21.54		
Fe	2.14 ^b ±0.22	28.41ª±1.68		
Р	177.14 ^b ±3.19	206.22ª±15.05		
Mn	3.94 ^b ±0.91	7.47°±1.48		

Values are given as means of three replicates with a standard deviation $(\pm SD)$. Values with different letters in the rows show significant difference at 5% level of significance.

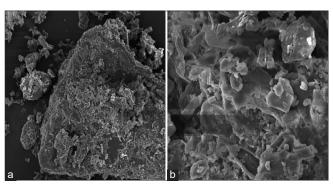


Fig 3. Microstructure of MLP (a) x 250 magnification (b) x 1500 magnification

folic acid and moringa leaves. The research concluded that the bioavailability of folate from moringa leaves was much higher than that of natural folate. The higher mineral content of MLP suggests its use in food to food fortification of staples.

SEM of control and supplemented biscuits

The microstructural analysis of the cross-section of the biscuit (Fig. 4) revealed that in control biscuit (micrograph 4a), the gelatinized starch granules can be seen entrapped in the protein matrix. As the level of enrichment increases the ability of the protein matrix to hold the starch granules in a network weakens. It can be very clearly seen in 10% enriched biscuits (micrograph 4f) that the continuity of the protein matrix is disrupted. Inclusion moringa leaves powder in pan bread also depicted disruption of protein matrix and appearance of cavities at 10% level of Moringa leaves substitution (Gammal et al., 2016). According to Rajiv et al. (2012), in high protein flours the gluten network is disrupted by less hydration, and the protein matrix does not enwrap the starch granules properly. While studying the microstructure of multigrain dough, Indrani et al.

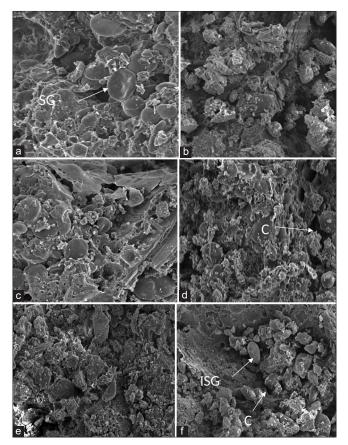


Fig 4. Microstructure of biscuits a: micrograph of biscuit with 100% WWF b: micrograph of biscuit fortified with 2% MLP c: micrograph of biscuit fortified with 4% MLP d: micrograph of biscuit fortified with 6% MLP e: micrograph of biscuit fortified with 8% MLP f: micrograph of biscuit fortified with 10% MLP. Where, SG: starch granules, ISG isolated starch granules, C: cavities

(2010) observed that protein matrix was disrupted. This effect can be seen very clearly in the micrographs. In the micrograph no 4f (biscuit with 10% MLP), individual starch granules can be seen that have been separated from the protein matrix. The biscuit structure depicts that the starch granules are somewhat flattened upon baking. Baking distorted the structure of granules. According to Adebiyi et al. (2016) baking process irreversibly alters the structure of the flour and forms an enveloping crust that stabilizes the structure of biscuits. Srivastava et al. (2014) studied the microstructure of control (wheat) cookies and cookie enriched with 7.5% pomegranate peel powder. He observed gelatinized starch granules embedded by protein matrix both in control and supplemented cookies. In enriched cookies he pointed the starch, protein and fibers.

Mineral composition of biscuit

Statistically the calcium content was significantly ($p \le 0.05$) increased by the addition of MLP (Table 2). In a similar study where bread was supplemented with drumstick leaves power, an increase was noticed in the calcium percentage (Premi and Sharma, 2018). They recorded a rise in the calcium content of the biscuit from 48.78mg/100g (control) to 99.41mg/100g (bread with 15% Moringa leaves powder). Tessera et al. (2015) also reported the increase in the calcium content of cookies from 69.73 to 296.65mg/100g while supplementing with 10% Moringa stenopetala leaves powder. Calcium is vital for the built-up of teeth. Moreover, it also helps in the maintenance of cell balance, contraction of muscle, blood clotting and the normal functioning of muscles, nerves and heart. It is also reported to participate in the activation of a number of enzymes (Sharif et al., 2009). It helps in the prevention of osteoporosis. Calcium is needed in higher amounts as compared to other nutrients and calcium consumption is very low in general populations. At present calcium fortification is gaining interest because of its immense health benefits (Elshehy et al., 2018). Findings of the current research reflects 84% enhancement in the Ca content of the biscuits.

Based on statistical analysis, the potassium content was significantly affected ($p \le 0.05$) by the addition of MLP in the biscuit (Table 2). Potassium along with sodium maintain the fluid balance by controlling acid balance. It is also involved in the enzyme systems in the body (Sharif et al., 2009). Our results for the potassium content of the biscuits are in agreement with those of Kumar et al. (2019). The authors prepared multigrain biscuits and reported 281.23 and 485.11mg/100g of potassium content for wheat and multigrain biscuits, respectively. Similar results of an increase in potassium content were also observed by El-Sharnouby et al. (2012) who prepared biscuits with wheat, wheat bran and date fruit composite flour. Moringa enrichment appreciably enhanced the potassium content of the biscuit.

Minerals (mg/100g)	WMB ₀	WMB ₂	WMB ₄	WMB ₆	WMB ₈	WMB ₁₀	% increase/ decrease
Mg	43.05 ^f ±1.38	51.28°±1.06	57.56 ^d ±0.56	64.26°±1.01	69.35 ^b ±1.16	74.67ª±0.50	42.34
Ca	24.66 ^f ±4.29	40.19°±2.83	55.06 ^d ±3.36	68.26°±3.67	84.23 ^b ±3.30	101.24ª±5.50	84.91
Na	53.39±0.72	56.42±6.61	55.31±4.70	57.20±1.68	52.49±3.92	57.25±3.44	-
К	281.67 ^f ±7.09	325.20°±5.00	372.81 ^d ±2.55	429.66°±3.10	478.50 ^b ±2.25	560.07°±4.76	49.82
Р	127.14 ^b ±14.66	137.38 ^{ab} ±16.46	143.06 ^{ab} ±37.02	158.72 ^{ab} ±42.68	174.93 ^{ab} ±21.22	187.68ª±28.38	32.32
Mn	2.73±0.54	3.19±0.17	2.74±0.28	2.73±0.31	2.92±0.37	2.74±0.28	-
Zn	1.90±0.26	2.05±0.20	2.01±0.10	2.11±0.09	1.82±0.09	1.85±0.11	-
Fe	1.37°±0.26	1.62 ^{de} ±0.25	1.94 ^{cd} ±0.02	2.22 ^{bc} ±0.21	$2.49^{ab} \pm 0.15$	2.78 ^{a±} 0.11	50.92

Table 2: Mineral composition of WWF biscuit fortified with MLP

Values with different letters in the rows show significant difference at 5% level of significance. WMB₂: biscuit with 100% WWF; WMB₂: biscuit with 98% WWF and 2% MLP; WMB₄: biscuit with 96% WWF and 4% MLP; WMB₆: biscuit with 94% WWF and 6% MLP; WMB₆: biscuit with 92% WWF and 8% MLP WMB₁₀: biscuit with 90% WWF and 10% MLP.

The fortification of whole wheat flour biscuit with MLP had a positive effect on the phosphorus content and a significant ($p \le 0.05$) increased was observed. In a research of cookie supplementation with *Moringa stenopetala* leaves powder an increase in the phosphorus content of cookies from 96.45 to 129.98mg/100g (Tessera et al., 2015). Galla et al. (2017) reported an increase in the phosphorus content of biscuit from 54.44 to 132.23mg/100g when supplemented with spinach powder.

The addition of MLP to WWF biscuit significantly $(p \le 0.05)$ increased the magnesium content of the biscuit. Similar results of an increase in magnesium content (26.54 - 60.21 mg/100g) of biscuit were also recorded by El-Sharnouby et al. (2012) who prepared biscuits with wheat, wheat bran and date fruit composite flour. Magnesium functions in the formation of teeth and bones and protein synthesis. It also helps in the conduction of nerve impulse, the release of energy from muscle glycogen and in muscle function (Sharif et al., 2009).

Statistically, a non-significant (p > 0.05) effect was observed on the Na, Mn and Zn content of the biscuit with the enrichment of MLP.

The iron content of the biscuits (Table 2) showed a significant increase ($p \le 0.05$) in the enriched biscuits. Premi and Sharma (2018) reported an increase in the iron content (1.30 - 1.94 mg/100g) of bread supplemented with drumstick leaves powder. Meanwhile, Tessera et al. (2015) also noticed increase in the iron content of cookies supplemented with *Moringa stenopetala* leaves powder. The authors noticed increase from 2.87 to 5.56mg/100g of the cookies with supplementation of 10% of the leaves powder. An increase of iron content (2.85 - 5.64mg/100g) of spinach powder supplemented cookies was also reported by Galla et al. (2017). Singh and Kawatra (2006) reported a significant increase in the iron content of biscuit (5.1%) and cake (10.2%) using Amaranthus leaves.

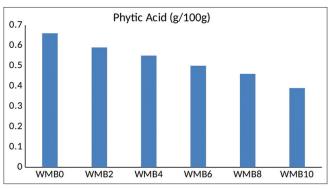


Fig 5. Phytic acid content of Biscuit. WMB0: biscuit with 100% WWF; WMB2: biscuit with 98% WWF and 2% MLP; WMB4: biscuit with 96% WWF and 4% MLP; WMB6: biscuit with 94% WWF and 6% MLP; WMB8: biscuit with 92% WWF and 8% MLP WMB,0: biscuit with 90% WWF and 10% MLP

Phytic acid content

The phytic acid content, which is considered as an antinutritional component, was also determined. The data regarding the phytic acid content is given in Figure 5. The phytic acid content of the biscuits was recorded as 0.66, 0.59, 0.55, 0.50, 0.46 and 0.39% for WMB, WMB, WMB, WMB, WMB and WMB₁₀ respectively. The phytic acid content decreased significantly ($p \le 0.05$) with increasing the level of enrichment of MLP. The findings of the present study are also confirmed by the results of Vitali et al. (2007) who noticed phytate content of 0.56% for standard samples and 0.14% and 0.78% for white flour and whole grain flour biscuits. In contrast to our study Singh et al. (2009) observed an increase in the phytic acid content of the biscuit from 0.12 to 0.13% in biscuits supplemented with 5% Amaranth leaves powder. Antinutritional factor are substances that reduce the nutritive value of the food by lowering the digestibility of the nutrients. The recommended intake of phytates is 250 - 500 mg/100 g(Adeola and Ohigura 2018). It has been reported that fermentation and baking significantly decrease the phytate content in bread making (Almana, 2000; Leenhardt et al., 2005). According to Daneluti and Matos (2013) phytic acid undergoes thermal decomposition when heated above 150°C. The inclusion of moringa leaves powder decreased the phytate content of the biscuits which is very significant as it will increase the bio accessibility of certain nutrients.

CONCLUSION

It can be concluded from the results observed that fortification of whole wheat flour biscuit with moringa leaves powder significantly increased the mineral content of the biscuit except zinc, sodium and manganese content. Interestingly the phytic acid content decreased which indicates a possibility that the bioavailability of the minerals will also be increased. MLP is nutrient dense local resource and its use in food to food fortification can be a promising and sustainable means in combating malnutrition especially in developing countries.

Conflict of interest

There are none to declare.

Author contribution

Aysha Riaz: methodolody, data analysis and original draft preparation; Said Wahab: conceptualization, supervision and validation; Muhammad Ayub: supervision and validation; Majid Suhail Hashmi: review and editing; Farkhanda Jabeen: methodolody.

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