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Biological, physiochemical and sensory evaluation of hard biscuit enriched with a powder of moringa leaves (*Moringa oleifera* L.)

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Abstract

Moringa oleifera is a tree of a not well-understood plant since it has not been fully studied all over the world. Thus, the aim of this study was to evaluate the effect of the addition of *Moringa oleifera* leaves powder (MOLP) on the biological, physiochemical, and sensory evaluation of hard biscuits. MOLP can be used as a functional food ingredient for both food and organic applications. The proximate analysis showed that Moringa leaves are rich in fiber, protein, carbohydrate, and ash (13.68, 21.60, 54.83, and 4.07 g/100 g, respectively). *Moringa oleifera* leaves are a reliable source of essential minerals such as Na (09.10), K (20.82), Mg (03.36), Ca (19.30) and Fe (18.64) mg/100 g. The amino acid composition of MOLP contained 8 essential and 9 nonessential amino acids, and the percentage of these was determined. The total phenolic content was 52 mg GAE/g. Meanwhile, the antioxidant activity was 85%. HPLC analysis of phenolic acids and flavonoids was performed. The proximate analysis and mineral composition of biscuits fortified with 0.5, 1, 1.5 and 2% MOLP showed that all treatments increased the mineral, protein, and amino acid contents by increasing the percentage of Moringa leaves powder compared with the control. Sensory accepts up to 1.5% of MOLP with a hard biscuit. MOLP decreased glucose levels at different concentrations compared to the control, while hemoglobin levels increased with increasing Moringa leaves. A similar test was observed for body weight. The results have shown the possibility of using dried *Moringa oleifera* leaves powder to improve the nutritional values of hard biscuits with sensory acceptability and biological effects.

Keywords: *Moringa oleifera*, antioxidant activity, amino acid, flavonoids, phenolics.

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1. Introduction

Moringa oleifera is a perennial tree that is still considered an underutilized plant and belongs to the Moringaceae family. All plant parts have a remarkable range of functional and nutraceutical properties (Singh *et al.,* 2013) making this plant a diverse biomaterial for food and related purposes. Associated with the high nutritional value of its edible portions pave the way for making this plant more popular as an important food source to combat the protein energy malnutrition problem prevailing in most underdeveloped and developing countries of the world. The presence of several types of antioxidant compounds makes this plant a valuable source of natural antioxidants (Anwar *et al.,* 2007). Functional foods resemble conventional foods; the first is consumed in a normal diet. In contrast to conventional foods, functional foods, however, have proven physiological benefits and can reduce the risk of chronic disease beyond basic nutritional functions, including maintenance of gut health (FAO., 2007). The Foundation for Healthy Food explained: "A functional food can be a natural product that contains useful biological components, or a food obtained through a technological intervention that increases its level of biologically active compounds. Biologically active compounds are components of foods that act positively on key body functions that are relevant to health. They reduce the risk of developing diseases such as atherosclerosis, hypertension, myocardial infarction, and diabetes (Aramesh Mand Ajoudanifar, 2017). *Moringa oleifera* has potent antioxidant activity, which can prevent and protect pancreatic cells from the oxidative stress associated with the hyperglycemic state (Coskun *et al.*, 2005). Natural antioxidants found in Moringa plants are known as phenolic compounds. The shikimic acid route is used to biosynthesize phenolic chemicals from phenylalanine or tyrosine. Phenolic compounds range in complexity from simple to conjugated or complicated. The antioxidant effects of the phenolic compound are due to the hydroxyl group on the benzene ring. Polyhydroxy phenolic compounds are those that include more than two hydroxyl groups. Polyphenolic compounds are defined as those that have more than one phenol group (Zeb, 2020). Phenolic and flavonoid compounds are natural antioxidants present in plant foods. Phenolic compounds ranged from simple to conjugated or complex compounds. The hydroxyl group on the benzene ring manages the antioxidant properties of the phenolic compound. More than two hydroxyl-containing compounds are thus termed polyhydroxy phenolic compounds. When there is more than one phenol moiety in a compound, it is termed a polyphenolic compound. Phenolic antioxidants are extensively studied in different plant foods such as vegetables, fruits, cereals, seeds, leaves, berries, tea, onion bulbs, wine, and

vegetable oils (Dimitrios, 2006). A biscuit, a popular example of a bakery product, has been an already-to-eat snack that possesses several attractive features including a wide consumer base, relatively low cost, high convenience, long shelf life, and the ability to serve as a vehicle for important nutrients (Akubor, 2003; Hooda and Jood, 2005). They are generally available in different forms, tastes, and shapes. The major ingredients are flour, fat, sugar, and water, while other ingredients, such as milk, salt and aerating agents can be included. They can also be rich or fortified with other ingredients to meet the specific nutritional needs of consumers. In recent years, numerous studies have shown the potential of utilizing natural plants such as green leaves vegetables in biscuit production. The objectives of this study were to study the chemical composition, antioxidant activity, phenolic, content, flavonoid content, and amino acid composition of *Moringa oleifera* leaves, to study the effect of supplementing hard biscuits with 0.5%, 1% and 1.5% *Moringa oleifera* leaves powder on the nutritional value and quality of the biscuits produced, and to study the effect of supplementing hard biscuits with 0.5%, 1% and 1.5% *Moringa oleifera* leaves on the biological characteristics of normal rats.

2. Materials and methods

2.1 Material

2.1.1 Moringa oleifera leaves

Fresh mature *Moringa oleifera* leaves were carefully harvested in the summer of 15/July 2020 from a Moringa farm in Suhag, Egypt. The moringa leaves were collected, and the stalks were removed, dried, ground and stored in dry conditions until use.

2.1.2 Reagents and chemicals

All chemicals and reagents used in this study were obtained from Sigma Chemical Co. (St. Lewis, MO. Egypt) and purchased from El-Gamhoria Trading Chemicals and Drugs Co. (Assiut, Egypt). Technological Materials: Wheat flour (72% extraction), sugar powder, butter, baking powder, milk powder, egg, iodized salt, and other general ingredients were purchased from the local market in Assuit, Egypt.

2.1.3 Experimental animals

Forty adult male albino rats weighing between 150-200 ±2 g was obtained from the animal house, Faculty of Medicine, Assuit University, Egypt.

2.2 Methods

2.2.1 Preparation of Moringa oleifera leaves powder (MOLP)

The leaves were washed with clean water at a 1:5 leaves to water ratio to remove dust and pests, and subsequently drained on a draining table for approximately one

hour before being transferred to drying sheets at an ambient temperature of 29 to 32°C and a relative humidity of 75 to 80% and dried for 2 to 4 days to reach 11 to 12% moisture content. Dried leaves were milled using a hammer mill with a 150 µm screen. The obtained *Moringa oleifera* leaves powder was stored in airtight plastic bags, which also protected the leaves from ultraviolet light to preserve nutrients (Saini *et al.*, 2013).

2.2.1.1 Chemical analysis

Percentages of moisture, crude protein, crude fiber, and ash were determined according to the method described by AOAC (2000). Furthermore, the percentages of calcium, magnesium, iron, manganese, and zinc were determined. Sodium and potassium were determined by a flame photometer (Corning 400, Serial No. 4889, UK) according to the method described by Houba *et al.* (1989).

2.2.1.2 Determination of antioxidant activity of M. oleifera leaves powder

The DPPH radical-scavenging capacity of a methanol extract of *Moringa oleifera* was evaluated according to the method of Ravichandran *et al.* (2013) An aliquot of 0.2 ml sample extract was added to 3.8 ml of DPPH solution in absolute ethanol (final concentration 0.1 mm). The mixture was shaken vigorously for 1 min by vortexing and allowed to stand at room temperature in the dark for 30 min. Subsequently, the absorbance of the sample was measured using a UV-visible spectrophotometer at 517 nm against an ethanol blank. EC50 values (µg/ml) were calculated by interpolation from linear regression analysis. A Multiple Range tests. The capability to scavenge the DPPH radical was calculated using the following equation: DPPH scavenging effect (%) = (A0 – A1/A0) × 100, where A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of the sample.

2.2.1.3 Quantitative phenolics and flavonoids using HPLC of MOLP

Total phenolic content (TPC) in MOLP was determined according to the method of Zielinski and Kozlowska, (2000). HPLC determination of phenolic compounds was determined according to the method of Bendini *et al.* (2008).

2.2.1.4 Determination of the amino acid composition of MOLP

The amino acids were prepared and determined in the National Research Center, Cairo, Egypt, according to the method described by AOAC (2012).

2.2.2 Biscuit Manufacturing

2.2.2.1 Preparation of biscuit blends

The preliminary investigation was carried out by preparing biscuits with 0.0% MOLP as the biscuit control, 0.5% MOLP as treatment (1), 1% MOLP as treatment (2), 1.5% MOLP as treatment

(3) and 2% MOLP as treatment (4). These samples were evaluated for sensory attributes in comparison with the control. The following blends were selected and used for further analysis based on the sensory evaluation results. The ingredients (wheat flour 72% extraction, sugar powder, butter, egg, sodium chloride, sodium bicarbonate, vanilla solution 2% and milk powder) were used for the preparation of biscuits according to Omoba and Omogbemile (2013). The wheat flour 72% extraction and other dry ingredients were mixed to obtain a uniform blend. Butter and sugar were mixed to obtain sweetened shortening cream. Then slowly mixed dry flour was added to shortening cream with the addition of water to prepare dough. The dough was prepared by manual kneading of all the dry and liquid ingredients to attain uniformity with desirable viscoelastic characteristics. When the dough was ready it was kept for 10-15 minutes as it is and then used for sheeting. Sheets were made by rolling balls of dough on a wooden platform. These sheets were cut by hand operated metal dye, arranged on a butter coated tray, and kept for baking. Baking takes place in three successive stages in an electric oven. In the beginning, structural changes take place due to the heating of dough. In the second stage, the greatest loss of moisture occurs. In the third stage, the color of the biscuit changes to the typical light brown color of the finished biscuit. Each lot required 10-20 minutes at 160^{o}C for baking (Omoba and Omogbemile, 2013).

2.2.2.2 Chemical composition of hard biscuit

Percentages of moisture, crude protein, crude fiber, and ash were determined according to the method described by AOAC (2000). Furthermore, the percentages of calcium, magnesium, iron, manganese, and zinc were determined. Sodium and potassium were determined by a flame photometer (Corning 400, Serial No. 4889, UK) according to the method described by Houba *et al.* (1989).

2.2.2.3 Sensory evaluation of hard biscuit

The samples were accessed in the Food sciences and technology staff, faculty of Agriculture Al-Azhar University, Assiut, Egypt using a 10-member sensory panel drawn from staff of the institution adjudged to be remarkably familiar with biscuits. The samples that were disguisedly coded as A, B, C, D and E were presented to the panel members in an analogous manner and form in a separated booth to maintain privacy and avoid biased judgment. They were provided with a glass of potable water to rinse their mouth between samples tasting. Each panelist was requested to score the samples for color, taste, smell, texture, and general acceptability on a 9-point hedonic scale, where 1 = Dislikes extremely and 9 = Like extremely. The scores for sample A, which represents the wheat flour biscuit (WFB), and B, C, D, E, which represents the Moringa wheat flour biscuit (MWFB) were collated

accordingly by Wichchukit *et al*. (2015).

2.2.2.4 Experimental animals

Forty adult male albino rats weighing 150–200 ±2 g was used in the present work. Rats were provided by the animal house, faculty of medicine, Assiut University. Animal house and bred in specific pathogen free conditions. They were maintained in an air-conditioned room (20–25° C) and subjected to a 12:12-h light/darkness cycle with free access to food and water. A total of 40 rats were randomly divided into 5 main groups. The first group contained 6 rats fed the basal diet for thirty days and served as the negative control (C -ve). The second group contained 10 rats fed biscuits without MOLP for thirty days and served as the positive control (C +ve). Then, twenty-four rats were randomly divided into three groups (T1, T2 and T3), eight rats each, fed a hard biscuit diet with *Moringa oleifera* leaves powder at various levels for thirty days as follows: (T1) Rats fed a biscuit diet enriched with 0.5% *Moringa oleifera* leaves powder. (T2) Rats fed a biscuit diet enriched with 1% *Moringa oleifera* leaves powder. (T3) Rats fed a biscuit diet enriched with 1.5% *Moringa oleifera* leaves powder.

2.2.2.5 Estimation of blood glucose

The blood glucose estimation (mg/dl) was performed on the same day using a glucose colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to Weissman and Klien (1958).

2.2.2.6 Estimation of blood hemoglobin

Various methods for hemoglobin determination (g/dl) that are employed in these automated instruments are the cyanometer hemoglobin technique and the oxyhemoglobin method according to Louderback and Fontana (1976).

2.2.2.7 Relative organs and body weight changes

After 30 days, livers and kidney were carefully excised and weighed for the evaluation of their ratios/body weight. The relative organ weight was calculated by Al-Attar (2010) using the following equation: Relative organ weight = (organ weight) / (body weight) ×100.

2.2.3 Statistical analysis

The statistical analysis was statistically analyzed by one-way analysis of variance (ANOVA) using the SPSS 11-software package. The sensory analysis was statistically analyzed. Duncan's multiple range test was applied to assess significant differences between means at the 5% level of probability Duncan. Each experiment (in triplicate) was repeated, and the values are presented as the means ± standard deviations.

3. Results

3.1 Chemical composition of MOLP

The obtained values for the chemical characteristics of MOLP are summarized in Table (1). The analyzed chemical attributes were moisture, ash, crude fats, crude protein, and crude fiber, which were 3.88, 4.07, 1.94, 21.60, and 13.68 respectively, while carbohydrate was 54.83.

3.2 Determination of some mineral contents of MOLP

As shown in Table (2), Moringa leaves powder contained elevated levels of sodium (9.10 mg/100 g), potassium (20.82 mg/100 g), magnesium (3.36 mg/100 g), iron (18.64 mg/100 g), zinc (1.0 mg/100 g), copper (1.05 mg/100 g) and calcium (19.30 mg/100 g).

3.3 Amino acid composition of MOLP

As shown in Table (3), *Moringa oleifera* leaves powder contains 17 amino acids. Essential amino acids accounted for 6.34% of the total amino acids. The highest percentage was Lucien 1.60%, followed by lysine 1.14% and the lowest was histidine 0.43%. The percentage of nonessential amino acids was 9.90% of the total amino acids. The highest is glutamine 2.34% followed by aspartic acid 1.71%, and arginine 1.19%, but the lowest amino acid is cysteine 0.63%.

Table (1): Chemical composition of *Moringa oleifera* leaves powder.

Constituents	g/100 g *
Moisture	3.88± 0.02
Ash	4.07±0.07
Crude Fats	1.94±0.02
Crude Protein **	21.60±0.05
Crude fiber	13.68±0.02
Total carbohydrates ***	54.83

* Calculated on a dry wet basis, and values are the mean ± standard deviation (triplicate each cultivar), **Crude protein = (N %) × 6.25, *** Total carbohydrates were calculated by differences.

Table (2): Mineral elements of *Moringa oleifera* leaves powder.

Mineral content	mg/100 g of MOLP
Sodium (Na)	09.10 ± 1.50
Potassium (K)	20.82 ± 0.08
Magnesium (Mg)	03.36 ± 0.06
Iron (Fe)	18.64 ± 0.07
Zinc (Zn)	01.00 ± 0.11
Copper (Cu)	01.05 ± 0.33
Calcium (Ca)	19.30 ± 0.02

Values are the mean ± standard deviation (triplicate for each cultivar).

Table (3): Amino acid content of *Moringa oleifera* leaves powder (MOLP).

Essential amino acid	g/100 g of MOLP	Nonessential amino acid	g/100 g of MOLP
Lucien	1.60	Glutamine	2.34
Isoleucine	0.82	Arginine	1.19
Vilene	1.10	Glycine	0.95
Lysine	1.14	Proline	0.90
Threonine	0.80	Tyrosine	0.75
Methionine	0.45	Aspartic	1.71
Histidine	0.43	Serine	0.67
Phenyl alanine	1.11	Alanine	1.16
		Cysteine	0.63

3.4 Qualitative and quantitative analysis of phenolic compounds and flavonoids using HPLC

As shown in Table (4), the major phenolic compounds identified were caffeic acid (0.536 mg/g), ellagic acid (0.189 mg/g), and chlorogenic acid (0.11 mg/g) while the major flavonoid compounds were quercetin (1.52 mg/g), kaempferols (1.412 mg/g), and rutin (0.82 mg/g), which expressed exceptionally strong antioxidant activity.

3.5 Antioxidant activity and total phenolic content of MOLP

Table (5) shows that the total phenolic content was 52 mg/g, and the antioxidant activity was 85%.

Table (4): Phenolic and flavonoids of MOLP using HPLC.

Parameter	Compounds	mg/g of MOLP
Phenolics	Gallic Acid	Not detected
	Chlorogenic acid	0.11
	Ellagic acid	0.189
	Caffeic acid	0.536
Flavonoids	Catechin	Not detected
	Rutin	0.82
	Hesperidin	Not detected
	Quercetin	1.52
	Kaempferols	1.412
	Apigenin	Not detected

Table (5): Antioxidant activity and total phenolic content of MOLP.

Parameters	Results
Antioxidant activity (%)	85 ± 0.22
Total phenolic content (mg GAE/g)	52 ±0.06

Values are the mean ± standard deviation (triplicate for each cultivar).

3.6 Chemical characteristics of hard biscuit enriched with a powder of Moringa oleifera leaves

Table (6) shows the nutrient contents of biscuits before and after adding a different ratio of Moringa leaves powder. Each 100 g control contained 4.74% moisture, 5.11% ash, 18.66% fat, 8.59% protein and 62.9% carbohydrate.

3.7 Changes in some mineral contents of hard biscuits enriched with powder of Moringa oleifera leaves

Table (7) shows the mineral compositions (mg/100 g) of biscuits that were nonenriched (control) with Moringa leaves powder and enriched with it (T1, T2, and T3). The results of (control, T1, T2, and T3) for sodium were 395.75, 395.79±1, 395.84±2, and 395.88±1.5 respectively. Potassium, 257.01±0.11, 257.1±1.3, 257.22±1, and 257.3±2.1 respectively. Magnesium, 41.60±0.12, 41.62±0.9, 41.63±2, and 41.65±3.9, respectively. Iron levels were 23.89±0.04, 23.9±1.7, 24.08±8, and 24.17±3.5, respectively. The zinc contents were 3.90±0.20, 3.905±0.2, 3.91±6, and 3.91±2.7, respectively. Calcium 135.89±0.1, 135.99±1, 136.08±5, and 136.18±1.8 respectively.

Table (6): Chemical characteristics of hard biscuit enriched with a powder of MOL.

Constituents (%)	Control	T1	T2	T3
Moisture	4.74^{c}±0.06	4.73^{c}±0.00	04.22^{a}±0.02	4.56^{b}± 0.66
Ash*	5.11^{a}±0.10	5.13^{a}±0.03	05.15^{a}±0.09	5.17^{a}±0.33
Crude fats*	18.66^{a}±0.04	18.67^{a}±0.01	18.67^{a}±0.04	18.68^{a}±0.2
Crude protein**	8.59^{b}±0.06	8.69^{ab}±0.08	08.80^{ab}±0.03	8.91^{a}± 0.08
Carbohydrates***	62.9^{d}	62.78^{c}	63.16^{b}	62.68^{a}

Values are means ± standard deviations, p≥0.05. *Calculated on a dry wet basis. **Crude protein = (N %) × 6.25. ***Total carbohydrates were calculated by differences. Control= Biscuit without MLP. T1= Biscuit with 0.5% Moringa leaves powder (0.5% MLP). T2= Biscuit with (1.0% MLP). T3= Biscuit with (1.5% MLP). Means in the same rows followed by the same letters are not significantly different.

Table (7): Changes in mineral contents of hard biscuits enriched with a powder of MOL.

Minerals (mg/100 g)	Control	T1	T2	T3
Sodium	395.75^{a}±0.02	395.79^{a}±1	395.84^{a}±2	395.88^{a}±1.5
Potassium	257.01^{a}±0.11	257.1^{a} ±1.3	257.22^{a}±1	257.3^{a}±2.1
Magnesium	41.60^{a} ± 0.12	41.62^{a} ±0.9	41.63^{a}±2	41.65^{a}±3.9
Iron	23.89^{a} ± 0.04	23.9^{a}± 1.7	24.08^{a}±8	24.17^{a}±3.5
Zinc	3.90^{a} ± 0.20	3.905^{a}±0.2	3.91^{a}±6	3.91^{a}±2.7
Calcium	135.89^{a} ± 0.1	135.99^{a}±1	136.08^{a}±5	136.18^{a}±1.8

Values are means ± standard deviations (p≥0.05). Control= Biscuit without Moringa leaves powder (MLP). T1= Biscuit with 0.5% MLP. T2= Biscuit with (1.0% MLP). T3= Biscuit with (1.5% MLP). Means in the same row followed by the same letter are not significantly different.

3.8 Sensory evaluation of hard biscuits enriched with a powder of Moringa oleifera leaves

Table (8) shows the comparison among the biscuits of their organoleptic quality factors, such as color, smell, texture, taste, and overall acceptability for the control and all treatments (T1, T2, T3, T4). The color results were 8.80±2.11, 7.80±2.02, 7.60±1.99, 6.00±2.09 and 5.40±2.14, respectively. Taste was 8.40±1.52, 8.40±2.45, 7.60±1.64, 6.60±1.33 and 7.00±3.10, respectively. Smell were 8.00±2.11, 7.80±2.00, 6.80±1.97, 6.60±2.55 and 5.60±1.85, respectively. The textures were 8.00±3.01, 7.20±2.33, 7.60±1.21, 7.80±2.35 and 7.80±1.23, respectively. The overall acceptability was 8.60±2.14, 8.60±1.26, 7.60±4.21, 7.40±3.14 and 5.60±2.17, respectively.

3.9 Relative body, liver, and kidney weights

As shown in Table (9), the liver weight and relative liver weight in T1 presented a slight decrease (3.33%) in comparison with those in control 2 (3.40%), while those in T2 were slightly increased (2.29%). However, T3 showed a higher decrease (2.95%). The relative kidney weight in Table 9 shows a decrease in T1 (0.48%) and T3 (0.54%) when compared with control 2 while T2 showed a slight increase (0.44%) when compared with control 2.

Table (8): Sensory evaluation of hard biscuits enriched with a powder of MOL.

Treatments	Color	Taste	Smell	Texture	Overall
Control	8.80^{a}±2.11	8.40^{a}±1.52	8.00^{a}±2.11	8.00^{a}±3.01	8.60^{a}±2.14
T1	7.80^{ab}±2.02	8.40^{a}±2.45	7.80^{ab}±2.00	7.20^{a}±2.33	8.60^{a}±1.26
T2	7.60^{b}±1.99	7.60^{b}±1.64	6.80^{abc}±1.97	7.60^{a}±1.21	7.60^{b}±4.21
T3	6.00^{c}±2.09	6.60^{c}±1.33	6.60^{bc}±2.55	7.80^{a}±2.35	7.40^{c}±3.14
T4	5.40^{c}±2.14	7.00^{bc}±3.10	5.60^{c}±1.85	7.80^{a}±1.23	5.60^{c}±2.17

Values are means ± standard deviations ($p \geq 0.05$). Control=Biscuit without Moringa leaves powder (MLP). T1= Biscuit with 0.5% MLP). T2= Biscuit with (1.0% MLP). T3= Biscuit with (1.5% MLP). T4= Biscuit with (2.0%MLP). Means in the same column followed by different letters are significantly different.

Table (9): Relative body and organ weight at the end of the experimental period.

Treatments	Body weight (g)	Relative liver weight		Relative kidney weight	
		L.W (g)	RLW%*	K.W. (g)	RKW%**
C-ve	223±1.95	7.14±2.01	3.20	1.28±0.26	0.57
C+ve	232±3.8	7.89±1.56	3.40↑	1.43±0.51	0.61↑
T1	215±5.2	7.17±2.13	3.33↓	1.03±0.94	0.48↓
T2	268±6.8	6.14±1.44	2.29↓	1.18±0.0	0.44↓
T3	225±6.4	6.65±1.20	2.95↓	1.22±0.66	0.54↓

Values are means ± standard deviations. C-ve = rats fed basal diets. C+ve = rats fed on biscuit without Moringa leaves powder (MLP). T1= rats fed 0.5% MLP biscuits. T2= rats fed biscuits with 1.0% MLP. T3= rats fed biscuits with 1.5% MLP. * Relative liver weight % = organ weight (g) / body weight (g) ×100. ** Relative kidney weight % = organ weight (g) / body weight (g) ×100.

3.10 Changes in blood glucose (mg/dl) level of experimental rats

Changes in blood glucose levels are shown in Table (10), treatments (1), (2) and (3) decreased glucose levels when compared with the control +ve. The highest decrease was observed in treatment (3), 14.96%, followed by treatment (2), 10.21%, and the lowest decrease was observed in treatment (1), 7.3%.

3.11 Changes in blood hemoglobin level (g/dl) of experimental rats

Changes in hemoglobin levels are shown in Table (11). During the experimental period, the control (+ve) group showed an approximately 3.62% increase in hemoglobin levels when compared with the control (-ve) group. Treatments (1), (2) and (3) increased the hemoglobin level when compared with the control (+ve). The highest increase was observed for treatment (3), 32.03%, followed by treatment (2), 27.18%, and the lowest was observed for treatment (1), 15.53%.

Table (10): Changes in blood glucose levels (mg/dl) of experimental rats.

Treatments	Experimental period					%
	0 day	7 days	14 days	21 days	28 days	
C-ve	119.55^{b}±2.1	118.9^{abc}±1.2	119.9^{b}±0.8	120^{c}±2.2	120.0^{c}±0.91	
C+ve	120.07^{a}±0.6	119.7^{c}±1.9	118.4^{b}±0.8	119.3^{c}±2.33	119.9^{c}±1.22	0.14↓
T1	120.07^{a}±0.6	117.5^{bc}±2.3	112.3^{a}±0.33	110.5^{b}±1.98	111.3^{b}±1.41	7.30↓
T2	120.07^{a}±0.6	114.9^{ab}±4.1	108.1^{a}±2.13	103.6^{a}±3.14	107.8^{b}±1.02	10.21↓
T3	120.07^{a}±0.6	113.2^{a}±0.9	110.7^{a}±2.51	100.1^{a}±4.22	102.1^{a}±0.98	14.96↓

Values are means ± standard deviations. Control -ve = rats fed basal diets. Control +ve = rats fed Biscuit without Moringa leaves powder (MLP). T1= rats fed biscuits with 0.5% MLP. T2= rats fed biscuits with 1.0% MLP. T3= rats fed biscuits with 1.5% MLP. Means in the same row or column by the different letters were significantly different ($p \geq 0.05$).

Table (11): Changes in hemoglobin level (g/dl) of experimental rats.

Treatments	Experimental period					%
	0 days	7 days	14 days	21 days	28 days	
C-ve	10.3±2.1	10.2±2.22	10.04±1.9	10.6±1.41	10.89±0.9	
C+ve	10.3±2.1	10.5±3.22	10.5±2.17	11.2±3.11	11.3±6.12	9.7↑
T1	10.3±2.1	10.7±2.41	11.3±2.13	11.8±1.87	11.9±3.15	15.53↑
T2	10.3±2.1	10.3±0.87	12.1±0.41	12.2±2.13	13.1±2.34	27.18↑
T3	10.3±2.1	10.4±2.54	12.4±0.33	12.8±2.02	13.6±5.13	32.03↑

The values are the means of triplicates ±SD. Control (-ve) = rats fed basal diets. Control (+ve) = rats fed biscuits without (MOLP). T1=rats fed biscuits with 0.5%. T2= rats fed biscuits with (1.0% MLP). T3= rats fed biscuits with 1.5% MLP. Means in the same row or column by the different letters were significantly different ($p \geq 0.05$).

4. Discussion

The obtained values for the chemical characteristics of *Moringa oleifera* leaves powder are summarized in Table (1). Similar results are very close to those reported by El–Massry *et al.* (2013) and Vaknin and Mishal (2017), who reported

that the protein contents in Moringa leaves ranged from 19 to 29.7 (g/100 g) while Monika *et al.* (2015) evaluated the chemical composition and nutritional values of dried *Moringa oleifera* leaves powder collected from two different regions in Mexico. All samples of *Moringa oleifera* exhibited moisture levels varying from 3.06 to 3.34%, lipids from 10.21 to 10.31%, fiber from 7.29 to 9.46%, ashes from 10.71 to 11.18%, crude protein from 10.74 to 11.48%, and carbohydrates from 54.61 to 57.61%. The presence of appreciable contents of protein, fiber and fat leads the potential of this biomaterial to have dietary purposes with associated nutritional aspects and optimal biomaterial to be used to make the functional food product (Singh *et al.*, 2013). The obtained results of some mineral contents of *Moringa oleifera* leaves powder (MOLP) agreed with those registered by Charles *et al.* (2011), who stated that the mineral contents of Moringa leaves powder for Ca, Mg, K, Fe, Zn and P were 2098.1, 406.0, 1922.0, 28.3, 5.4 and 351.1 mg/100 g (dry weight basis) in dried *Moringa oleifera* leaves, respectively. Methionine and cysteine are powerful antioxidants that help in the detoxification of harmful compounds and protect the body from radiation (Brisibe *et al.*, 2009). Proline is a major component of collagen protein and plays a key role in collagen stability. Isoleucine builds proteins and enzymes; Lucien works with isoleucine to enhance the energy level. Lysine helps in the accurate absorption of calcium and aids in antibody production. Methionine predominantly supports sulfur provision for the body, therefore decreasing the irritation in the bladder. Threonine is an integral portion of fibrous proteins. Tryptophan supports the immune system and reduces cholesterol levels while valine helps in muscle coordination. Arginine and histidine are essential amino acids for infants' growth and development (Adeyeye, 2004; Aremu *et al.*, 2006). These values were founded by Meyers *et al*. (2006), Brisibe *et al*. (2009), and Steve and Babatunde, (2013). Flavonoids promote antioxidant activity, cellular health and normal tissue growth and renewal throughout the body. They also work with vitamin C to reduce oxidative stress for the water-based portion of the cell and may slow down some of the effects of aging (Sunil, 2014). Phenolics and flavonoids are active antioxidant components in the leaves of *Moringa oleifera*. The provided separation of the compounds and the system minimized running time and costs in routine analysis (Amaglo *et al.*, 2010; Verma *et al.*, 2009). Various reports also indicate that *Moringa oleifera* is a rich source of phenolic compounds (Alhakmani *et al.*, 2013) and thus is widely used in traditional systems of medicine (Wang *et al.*, 2004). Phenolics and flavonoids have at least one hydroxyl ion substituted with an aromatic ring and can form chelate complexes with the metal ions, thereby being easily oxidized and are the means for donating electrons

to scavenge free radicals (Siddhuraju *et al.*, 2003; Sreedam *et al.*, 2010). Higher phenolic content in *Moringa oleifera* is also correlated with increased antioxidant activity (Kostyuk *et al.*, 2001). Natural antioxidants have attracted considerable interest in recent years for their role in preventing the autooxidation of fats, oils or food products holding fat. Antioxidants are either organic or inorganic compounds. They are categorized as primary and secondary antioxidants. This classification is based on the mechanism of action of antioxidants. For example, primary antioxidants neutralize free radicals either by donating an H-atom (hydrogen atom transfer, abbreviated HAT) or by a single electron transfer (SET) mechanism. These antioxidants are very efficient and normally required in extremely limited amounts to neutralize many free radicals (Siddhuraju *et al.*, 2003; Sreedam *et al.*, 2010). The results in Table 5 are close to those of Mushtaq *et al.* (2018), who recorded that *Moringa oleifera* leaves powder (MOLP) had values for total phenolic content (TPC), and antioxidant activity of 88.63 mg GAE/g, and 89.27%, respectively. Chemical characteristics of hard biscuit enriched with a powder of *Moringa oleifera* leaves according to the results shown in Table 6. The nutritive values were increased by increasing Moringa leaves powder. There were no significant ($p \leq 0.05$) differences between treatments for ash and fat when compared with the control while there were significant ($p \geq 0.05$) differences between treatments for moisture, protein and carbohydrates when compared with the control. Thus, Moringa leaves can be successfully incorporated into biscuit wheat flour up to a level of 1.5% to enhance nutritional quality with acceptable sensory attributes. These values ranged from 5.13 to 5.17 for ash, 18.67 to 18.67 for fat, 8.69 to 8.91 for protein and 62.68 to 63.16 for carbohydrate. These data are very close to those of Alam *et al.* (2014) who concluded that Moringa leaves powder can be successfully incorporated into biscuits wheat flour up to a level of 1% to yield biscuits of enhanced nutritional quality with acceptable sensory attributes. Hence, the development and utilization of such functional foods will not only improve the nutritional status of the population, but also help those suffering from degenerative diseases. Table (7) shows the changes in some mineral contents of hard biscuits enriched with a powder of *Moringa oleifera* leaves. Based on the statistical analysis, there were no significant differences between all the treatments compared to the control. Sodium (Na) is regarded as the most abundant mineral in biscuits, and its content in the control was 395.75 mg/100 g. This value increased to 395.79 in treatment 1. In treatment 2 (biscuit with 1% MLP) 395.84 mg/100 g was recorded. Treatment 3 (biscuit with 1.5% MLP) recorded 395.88 mg/100 g. However, it was 395.75 for the biscuit control. The same training was observed

with potassium values of 257.11, 257.22 and 257.33 compared to 257.01 for the control. The recommendations for Zn are 15 mg for men and 12 mg for women (Kirssel and Prentice, 1979). Furthermore, when comparing all treatments to the control, the values of Zink increased, and the highest increases were observed in T3 (0.38%), followed by T2 (0.26%), and T1 (0.13%). This indicated that the zinc content of the biscuits increased with the increasing quantity of MOLP. These findings are in accordance with those obtained by Ajibola *et al.* (2015), who investigated the effect of *Moringa oleifera* leaves powder on the quality characteristics and antioxidant properties of biscuits. The biscuit could therefore not be taken as a useful source of these trace minerals. This implies that other dietary sources must be explored to meet the daily requirements. The highest value of iron was observed in treatment 3 (1.18%), followed by treatments 2 (0.78%) and 1 (0.39%) when compared with the biscuit control. Ca content (mg/100 g), when comparing all treatments to the biscuit control, the change in the level of calcium was increased by increasing the addition of MOLP, and the highest percentage increase was observed for T3 (0.21%), followed by T2 (0.14%) and T1 (0.071%). This finding agrees with those (Ajibola *et al.*, 2015; Anwar *et al.*, 2007), who reported that the calcium content of biscuits increased from 29.17% when Moringa leaves powder was added to the biscuits. The significant increase in the calcium content could be due to the presence of a higher calcium content in Moringa leaves powder. On the other hand, the increase in the level of Mg for all treatments compared with the biscuit control was T3 (0.13% ↑), followed by T2 (0.11% ↑) and T1 (0.042% ↑). Shivani *et al.* (2006) reported that MOLP supplemented biscuits possess a slightly higher nutritive profile than the control with a significant increase in protein, fiber, iron, and calcium. Similar improvements in nutritional characteristics, such as dietary fiber, protein, iron, and calcium have been reported for green leafy vegetable supplemented cookies and biscuits. Comparison of organoleptic or sensory qualities of hard biscuits depend on their first appearance, color, flavor, smell, texture, and overall taste of the sample. Table (8) shows the comparison among the biscuits of their organoleptic quality factors, such as color, smell, texture, taste, and overall acceptability, which are very important for the food industry because consumer acceptance depends largely on these attributes (Mamta *et al.*, 2017). The results in Table (8) show that the significant ($p \geq 0.05$) differences in the color rating of the wheat flour biscuit control from 8.80 to T1, T2, T3 and T4 were 7.80, 7.60, 6.00 and 5.40, respectively. Fortification with Moringa leaves powder similarly led to a decrease in the color of gluten-free biscuits (Hayat *et al.*, 2018) and whole wheat biscuits (Obichili and Ifediba, 2019). The inferior color of the fortified sample might be due

to the dulling effect of Moringa leaves powder (Obichili and Ifediba, 2019), which is attributable to the deep green color, which is related to the high chlorophyll content of Moringa leaves (Karim *et al.*, 2015). There was no significant ($p \leq 0.05$) difference in the taste of the control and T1 (8.40 and 8.40). There was a significant difference between the other treatments T2, T3 and T4 at 7.60, 6.60 and 7.00, respectively. These findings agree with those of Igbabul *et al*. (2018), who reported a wider increase in the taste of cookies from 4.40 of the ordinary sample to 7.60 of the sample fortified with moringa leaves powder. The improved taste of the Moringa substituted sample may be due to the versed phenolic and bioactive compounds in Moringa leaves which combine with other substances in the recipe to form aromatic complexes with a pleasant taste. There was no significant ($p \leq 0.05$) difference in the texture rating of all treatments compared with the control. The finding of texture is in line with those obtained by Igbabul *et al.* (2018), who reported similar improvement in the texture of cookies made from sweet deter, moringa leaves powder and wheat composite flour. The grainy matrix of imbedded Moringa leaves powder might have resulted in a crispy bake, and crispiness can be complementary to the desirable textural property of biscuit. The biscuit control and T1 biscuits were not significantly ($p \leq 0.05$), with general acceptability scores of 8.60 and 8.60 while the highest scores of the other biscuits were 7.60, 7.40 and 5.60 for T2, T3 and T4, respectively. The results obtained were like those obtained by Igbabul *et al.* (2018), who reported similarly higher general acceptability of cookies fortified with Moringa leaves powder from 4.66 to 8.13. The higher general acceptability of the Moringa wheat flour biscuit is expected since it excelled in three out of other attributes considered. From Table 7, it becomes clear that the best treatment is T1 (0.5%) followed by T2 (1%), T3 (1.5%) and T4 (2%). The relative body and organ weights at the end of the experimental period are shown in Table 9. Liver weight and relative liver weight in T1 presented a slight decrease (3.33%) in comparison with control 2 (3.40%), while T2 was slightly increased (2.29%). However, T3 showed a higher decrease (2.95%). The relative kidney weight in Table 9 shows a decrease in T1 (0.48%) and T3 (0.54%) when compared with control 2, while T2 showed a slight increase (0.44%) when compared with control 2. In accordance with previous studies (David *et al.*, 2002; Nkukwana *et al.*, 2014; Safa *et al.*, 2014) the results revealed that feeding with *Moringa oleifera* leaves meal could improve dressing weight. In contrast, the current findings are consistent with those of Zanu *et al.* (2012), who reported that inclusion of *Moringa oleifera* leaves meal at various levels (5, 10, and 15%) in the diet of broilers has no effect on dressing weight. Moreover, the dressing percentage was not affected by the

inclusion of *Moringa oleifera* leaves meal. Changes in blood glucose levels are shown in Table (10) during the experimental period. Treatments (1), (2) and (3) decreased glucose levels when compared with the control +ve. The highest decrease was observed in treatment (3), 14.96% followed by treatment (2), 10.21%, and the lowest decrease was observed in treatment (1), 7.3%. In the present study, the reduction in blood glucose levels in treatments (1, 2 and 3) may be due to the substances present in the *Moringa oleifera* leaves powder that stimulate insulin secretion or protect the intact functional β-cells from further deterioration. The reduction in blood glucose levels by biscuits supplemented with *Moringa oleifera* leaves powder may be due to its numerous bioactive compounds such as iron, flavonoids, and phenols. The results indicated that *Moringa oleifera* had an ameliorating effect on glucose intolerance. Some of these bioactive compounds may exert their hypoglycemic effects by, reducing insulin resistance, increasing release, and decreasing glucagon secretion, slowing the digestion and absorption of carbohydrates, or decreasing hepatic glucose production (Katzung *et al.,* 2009). In one study of Wistar rats, researchers found that *Moringa oleifera* relieved swollen mitochondria and increased glucose storage as glycogen granules in iron-deficient liver cells (Ndong *et al.,* 2007). These results agree with Jaiswal *et al.* (2009), who scientifically validated the widely claimed use of *Moringa oleifera* as an ethno-medicine to treat diabetes mellitus. The dose of 200 mg/kg *Moringa oleifera* leaves aqueous extract was found to decrease the blood glucose level (BGL) of normal animals by 26.7% and 29.9% during fasting blood glucose (FBG). Furthermore, Kushwaha *et al.* (2012) studied 30 postmenopausal women who were supplemented daily with 7 g of *Moringa oleifera* leaves powder for a period of 3 months. The control group also consisted of 30 postmenopausal women. The data revealed a significant decrease in fasting blood glucose levels (13.5%). Changes in hemoglobin levels are shown in Table (11). During the experimental period, the control (+ve) group showed an approximately 3.62% increase in hemoglobin levels compared with the control (-ve) group. Treatments (1), (2) and (3) increased the hemoglobin level when compared with the control (+ve). The highest increase was observed for treatment (3), 32.03% followed by treatment (2), 27.18%, and the lowest was observed for treatment (1), 15.53%. The normal range of hemoglobin is 10-14 g/dl (David *et al.,* 2002). These changes might be due to iron, and the function of iron is paramount in the formation of hemoglobin, which is synthesized in immature red blood cells in the bone marrow. Hemoglobin works in two ways: (1) iron-containing heme combines with oxygen in the lungs and then releases oxygen in the tissues, and (2) it picks up carbon dioxide in the

tissues and then releases it in the lungs (Mary *et al.*, 2007). These results were similar to those obtained by Kushwaha *et al.* (2012).

5. Conclusion

From this study, we can conclude that wheat flour enriched with increasing levels of MOLP from 0 to 1.5% in biscuit manufacturing affects the increasing nutritional value, and blood hemoglobin level and decreases blood glucose levels. The protein, crude fiber, and mineral contents of biscuits increased with increasing amounts of MOLP. The results have shown the possibility of utilizing dried *Moringa oleifera* leaves powder to improve the nutritional values of biscuits with acceptable sensory and biological effects.

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