J. Sus. Agric. Sci. **Vol. 48**, No. 4. pp: 41-52 (2022)

Journal of Sustainable Agricultural Science

http://jsas.journals.ekb.eg/

Nutritional evaluation of Moringa leaves and seeds flour and quality characteristics of fortified biscuits

Maalem H. Al-Moalem

Prince Sattam Bin Abdulaziz University, College of Education-Delam, Department of Home Economics, Kingdom Saudi Arabia

THE AIM of this work to study influence of addition of *Moringa Oleifera* leaves or seeds on the nutritional value, chemical composition، and sensorial properties of biscuits suitable for all customers.

Replacement of wheat flour at different ratios (4, 8, 12, and 16%) with defatted Moringa leaves powder (DMLP) and defatted Moringa seed powder (DMSP) to prepare biscuits. (DMLP) and (DMSP) are high in crude protein, ash, and fiber compared with wheat flour (72% ext.). The MLP had higher levels of sodium, calcium, magnesium, manganese, and iron than the MSP. MLP was richer in indispensable amino acids than MSP and wheat flour (72% extra.). Moisture, crude protein, ash, and crude fiber increased in biscuit products in contrast to carbohydrate values. The results showed that replacement of (DMLP) and (DMSP) until 16% is suitable. Based on the obtained results, the biscuit prepared from (DMLP) or (DMSP) covers proteins and minerals for children's nutritional needs in developing countries and could be suggested in institutional feeding programs.

Keywords: Moringa leaves and seeds, nutritive value, fatty acids, amino acid profile, biscuits.

Introduction

The *Moringa Oleifera* tree is an Indian plant, adapted to grow in different countries, including Egypt. Many parts of this plant have various benefits, such as seeds, leaves, and flowers. It has a lot of uses, like foods and drinks, to try to get its highest advantages. The high content of minerals, especially iron and protein, antioxidants, and vitamin A and B group are some of its benefits. It is a significant source of fats, proteins, beta-carotene, vitamin C, iron, potassium, and other nutrients with low toxicity of seeds and leaves **(Mahmoud et al., 2010, Gopalakrishnanb et al., 2016).** *Moringa Oleifera* is widely called the miracle tree, which includes 46 natural antioxidants, 539 biochemical activities, and 36 anti-inflammatories. It's an exceptionally good source of minerals, amino acids, and vitamins **(Amjad et al., 2015, Lin et al., 2018).** There is a global need to increase *Moringa Oleifera's* cultivation area worldwide because it is a highly nutritious plant. The leaves include large and different quantities of vitamins, antioxidants, phytochemicals, flavonoids, minerals, and high-quality protein as they resemble milk proteins (containing eight necessary amino acids), **Fahey et al., (2005).** Moringa leaves and seeds are an excellent source of high nutritional value, such as crude protein, total flavonoid content, total phenolic content, vitamins (A, B, and C), and minerals (Ca, Fe, and Mg), essential amino acids, and natural antioxidants. They act as antidiabetic and anticancer. It is used to prevent various diseases, including liver disease, and malaria, and it helps the system with immunology and digestion. It is cultivated in remote countries and malnourished areas for its various medicinal and nutritional benefits. **(Vyas et al., 2015, Udikala et al., 2017).** Moringa leaves are eaten fresh or cooked as side dishes or as the main dish. They are also dried and used in soups and sauces as a powder **(Kiin-Kabari et al., 2017).** Moringa seeds are used for food seasoning or eaten as roasted nuts in some places and are used in water purification **(Ogunsina et al., 2011,**

*Corresponding author e-mail: maalem.h.m@hotmail.com
Received: 10/06/2022; Accepted: 17/07/2022
DOI: 10.21608/JSAS.2022.143903.1359

James and Zikankuba, 2017). Baked products can be fortified in various ways to provide consumers with therapeutic needs **(Dachana et al., 2010).** Cookies are among the popular bakery products in which ready-to-eat snacks are consumed extensively all over the world because they are ready to eat, cheap, rich nutritionally, available in different tastes, and have a longer shelf life. The major ingredients are sugar, fat, and water **(Mohsen et al., 2009). (Salem et al., 2021)** found that processing a type of bakery product such as cookies using moringa seeds had high nutritive values, especially in protein, crude fibers, and minerals. **Asensi, et al., (2017)** and **Oyeyinka, and Oyeyinka (2018)** reported that *Moringa Oleifera* is used almost exclusively in bread, biscuits, and meat products for nutritional, technological, and preservative purposes, respectively. also, it can provide other benefits for the product, such as improving digestion, dough stability, antioxidant capacity, and preservation, among other benefits associated with the plant. The nutritional content of bakery products fortified in various quantities with *Moringa Oleifera* leaves and seed powders may be significantly increased and nutritionally significant for people's health, such as dietary fiber minerals, Protein, and Lipids. Also, adding MLP and MSP to cookies can increase essential and non-essential amino acids, which have a significant nutritional advantage for developing countries and can help reduce malnutrition diseases **(Bolarinwa et al., 2017, Rabie et al., 2020)**. Therefore, the present work aimed to evaluate the nutritional value and bioactive compounds of moringa leaves and seeds and to utilize them (DMLF) and (DMSF) in biscuit processing as new food products in the market-rich in protein and minerals.

Materials and Methods

Materials:

Moringa (*Moringa Oleifera*) leaves (ML) and seeds (MS) were obtained from the Crop Research Institute, Agricultural Research Center, Sakha, Kafr- Al-Sheikh City, Egypt in the season of 2019-2020. The wheat flour (72%) was purchased from Delta Middle and West Milling Company, Tanta, Egypt. The sugar (sucrose), shortening, baking powder, and whole eggs were purchased from the local market in Tanta, EL-Gharbia Governorate, Egypt. All of the chemicals used in this study were obtained from EL-Gomhourya pharmaceutical company of Tanta, EL-Gharbia Governorate, Egypt. All the other chemicals were analytical grads.

Sample preparation:

The leaves were harvested in their green form; the seeds were collected from mature dried pods. The leaves and seeds were washed with distilled water and dried at 45± 2°C overnight, in an oven, then milled and passed through a 40-mesh sieve and packed in polyethylene bags, and stored at-18°C until usage. As mentioned by **Bolarinwa *et al.*, (2017).**

Chemical analysis:

Gross chemical composition and caloric value of samples:

Moringa leaves, seed powder, and biscuit samples were analyzed for moisture, ash, ether extract, crude protein, and crude fiber content were determined according to the methods of **A.O.A.C. (2005).** Total and available carbohydrates were calculated by difference**.** The energy value for biscuit samples was calculated using the Atwater factors of 4, 9، and 4 for protein, fat and total carbohydrates **(Chaney, 2006).**

Determination of minerals:

Samples were prepared for mineral determination according to the method of the **A.O.A.C. (2005).** Phosphorus content was determined according to the methods described by **Murphy and Riley (1962).** While potassium and sodium contents were measured using a flame photometer **(Pearson 1976).** The iron, magnesium, manganese, copper, zinc, and calcium contents of samples were followed by **Pearson (1976).**

Determination of amino acids:

Fifty milligrams of the samples were mixed with 10 ml of 6 N hydrochloric acid containing 50 µl mercaptethanol in a heat-resistant tube. The tubes were sealed, heated in an oven at 110°C for 24 h., then cooled to room temperature and filtered through Whitman No.1 filter paper. Both the tube and the precipitate were washed with distilled water. The washed water was added to the previous filtrate and then completed to 25 ml in a volumetric flask. Five ml of the filtrate were transformed into a 25 ml beaker and placed in a vacuum desiccator until dry in the presence of potassium hydroxide. The dried residue was dissolved in one ml of sodium citrate buffer (PH 2.2) and analyzed by (Beckman amino acid analyzer, Model 119 CL) as described by **Sadasivam and Manickam (1992)** method.

Determination of Tryptophan:

The tryptophan content of samples was determined colorimetrically after subjecting them to alkaline hydrolysis as outlined by **Blauth *et al.* (1963).**

Extraction of total phenolic compounds (TPC):

The prepared ground materials (10 g) of each sample were soaked in 100 ml of Ethanol (80%), overnight in a shaker at room temperature according to **Salem *et al.* (2018).** The extracts were filtrated through Whatman No.1 filter paper. The filtrates were evaporated under vacuum in a rotary evaporator below 40^{o}C. The extracts obtained after evaporation of organic solvents were stored at-18±2^{o}C until further analysis.

Determination of total phenolic compounds:

The total phenolic compounds of the extracts were determined according to the method given by **(Singleton *et al.*, 1999**) using Folinciocalteau reagent and used to estimate the phenolic acid content using a standard curve prepared using gallic acid.

Determination of total flavonoid content:

The total flavonoid content was measured using colorimetric aluminum chloride (AlCl3) assays based on **Meda *et al.* (2005).** As quercetin equivalents (QE) in mg/g of sample (mg of QE/g).

Determination of DPPH radical scavenging capacity:

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of sample extracts was determined by spectrophotometer according to a modified method described by **(Lee *et al*., 2003)** at 517 nm (HITACI, U-1900). The total antioxidant activity (TAA) is expressed as a % reduction of DPPH.

Preparation of DMLP and DMSP:

The Moringa leaves, and seeds powder weights were soaked for 48 hours at room temperature in an n-hexane solvent (40-60^{o}C), and then filtered. This process was repeated three times, using fresh solvent each time to extract most of the oil from the samples. Then the obtained solution was filtered and the solvent was removed by a rotary evaporator according to **Kahlon *et al.* (1992)**. The defatted moringa leaves and seed powder were milled using a laboratory scale hammer mill. The resulting powder was sieved through a 40 mesh screen and was kept in polyethylene bags and stored at-18±2^{o}C until used.

Preparation of biscuits:

The method of **(Nnam and Nwokocha 2003)** was used to prepare the biscuit samples. Blends containing 4, 8, 12, and 16% of DMLP and DMSP were used as replacements for wheat flour (72% extraction). The biscuit formulas were prepared using 300g of the powder mixture, 100g of margarine, 100g of ground sugar, 35ml of water, 6g of double-acting baking powder, and 12g of the whole egg. All the dry ingredients were blended by stirring 12 strokes with a wooden spoon. Fat was rubbed into the powder mixture until the consistency of bread crumbs was achieved. The egg was whisked for 3 min. and folded into the powder mixture. Water was added to the mixture and a wooden spoon was used to stir 10 strokes to get a homogenous dough. The dough was rolled on a pasty board with a rolling pin to a 3 mm thickness. The flat dough was cut with a biscuit cutter. The biscuits were placed on an aluminum baking pan greased with a little margarine and baked in a gas oven at 200^{o}C for 15 min. After removing from the oven, biscuits were allowed to cool at room temperature for 2 hr., then divided into two lots; the first lot was used immediately for the measurement of sensory evaluation. The second was used for chemical analysis.

Color and Hardness evaluation of biscuits:

Color: The surface color of biscuit samples was determined by the tristimulus color system using a spectrophotometer (MOM, 100D, Hungary). Color coordinates X, Y, and Z were converted to corresponding Hunter L*, a*, and b* color coordinates according to the formula described by **Francis (1983).**

Hardness: The force required to break the biscuits was measured according to the method of **Sharma *et al*.** (**2016**).by using the digital force gauge (model FGN-50, Japan). The probe was carried out using a chisel knife. The biscuits were placed on a plate with a hole located at the center of it. The maximum force required to break the biscuits was reported in Newton (N).

Sensorial evaluation of biscuits:

A semi-trained panel of twenty members used ten-point hedonic-scale ratings for color, taste, odor, texture, and overall acceptability to provide organoleptic characteristics for different repaired biscuits, **Watts *et al*. (1989).** It graduated as the following: extremely liked (9), liked very much (8), moderately liked (7), slightly liked (6), neither liked nor disliked (5), slightly disliked (4), moderately disliked (3), disliked very much (2), and extremely disliked (1).

Statistical analysis:

Data were analyzed using A one-way analysis of variance (ANOVA) that was used to show the significance of treatments. The statistical software SPSS (Version 17.0, SPSS Inc., Chicago, IL) was used for analysis (**Steel and Torrie, 1980).**

Results and discussion

Nutritional composition and bioactive compounds of raw materials:

The results in Table (1) show significant differences between MLP, MSP, DMLP, DMSP, and wheat flour

(72% ext.) concerning their content of moisture, crude protein, crude ether extract, crude fiber, total carbohydrates, available carbohydrates, total phenolic, total flavonoids, and antioxidant activity. The results revealed that DMLP and DMSP are considered good sources of crude protein, crude fiber, and ash compared with these compounds in MLP, MSP, and wheat flour72%, where they recorded (27.03 and 49.67%), (16.20 and 11.70%), and (7.30 and 8.29%). This means adding DMLP and DMSP to bakery products would improve their protein and mineral contents. The data in this respect was in agreement with the findings of numerous investigators **(El-Gammal et al., 2016; Khalaf et al., 2018, Rabie et al., 2020).** It could also be observed that MLP and MSP are significantly higher in total phenolic, flavonoid, and antioxidant activities than DMLP, DMSP, and wheat flour72%. On the contrary, wheat flour 72% had the highest content of total carbohydrates and available carbohydrates (88.88 and 88.33%). Similar data was previously reported by **Hasab- Allah et al., (2017); Khalaf et al., (2018), and Salem *et al.*, (2021).**

Table 1. Nutritional composition and bioactive compounds of raw materials.

Samples	Dry weight basis				
Components	MLP	MSP	DMLP	DMSP	WF (72%ext.)
Moisture%	8.03±0.19[b]	7.23±0.12[c]	6.15±0.13[d]	5.13±0.05[e]	10.11±0.11[a]
Dry matter	91.97±0.18[d]	92.77±0.20[c]	93.85±0.32[b]	94.87±0.24[a]	89.89±0.29[e]
Crude Protein%	26.02±0.31[d]	29.99±0.17[b]	27.03±0.19[c]	49.67±0.25[a]	9.33±0.11[e]
Ether extract%	2.85±0.13[b]	33.65±0.32[a]	0.65±0.11[d]	0.81±0.09[d]	1.28±0.06[c]
Ash%	7.00±0.11[b]	6.32±0.08[c]	7.30±0.10[b]	8.29±0.16[a]	0.51±0.14[d]
Crude fibre%	15.70±0.19[ab]	8.83±0.14[c]	16.20±0.12[a]	11.70±0.07[b]	0.55±0.03[d]
Total Carbohydrates%	64.13±0.41[b]	30.04±0.56[d]	65.02±0.37[b]	41.23±0.27[c]	88.88±0.35[a]
Available Carbohydrates%	48.43±0.33[b]	21.21±0.34[d]	48.82±0.19[b]	29.53±0.14[c]	88.33±0.51[a]
Caloric Value (K. cal./100g)	386.25±0.35[b]	542.97±0.27[e]	374.05±0.21[c]	370.89±0.34[d]	404.36±0.21[a]
TPC (mg GAE/g)	31.02±0.23[a]	13.31±0.20[c]	27.93±0.63[b]	12.19 ±0.25[d]	0.56±0.09[e]
TFC (mg QE/g)	19.80±0.14[a]	1.06±0.10[c]	16.17±0.17[b]	0.98±0.22[c]	0.08±0.07[d]
Antioxidant activity (%)	87. 51 ±0.27[a]	83.43 ±0.11[b]	82. 40 ±0.16[c]	80.22 ±0.19[d]	45.33±0.07[e]

Each value is an average of three determinations ± standard division.

Values followed by the same letter in rows are not significantly different at P<0.05.

TPC: Total phenolic content; TFC: Total flavonoid content; MLP: Moringa leaves powder; MSP: Moringa seeds powder; DMLP: Defatted Moringa leaves powder; DMSP: Defatted Moringa seeds powder.

Mineral content:

Among the functional food ingredients, minerals have a fundamental function in their indispensable role in a healthy life. Minerals, including trace minerals, are important for helping body metabolism, water balance, bone health, helping heart function, preventing fatigue and muscle spasms, and helping transport oxygen throughout the body. It is very important to include the right amount of minerals in the body **(National Academy of Sciences, 2001).**

Data in Table (2) shows that wheat flour (72%) contains lower values for all determining elements compared to MLF and MSF. It is also apparent from the same tables that, there was a significant enhancement in the mineral content of MLP and MSP. Furthermore, the MLP had high levels of sodium (200.56 mg/100g), calcium (1100.12 mg/100g), magnesium (51.5mg/100g), manganese (4.11a mg/100g), and iron (41.33a mg/100g) than those of MSP, while MSP had higher values of potassium and phosphorus than those of MLP. In addition, the iron content of MLP is twenty-five times higher than that of wheat flour. Iron is important for schoolchildren, who mostly need more iron to avoid anemia, especially in developing countries. Therefore, it could be mentioned that fortification of wheat flour with MLP and MSP can produce bakery products with high levels of minerals, especially iron. Also, magnesium and copper contents were nearly the same for MLP and MSP. These results are supported by (Ilyas et al., 2015; El-Gammal et al., 2016; Hasab- Allah et al., 2017; Khalaf et al., 2018; Rabie et al., 2020, and Salem *et al.*, 2021). They reported that some observed differences may be attributed to plant variety, soil structure, environmental conditions, and agricultural treatments of Moringa farming.

Table 2. **Mineral contents (mg/100g) of raw materials.**

Minerals	MLP (mg/100g)	MSP (mg/100g)	Wheat flour (72% ext.) (mg/100g)
Potassium (K)	1380.0±4.38[b]	3840.90±4.9[a]	142.11±0.96[c]
Sodium (Na)	200.56±1.20[a]	60.20±0.85[b]	25.63±0.28c
Calcium (Ca)	1100.12±4.00a	254.11±0.79[b]	14.09±0.12c
Phosphorus (P)	223.11±3.40b	421.40±4.7[a]	121.99±3.38c
Magnesium (Mg	51.5±0.65[b]	53.79±0.32[a]	22.6±0.38[c]
Copper (Cu)	0.67±0.10[a]	0.73±0.06[a]	0.72±0.08a
Zinc (Zn)	6.35±0.11[a]	5.97±0.13[a]	0.25±0.06[b]
Manganese (Mn	4.11±0.09[a]	1.30±0. 09[b]	0.85±0.07[b]
Iron (Fe)	41.33±0.42[a]	6.95±0.26[b]	1.51±0.09[c]

Each value is an average of three determinations ± standard deviation.

Values followed by the same letter in rows are not significantly different at P<0.05.

MLP: Moringa leaves powder; MSP: Moringa seeds powder.

Amino acids composition:

The nutritive value of food, especially protein, depends not only on its amino acids profile in general but also on the quantities of indispensable amino acids in particular **(Afify *et al.*, 2012).** The amino acids composition of MLP, MSP, and wheat flour (72% ext.) are given in Table (3). The results show that MLP was richer in indispensable amino acids than MSP and wheat flour (72% ext.). The total amino acids of MLP, MSP, and wheat flour (72% ext.) were (93.13, 80.26, and 88.60 g/100g crude protein) respectively.

As for indispensable amino acids, it could be observed that leucine was the dominant acid in MLP, MSF, and wheat flour (72% ext.). They recorded (7.34, 4.99, and 5.88 g/100g protein). Meanwhile, cysteine was the lowest amino acid in MLP recorded (1.44 g/100g protein) while tryptophan was the lowest amino acid in MSP and wheat flour (72% ext.) recorded (0.56 and 1.14 g/100g protein). These results agree with **(Rabie *et al.*, 2020 and Salem *et al.*, 2021**), who reported that leucine was the dominant acid in moringa parts. Concerning non-indispensable amino acids reflected that glutamic acid was the highest amino acid among all of the other acids in MLP, MSP, and wheat flour (72% ext.) that were recorded (13.33, 16.40, and 11.60 g/100g protein respectively). Meanwhile, aspartic acid was the second amino acid in MLP that was recorded (8.69 g/100g protein). While arginine was the second amino acid in MSP that was recorded (11.70 g/100g protein). Furthermore, proline was the second amino acid in wheat flour (72% ext.) that was recorded (9.34g/100g protein).

Table 3. **Amino acids profile (g/100g protein) of raw materials.**

Amino acids	**MLP**	**MSP**	**Wheat flour (72%)**	**FAO/WHO** ** **(1973)**
		Indispensable amino acid		
Valine	4.64	3.47	3.92	5.0
Leucine	7.34	4.99	5.88	7.0
Isoleucine	4.18	2.75	3.00	4.0
Metheonine	1.50	1.94	1.80	
Cycteine	1.44	2.89	1.58	
Metheonine+ Cycteine	2.94	4.83	3.38	3.5
Phenylalanine	5.46	4.05	3.99	
Tyrosine	3.50	2.09	2.54	
Phenylalanine+Tyrosine	8.96	6.14	6.53	6.0
Therionine	4.80	2.83	5.14	4.0
Lysine	4.97	2.27	2.86	5.50
Tryptophan*	1.99	0.56	1.14	1.0
Total Indispensable amino acids	41.81	28.06	32.99	36.0
		Non Indispensable amino acid		
Glycine	4.81	4.92	7.25	
Alanine	6.22	4.41	5.31	
Serine	4.24	2.98	4.42	
Aspartic acid	8.69	4.82	7.71	
Glutamic acid	13.33	16.40	11.60	
Proline	5.58	4.67	9.34	
Arginine	6.25	11.70	6.90	
Histidine	2.42	2.30	3.08	
Total Non-Indispensable amino acids	51.54	52.20	55.61	
Total amino acids	93.13	80.26	88.60	

MLP: Moringa leaves powder; MSP: moringa seeds powder.

* Tryptophan was determined calorimetrically.

** FAO/WHO (1973).

The quality of proteins as a source of amino acids can usually be adequately assessed by comparison with the recommended pattern of indispensable amino acids. MLP had higher total indispensable amino acids than the **FAO/WHO (1973)** reference pattern following wheat flour (72%) and MSP. Furthermore, MLP reported higher Phenylalanine+ Tyrosine content, compared with MSP and the FAO/WHO (1973) recommended pattern. Meanwhile, Methionine + Cysteine were higher amino acids in MSF compared with MLP and the **FAO/WHO (1973)** recommended pattern. MLP and MSP in food products fortified with the IAAs, especially cereal products. These results are nearly in conformity with those found by **Ijarotimi et al., (2013) and Khalaf et al., (2018).**

Phenolic compounds of MLP and MSP identified by HPLC :

Phenolics are antioxidants, and there is a general belief that the phenolics present in plant food contribute to preventing the oxidative damage that is implicated in a range of diseases, including cancer, cardiovascular diseases, and aging **(Scalbert et al., 2005).**

Table 4. Phenolic compounds (mg/100g) of MLP and MSP identified by HPLC.

Phenolic compound	MLP (mg/100g)	MSP (mg/100g)
Gallic acid	0.37	1.89
Caffeic acid	3.77	0.1
Caffeine	1.33	0.23
Ferulic acid	2.61	0.16
Vanilic acid	1.83	0.15
Chlorogenic acid	5.10	0.67
Ellagic acid	4.67	0.12
P- coumaric acid	1.44	0.26
P-OH-benzoic acid	13.88	0.90
benzoic acid	11.63	0.37
Catechol	6.11	0.29
catechin	5.33	0.94
Cinnamic acid	1.77	0.12
Salycilic acid	7.87	0.21
pyrogallol	13.03	46.09
qumarin	8.50	0.19
Protocatchuic acid	16.02	0.95
α-cumaric acid	1.27	0.04
Iso-ferulic acid	1.92	0.25

MLP: Moringa leaves powder; MSP: moringa seeds powder.

Nineteen phenolic compounds were identified from MLP and MSP by High-Performance Liquid Chromatography (HPLC) analysis. The detected phenolic compounds were Gallic acid, Caffeic acid, Caffeine, Ferulic acid, Vanillic acid, Chlorogenic acid, Ellagic acid, P- coumaric acid, P-OH-benzoic acid, benzoic acid, Catechol, catechin, Cinnamic acid, Salicylic acid, pyrogallol, qumarin, Protocatechuic acid, α-coumaric acid, and Iso-ferulic acid are shown in Table (4). The obtained data indicated that MLP showed the highest content of Protocatechuic acid (16.02 mg/100g) followed by P-OH-benzoic acid (13.88 mg/100g) followed by pyrogallol (13.03 mg/100g), while MSP showed the highest content of pyrogallol (46.09 mg/100g) followed by Gallic acid (1.89%) followed by Protocatechuic acid (0.95 mg/100g). These results were near to those reported by **El-Massry et al. (2013); Abdel-Nabeh et al., *(*2015) and Hasab- Allah et al., (2017).** However, it is important to consider that phenolic compound yields are strongly dependent not only on the season, weather conditions, and the application of fertilizers, but also on the cultivar and genetic variability, which could be the most relevant factors for the phytochemical composition of *Moringa Oleifera* **(Nouman *et al.*, 2016).**

Chemical composition of biscuits:

The results of the chemical composition of biscuits made from different levels of DMLP and DMSP are recorded in Table (5). The obtained results showed that the moisture, crude protein, ash, and crude fiber contents of biscuits increased. While, the carbohydrate value decreased gradually by increasing the substitution levels of DMLP and DMSP. These results are in harmony with the findings of **Mouminah (2015).** They reported that replacing wheat flour with MLP and MSP ~~It~~ increased protein, fat, crude fiber, and ash. Conversely, carbohydrates were decreased. In the same table, there were significant differences between control and substitution biscuits at different levels of DMLP and DMSP in moisture content. The moisture content ranged between 7.90% in control to 8.51and 8.35% in substitution biscuits with 16% DMLP and DMSP. The increased moisture content can be explained by the higher content of protein and fiber, which also increases the water-binding capacity of the dough with higher levels of DMLP and DMSP **(Farahat *et al.*, 2020, Salem *et al.*, 2021).** As shown in the same table, the substitution of DMLP and DMSP for wheat flour leads to increased protein content from 10.45% in control to 13.16 and 16.65% in biscuit substitutions with 16% DMLP and DMSP respectively. This increment may be due to the DMLP and DMSP are high protein content as compared to wheat flour. The data of the present study is in agreement with those found by **Ogunsina *et al.*, (2010), El-Gammal *et al.*, (2016), Salem *et al.*, (2021).** Therefore, DMLP and DMSP tended to improve the protein content of biscuits. Meanwhile, there was an increase in ash and crude fiber content with an increasing level of substitution of DMLP and DMSP in all biscuit samples. This increment may be due to the incorporation of DMLP and DMSP.

Table 5. Chemical composition (% on a dry weight basis) of biscuits made of different substitution levels of defatted moringa leaves and seed powder.

Component % / Samples	Moisture (g/100g)	Protein (g/100g)	ether extract (g/100g)	Ash (g/100g)	Crude Fibre (g/100g)	*T. C (g/100g)	Energy value (K. cal./100g)
Control	7.90 ±0.19^{e}	10.45 ±0.19^{h}	23.20 ±0.19^{a}	0.98 ±0.19^{g}	1.19 ±0.19^{h}	65.37 ±0.19^{a}	512.08a
4% D MLF	8.07 ±0.19^{cde}	11.03 ±0.19^{g}	23..14 ±0.19^{a}	1.18 ±0.19^{f}	1.71 ±0.19^{f}	64.63 ±0.19^{b}	511.01^{ab}
8% DMLP	8.19 ±0.19^{bcd}	11.72 ±0.19^{f}	23.09 ±0.19^{a}	1.43 ±0.19^{e}	2.32 ±0.19^{d}	63.75 ±0.19^{c}	509.73^{bc}
12% DMLP	8.29 ±0.19^{abc}	12.44 ±0.19^{e}	23.03 ±0.19^{a}	1.59 ±0.19^{d}	2.99 ±0.19^{b}	62.94 ±0.19^{d}	508.79^{cd}
16% DMLP	8.51 ±0.19^{a}	13.16 ±0.19^{d}	23.01 ±0.19^{a}	1.94 ±0.19^{b}	3.50 ±0.19^{a}	61.89 ±0.19^{e}	507.29^{e}
4% DMSP	8.04 ±0.19^{de}	12.00 ±0.19^{f}	23.14 ±0.19^{a}	1.23 ±0.19^{f}	1.52 ±0.19^{g}	63.62 ±0.19^{c}	510.78^{ab}
8% DMSP	8.13 ±0.19^{bcde}	13.58 ±0.19^{c}	23.08 ±0.19^{a}	1.51 ±0.19^{de}	1.93 ±0.19^{e}	61.82 ±0.19^{e}	509.39^{c}
12%DMSP	8.22 ±0.19^{bcd}	15.09 ±0.19^{b}	23.01 ±0.19^{a}	1.8٠ ±0.19^{c}	2.43 ±0.19^{d}	60.10 ±0.19^{f}	507.85^{de}
16% DMSP	8.35 ±0.19^{ab}	16.65 ±0.19^{a}	23.07 ±0.19^{a}	2.08 ±0.19^{a}	2.81 ±0.19^{c}	58.19 ±0.19^{h}	507.06^{e}

Each value was an average of three determinations ± standard division.
Values followed by the same letter in columns are not significantly different at $p \leq 0.05$.
Control: Biscuits with 100% Wheat flour; DMLP: Defatted Moringa leaves powder; DMSP: Defatted Moringa seeds powder.
*T. C: Total Carbohydrates.

On the other hand, it could be noticed that there were no significant differences in the ether extract contents of the control and substitution biscuits. Conversely, carbohydrates were decreased progressively when the DMLP and DMSP levels increased in all biscuit samples, which reached (61.89%) at 16.0 % of DMLP and (58.19%) at 16.0% DMSP as compared to (65.37%) for the control sample. These results are following those found by **Ogunsina *et al.*, (2011), Ashoush and Mahdy (2017), Salem *et al.*, (2021).** Furthermore, the energy value of biscuit samples was significantly decreased by increasing DMLP and DMSP. It was decreased from 512.08 kcal/100g in control to 507.29 and 507.06% kcal/100g in biscuit substitution with 16.0 % in DMLP and DMSP. These results were in agreement with **Salem *et al*., (2021).**

Color and Hardness evaluation of biscuits:

Color is an important trait because it can stimulate an individual's appetite. As presented in Table (6). Significant differences ($p < 0.05$) in color measurements were reported. The biscuits made from different levels (4, 8, 12, and 16 %) of DMLP and DMSP were darker than biscuits made from wheat flour. where L* considerably decreased from 63.34 to 57.38 in the case of DMLP biscuits, but DMSP biscuits decreased from 69.30 to 57.20 **(Abdel-Samie and Abdulla, 2014; Khalaf *et al.,* 2018).** While biscuits made level (16%) of DMLP had negative a* values, this may be due to the sharp green color of the moringa leaves, a* values gradually decreased with the increase of DMLP. Also, a* slightly decreased in DMSP with increasing substitution.

Table 6. **Color and textural attributes of biscuits substituted with different levels of D.MLF and D.MSF.**

Parameters	L (Lightness)	A (Redness∕green-ness)	b (Yellowness∕blue-ness)	Hardness (N/cm2)
Samples				
control	78.76 ±1.15 [a]	6.69 ±0.50 [a]	32.92 ±1.05 [a]	45.79 ±0.16 [i]
4% D. MLF	63.34 ±0.35[d]	3.14 ±0.14[c]	23.26 ±0.30[c]	107.50 ±0.11[h]
8% D.MLF	64.44 ±0.44[e]	2.10 ±0.11[d]	20.31 ±0.32[d]	112.24 ±0.19[f]
12% D.MLF	58.89 ±0.17[f]	1.07 ±0.06[e]	14.96 ±0.34[f]	117.34 ±0.22[d]
16% D.MLF	57.38 ±0.38[g]	- 0.19 ±0.31[f]	13.06 ±0.30[g]	124.09 ±0.11[b]
4% D.MSF	69.30 ±0.44[b]	4.11 ±0.11[b]	24.76 ±0.15[b]	110.37 ±0.13[g]
8% D.MSF	64.28 ±0.40c	3.25 ±0.22[c]	22.08 ±0.27[c]	115.65 ±0.11[e]
12%D.MSF	59.47 ±0.46[f]	2.14 ±0.16[d]	20.12 ±0.14[d]	122.79 ±0.18[c]
16%D.MSF	57.20 ±0.25[h]	1.89 ±0.09[d]	17.47 ±0.51[e]	129.75 ±0.22[a]

Each value was an average of three determinations± standard division.
Values followed by the same letter in columns are not significantly different at $p \le 0.05$.
Control: biscuits with 100% Wheat flour; DMLP: Defatted Moringa leaves powder; DMSP: Defatted Moringa seeds powder.

In addition, the b* value gradually decreased ($p < 0.05$) as the level of DMLP and DMSP increased in biscuits. These changes were expected since the DMLP is characterized by a dark green color. Similar results have been reported in Moringa Oleifera L. leaf-enriched maize-based tortillas **(Paramo-Calderon *et al.,* 2019).** In addition, previous indications suggested that protein content can be negatively related to the lightness of biscuits, indicating that the Maillard reactions also play a role during color formation **(Giuberti *et al.,* 2018).** As shown in the same table, a significant increase in the biscuit hardness was observed with increasing substitution of D.MLP and D.MSP. The above results indicate that the DMLP and DMSP having high protein, dietary fiber, calcium, and iron content diluted the gluten. The data of the present study is in agreement with those found by **Dachana *et al.,* (2010), Giuberti *et al.*, (2018), and Giuberti *et al.*, (2021).**

Sensory characteristics of biscuit samples

The data of the sensory evaluation of biscuit substitutions with DMLP and DMSP are presented in Figure 1. The higher score of taste was recorded in control biscuits (9.0), followed by substitution biscuits with 4% DMLP and DMSP. The lowest score of taste was found in biscuit substitution with 16% DMLP and DMSP. This may be due to the taste bitterness of defatted Moringa seeds which was rated as a decrease acceptable by panelists (**Ogunsina *et al.,* 2011).** The color of any kind of food is an important parameter that gives the first sight impression on consumers and affects its acceptability **(Reyes-Caudillo *et al.,* 2008).** A significant decrease ($p<0.05$) in color could be observed. This may be due to the green color of the biscuits imparted by the chlorophyll content of DMLP, which affects negatively consumers. From the same table, it could be observed that there was no significant difference in odor and texture score between the control sample and substitution biscuits with 4.0 and 8.0% of DMLP and DMSP. Meanwhile, significant differences were noticed in odor and texture scores among substitution biscuits with 12.0 and 16.0% of DMLP and DMSP.

Also, from the same Figure, significant differences were noticed in the overall acceptability score between the control sample and substitution biscuits DMLP and DMSP. The lowest overall acceptability score was found in biscuit substitution with 16% of DMLP and D.MSP. All samples were acceptable. These results were in agreement with **Ogunsina *et al.*, (2011); Mouminah, (2015)**. **and Salem *et al.*, (2021).** They reported that the incorporation of MLP in cookies caused the relatively greenish and dark color of the crust and crumbs.

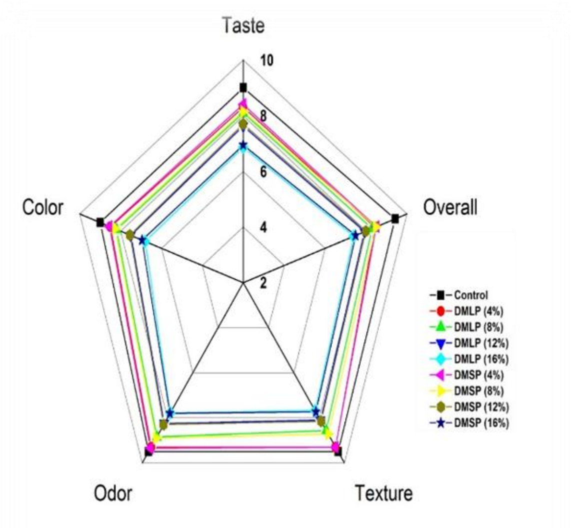

Fig 1. Sensory characteristics of biscuits made with different substitution levels of defatted moringa leaves and seed powder.

Conclusion

Through all the data concerning different compounds existing in *Moringa Oleifera*, The research concluded that Moringa leaves and seeds are very rich in many important nutrients for human health, such as proteins, fiber, calcium, iron, zinc, and bioactive compounds. It has been proved that it could be practical to utilize different parts of Moringa in producing very important and palatable economic products, such as biscuits made from defatted Moringa leaves and seeds. It is also recommended to expand the area cultivated with moringa in the future.

Conflict of Interest

I, the author of this paper, earnestly declare that there is no conflict of interest or relationship, financial or otherwise (between me and any individual, organization, or a group of people) that might be perceived as influencing my objectivity.

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