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Nutritional Evaluation of Chia and Moringa Seeds Flour and Quality Characteristics of Fortified Cookies

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ABSTRACT

This study was carried out to investigate the utilization of defatted Chia seeds flour (DCSF) and defatted Moringa seeds flour (DMSF), as replacement of wheat flour at different levels (5, 10 and 15%) to prepare cookies rich protein and minerals. The obtained results revealed that, DCSF and DMSF, are very rich in protein, ash and fiber compared with CSF, MSF and wheat flour 72%**.**Wheat flour (72%) contains lower values in all determined elements excepted for sodium compared to chia and moringa seeds in addition, moringa seeds had higher values of potassium, phosphorus, copper, than those of chia seeds. The total amino acids of chia and moringa seeds were (56.97 %) and (79.13 %) respectively. Furthermore, moringa seeds were rich in essential and non- essential amino acids than chia seeds. Total saturated fatty acid of moringa seeds oil were higher (23.43%) than that of chia seeds oil (9.53%).High amounts of extracted polyphenolic compounds by ethanol from chia and moringa seeds were (1.63 and 1.34 mg gallic acid/g of DW) respectively. Moisture, crude protein, ash and crude fiber contents increased in cookies products in contrast, carbohydrates value decreased gradually with increasing the substitution levels of DCSF and DMSF. From the results of sensory evaluation, it should be noted that, fortification of DCSF and DMSF until 15% is acceptable for the sensory evaluation of cookies. Based on the obtained results, the new product of cookies contained DCSF and DCMF can be covered protein and minerals of nutritional needs of schoolchildren in developing countries and could be recommended as a food aid in institutional feeding programs for pupils in different school stages.

Keywords: Moringa seeds, Chia seeds, Minerals, Fatty acids; Amino acid profile; cookies

1. Introduction

Chia (*Salvia hispanica* L.) is an annual herbaceous plant belonging to the Lamiaceae (Mint) family (Ixtainaa *et al.,* 2008).Chia seeds are a well-known food, their global production is renowned nowadays for their potential health properties. Recently, Lately, They were studied largely due to their rising success and recognition as a healthful food alternative (Capitani *et al.,* 2017).The plant's seeds are a healthy source of polyunsaturated fatty acids (PUFA) at higher concentrations (equivalent to 83% of extracted oil), with high amounts of omega 3 fatty acids, dietary fiber (34.4 g/100g), total protein (16.54 g/100g), and natural antioxidants (Giaretta *et al.,* 2018).Chia seed has a high fat content of 25-40%, out of which omega-3 and omega-6 fatty acids constitute 68% and 20% of the total mass, respectively (Goyat *et al.,* 2018). Chia protein contains high amounts of glutamic acid (123 g kg−1 raw protein), arginine (80.6 g kg^{-1} raw protein), and aspartic acid (61.3 g kg−1 raw protein). Its amino acid profile has no limiting factors in the adult diet, but threonine, lysine, and leucine were the limiting amino acids in a preschool child's diet. (Kulczynski *et al.,* 2019). Moreover, chia seeds contain a high amount of phenolic compounds and natural antioxidants such as chlorogenic and caffeic acids, quercetin, and kaempferol (Ixtaina *et al .,* 2008)which protects consumers from unhealthy effects, such as certain

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cardiovascular diseases , and certain types of cancer and providing vitamins and minerals(Craig and Sons,2004).After oil extraction, residual chia meals included high amounts of fiber (19–23%), protein (33.9–39.9%), and antioxidant compounds which are comparable to other oil seeds used in the food industry (Marineli *et al .,* 2014).The cookies flavored by chia seed and date syrup have been enhanced by incorporating additional nutrients. Cookies fortified by Chia seeds are a healthy source of protein, fat, omega 3, and antioxidants (Chelladurai *et al.,* 2019).

Moringa oleifera (also known as the "miracle plant") is one of West African regions's newest crops.The plant is entirely edible from leaves to roots. Moringa seeds contain significant sources of minerals (calcium, phosphorus and iron) and vitamins (A, B and C). It is rich in protein and fat but low in carbohydrates (Olushola, 2006). However, the protein, fat and mineral (especially magnesium) content of moringa seed has been reported to be significantly higher than that of moringa leave (Gopalakrishnanb *et al.,* 2016).Moringa seeds has also been found to contain 36.18, 43.58, 3.73 and 16.51% protein, fat, ash, carbohydrate, respectively. In addition, it contain a profile of important minerals, and are a good source for protein, vitamins, β – carotene, amino acids and various phenolics as well as antioxidants, anti-inflammatory nutrients and omega 3 and 6 fatty acid (Kasolo*et al*., 2010).The major saturated fatty acids present in the Moringa seeds are palmitic, stearic, and benic acids. Oleic acid is the main unsaturated fatty acid (67.9- 70.0ability during cooking and frying (Abdulkarim *et al.*, 2005).

Historically, Moringa (*Moring aoleifera* L.) enriched nutritional supplement with major medical and therapeutic values.Literature reveals multipurpose applications of different parts of moringa such as leaves, flowers, pods, seeds and roots. Fruits of moringa are a major source of vitamin C, i.e., 120 mg / 100 g fresh sliced pods. Furthermore, It is used in functional and traditional foods, e.g., in making soups, weaning foods, amala, biscuits, bread, cake and yoghurt as well as cheese and has the ability to preserve foods. In various medicinal applications, it works as antioxidant, anticancer, anti-inflammatory, antiulcer, anti hyperglycemic, anti diabetic and antimicrobial agent and in agricultural uses as animal feed, forage crop, (Masih *et al.,* 2019).Cookies are the most common category among the numerous bakery products. Cookies are a dried food that having low moisture content.Cookies are nutritional, tasty, palatable, light weight and convenience. It has low moisture content than cakes and bread; cookies are safer from microbiological spoilage and have a long shelf-life. (Divya, 2012).

Cookies are widely accepted and consumed in many countries and therefore offer a valuable supplementation vehicle for nutritional improvement. Cookies have been suggested as a good way to use composite flours as they are ready-to-eat, provide a good source of energy, and are consumed widely throughout the world (Arshad *et al.,* 2007).The term cookies or biscuits refers to a baked product generally containing the three main ingredients flour, sugar and fat, as they are called in many parts of the world. These are mixed together with other minor ingredients to form the dough (Mamat *et al.,* 2010).

This study was carried out to study nutritional evaluation of Chia and Moringa seeds flour and to utilize of (DCSF) and (DMSF), in cookies processing as new food industries product in the Egyptian market rich protein and minerals.

2. Materials and Methods

2.1. Materials

Chia seed (*Salvia hispanica* L.) was purchased from a local market at El-Mansura, Al-Dakahlya Governorate, Egypt at season 2018-2019.

a- Moringa (*Moringa oleifera*) seeds was obtained from crop Research Institute, Agriculture Research Center, Sakha at the Governorate of Kafr El-Sheikh, Egypt, at season 2018-2019.

b- The seeds were cleaned manually and foreign matters, such as stones, dirt and broken seeds were removed. Afterwards, the seeds were packed in polyethylene bags and stored at -18±2°C until used.

Other ingredients:

Wheat flours (72%) were purchased from Delta Middle and West Milling Company, Tanta Egypt, sugar, shortening, baking powder, and whole egg were purchased from the local market of Tanta city at EL-Gharbia Governorate, Egypt.

Chemicals:

All of chemicals used in this study were obtained from EL-Gomhouria pharmaceutical company,of Tanta city at EL-Gharbia Governorate, Egypt. All other chemicals were analytical grads.

Chemical analysis:

Gross chemical composition and caloric value of samples:

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 according to the methods of A.O.A.C. (2005).

The energy value was calculated according to James (1995).

Energy value (kcal.100 g^{-1}) = (g of protein × 4) + (g of lipids × 9) + (g of carbohydrates × 4).

Determination of minerals: samples were prepared for mineral determination according to the method of the A.O.A.C. (2005).

- **a-** Phosphorus (mg/100 g): Total phosphorus was determined by ascorbic acid technique using the colorimetric method that described by Murphy and Riley (1962).
- **b-** Potassium and sodium (mg/100 g): Potassium and sodium contents of samples were estimated using flame photometer as given by Pearson (1976).
- **c-** Iron, Magnesium, Manganese, Copper, Zinc and Calcium contents of samples were conducted using the atomic absorption spectrophotometer Perken Elmer Model 2180 and following the methods of Pearson (1976).

Determination of amino acids:

Fifty milligrams of the samples were mixed with 10 ml of 6 N hydrochloric acid containing 50 µl marcapto ethanol in heat-resistant tube. The tubes were sealed, heated in oven at 110ºC for 24 h., then cooled to room temperature and filtered through What man No.1 filter paper. Both tube and the precipitate were washed with distilled water. The washed water was added to the previous filtrate then completed to 25 ml in a volumetric flask. Five ml of the filtrate were transformed to 25 ml beaker and placed in a vacuum desiccator until dryness in 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:

Tryptophan content of samples was determined colourimetrically after subjecting to alkaline hydrolysis as outlined by Blauth *et al*. (1963).

Computed protein efficiency ratio (C-PER): was calculated from the amino acid composition using the equation developed by Alsmeyer, (1974), C-PER = -o.468+0.454 (Leu) +0.105 (Tyr).

Computed biological value of protein (C-BV): Biological value of chia and moringa seeds protein were calculated according to Farag *et al.* (1996), using the following regression equation: BV(%)=49.9+10.53(C-PER).

Extraction of total phenolic compounds (TPC):

The prepared ground materials (10 g) of each sample were soaked in 100 ml of each solvent (Ethanol, Ethyl acetate, Acetone and Diethyl ether) overnight in a shaker at room temperature according to Mohdaly *et al.* (2010) .The extracts were filtrated through Whatman No.1 filter paper. The residues were re-extracted three time under the same conditions. The combined filtrates were evaporated under vacuum in a rotary evaporator below 40ºC. The extracts obtained after evaporation of organic solvents were stored -18±2ºC until further analysis.

Determination of total phenolic compounds:

Total phenolic compounds of the extracts were determined spectrophotometrically using Folin-ciocalteau reagent according to the method described by (Singleton *et al*., 1999) and used to estimate the phenolics-acid content using a standard curve prepared using gallic acid.

Determination of DPPH radical scavenging capacity:

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Lee *et al.*, 2003) was performed with some modifications. The stock reagent solution ($1x10^{-3}$ M) was prepared by dissolving 22 mg of DPPH in 50 ml of methanol and stored at -20°C until use. The working solution ($6x10^{-5}$ M)was freshly prepared by mixing 6 ml of the stock solution with 100 ml of methanol. Antioxidants extracted from different samples were vortexed for 30 sec with 3.9 ml of DPPH solution and left for 30 min at room temperature , after which the absorbance at 515 nm was recorded . A absorbance of control without extract was also recorder. Scavenging activity was calculated as follows:

DPPH radical-scavenging activity (%) =(A control – A sample) / (A control) ×100

Where A is the absorbance at 515 nm .

Preparation of defatted chia and moringa seeds:

Chia and moringa seeds mill weight was soaked for 48 hours at room temperature in an n-hexane solvent(40-60ºC), then filtered. This process was repeated three times using fresh solvent each time to extract most of the oils from the samples. Then the obtained solution was filtered and the solvent was removed by rotary evaporator according to Kahlon *et al.* (1992).The crude oil, obtained have a dark greenish color. While,the defatted chia and moringa were milled using a laboratory scale hammer mill. The resulting flour were sieved through a 60-mesh screen and was kept in polyethylene bags and stored at -18±2ºC until used.

Determination of Physical and Chemical Properties of Extracted Oils:

Refractive index, Specific gravity, acid value, peroxide value, iodine value, saponification value and unsaponifiable matters of chia and moringa seeds oils were determined according the methods of A.O.A.C. (2005).

Fatty acids composition of oils:

The methyl esters were prepared using benzene: methanol: concentrated sulfuric acid (10:86:4) and the methylation process was carried out for one hour at 80-90 ºC according to Stahl, (1967).Identification of the fatty acid methyl esters were performed by G.L.C A pyeunic'am Gas-Liquid Chromatography (model 4550) equipped with a flame ionization detector and coiled glass Colum (1.6m × 4mm) packed with 10% PEGA (polyethylene glycol adipate) supported on chromosorb W-AW 100-200 mesh. Samples (1-1.5 ul) in to the column using ahamilton microsyringe. Gas chromatographic conditions used for isothermal analysis were column 190 ºC Flow rates: Hydrogen 33ml/min. nitrogen 30 ml/min. and air 330ml/min. Peak areas were measured using specttophysic integrator. A.O.A.C. (2005).

Preparation of the cookies:

Method of Alobo (2001) was used to prepare the cookies samples. Blends containing 5, 10 and 15% of (DCSF) or (DMSF), were used as replacement of wheat flour (72% extraction). Dry ingredients (sugar, shortening, baking powder and sodium chloride) and eggs were blended with the flour blends and dough thoroughly kneaded Fig 1. The dough was then placed on a cutting board, and then rolled out to achieve a suitable thickness and texture. Cookies cutter was used to cut the sheet of rolled dough into desired shapes and sizes, then baked in oven at 175 ± 5 ºC30 min. The cooked cookies was allowed to cool at room temperature, packed and store into two lots. Initially, one lot was used for sensory evaluation and the other lot for chemical analysis.

Sensory evaluation of cookies:

A semi-trained panel of twenty members using ten-point hedonic-scale ratings for color, taste, odor, texture and overall acceptability in order to provide organoleptic characteristics for different repaired cookies, Watts *et al*. (1989). Liked extremely 9, Like very much 8, Liked moderately 7, Liked slightly 6, Neither liked nor disliked 5, Disliked slightly 4, Disliked moderately 3, Disliked very much 2 and Disliked extremely 1.

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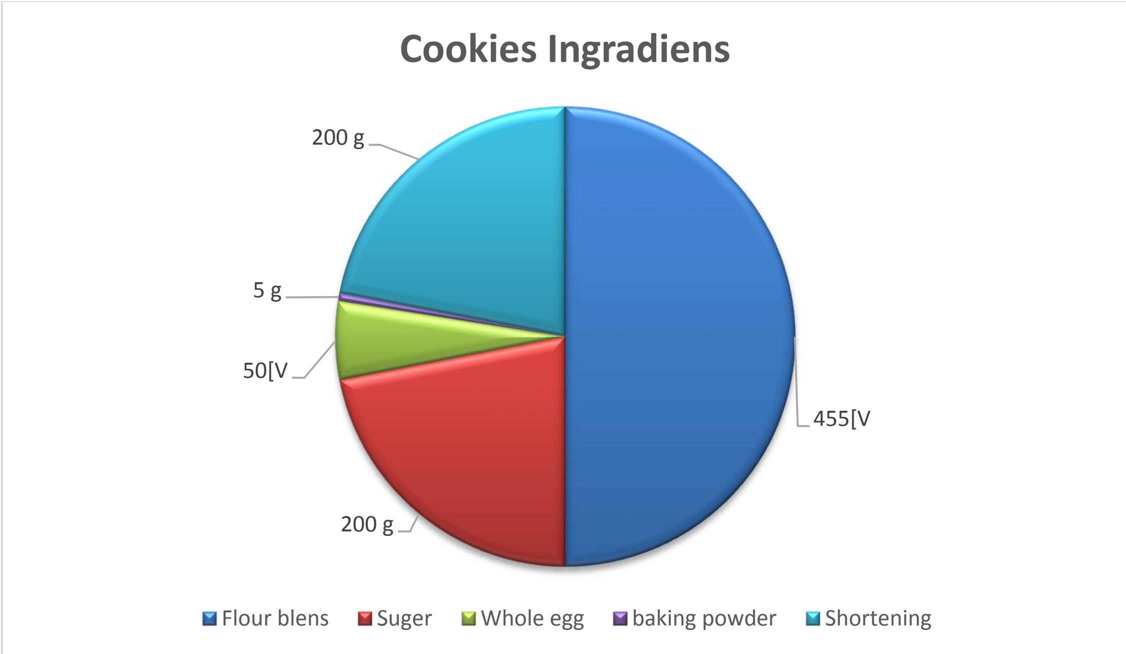

Fig. 1: Illustrates the composition of cookies

Statistical analysis:

Data were analyzed statistically using the analysis of variance and the means were further tested using DMART test outlined by Steel and Torrie (1980).

3. Results and Discussion

3.1. Proximate composition:

The gross chemical composition of chia (CSF), moringa seeds flour (MSF), defatted chia seeds flour (DCSF), defatted moringa seeds flour (DMSF), andwheat flour72% are recorded in Table (1). The obtained results show that, DMSF contain a significant high content of crude protein 40.47% compared with that of MSF 30.67, DCSF 30.20 % , CSF 20.78 and wheat flour (72%) 10.16 %.CSF contains highest content of ether extract (36.07%) followed by MSF(34.99), DCSF 1.19, DCSF 1.02, then wheat flour 72% (0.95%). Furthermore, DCSF and DMSF, are very rich in protein, ash and fiber compared with CSF, MSF and wheat flour 72%.

Table 1: Proximate chemical composition (%), of raw samples (on dry weight basis).

Samples / Component	Chia seeds	Moringa seeds	DCSF	DMSF	WF (72%)
Moisture	7.60±0.05^{b}	7.07±0.11^{c}	7.2±0.22^{c}	6.7±0.25^{d}	11.22±0.13^{a}
Dry matter	92.40±0.05^{c}	92.93±0.10^{b}	92.8±0.22^{b}	93.3±0.25^{a}	88.78±0.13^{d}
Protein	20.78±0.20^{c}	30.67±0.50^{b}	30.20±0.25^{b}	40.47±0.29^{a}	10.16±0.14^{d}
ether extract	36.07±0.7^{a}	34.99±0.4^{b}	1.19±0.24^{c}	1.02±0.21^{c}	0.95±0.16^{c}
Ash	4.81±0.04^{c}	4.29±0.10^{d}	8.2±0.26^{a}	7.84±0.30^{b}	0.65±0.15^{e}
Crud fiber	26.70±0.9^{b}	9.70±0.07^{d}	40.54±0.25^{a}	19.87±0.22^{c}	0.78±0.15^{e}
Total carbohydrats	38.34±0.5^{d}	30.05±0.4^{e}	60.41±0.0.75^{b}	50.67±0.80^{c}	88.24±0.45^{a}
Available carbohydrats	11.64±0.8^{d}	20.35±0.7^{c}	19.87±1.0^{c}	30.8±1.02^{b}	87.46±0.60^{a}
Total energy (Kcal/100)	561.11±0.52^{a}	557.79±081^{b}	373.15±0.16^{d}	373.74±0.15^{d}	402.15±0.20^{c}

Each value was an average of three determinations.
Values followed by the same letter in row are not significantly different at $p \leq 0.05$.
WF 72%: wheat flour 72%, DCSF: defatted chia seeds flour,
DMSF: defatted moringa seeds flour.

This means, the DCSF, DMSF when is added to bakery products would be improved their protein and minerals content. Data in this respect were in agreement with the findings of numerous of investigators (Ogunsina *et al.,* 2010; Amato *et al.,* 2015; Fernandes and Salas-Mellado, 2017; Hammad, 2018; Mohammed, 2019 and Salama , 2019)

3.2. Minerals content:

Minerals or elements play an important role in human nutrition, some are essential for much component as hem for blood(National Academy of Sciences, 2001).The data presented in Table (2)observed that, wheat flour (72%)) contains lower values in all determined elements excepted for Sodium compared to chia and moringa seeds .

Apparent also from the same Tables that, the chia seeds had high levels of calcium (621 mg/100g), magnesium (290.25 mg/100g) , sodium (22.4 mg/100g), iron (7.7 mg/100g) and Zinc (5.35 mg/100g), than those of moringa seeds. While moringa seeds had higher values of potassium, phosphorus, copper, than those of chia seeds. In addition, the iron content of chia seeds is higher five times than that of wheat flour. The iron is important for the schoolchildren, which mostly needs more iron to avoid the anemia especially in developing countries. The results indicated that, the chia and moringa seeds when added to bakery products would improve their minerals content. These results are supported by European Food Safety Authority (2009), El-Massry *et al*. (2013), Ullah *et al.* (2015), Salha (2018), Mohammed (2019) and Salama (2019).

Table 2: Mineral contents (mg/100g) of chia, moringa seeds and wheat flour 72%.

Minerals	Chia seeds	Moringa seeds	Wheat flour 72%
Potassium(K)	470.00±3.6^{b}	840.90±4.9^{a}	140.0±2.1^{c}
Sodium(Na)	22.40±0.25^{b}	7.20±0.08^{c}	25.89±0.27^{a}
Calcium(Ca)	621.00±4.2^{a}	254.11±2.7^{b}	37.0±0.34^{c}
Phosphorus(P)	645.00±4.5^{b}	721.40±4.7^{a}	316.0±3.2^{c}
Copper(Cu)	0.80±0.04^{b}	1.10±0.06^{a}	0.004±0.001^{c}
Zinc(Zn)	5.35±0.09^{a}	4.57±0.07^{b}	2.12±0.05^{c}
Manganese(Mn)	1.40±0.02^{a}	1.30±0.009^{b}	0.03±0.001^{c}
Magnesium(Mg)	290.25±2.8^{a}	281.00±2.7^{b}	132.0±2.03^{c}
Iron(Fe)	7.70±0.09^{a}	6.90±0.06^{b}	1.60±0.008^{c}

Each value is an average of three determinations ± standard deviation.
Values followed by the same letter in rows are not significantly different at $P<0.05$.

3.3. Amino acids composition:

The nutritive value of food, especially protein mostly would depend not only on its amino acids profile in general but also on the quantities of the essential amino acids content in particular (Afify *et al.,* 2012). Amino acids composition of chia and moringa seeds are given in Table (3).The results show that, the moringa seeds were rich in essential and non- essential amino acids than chia seeds. The total amino acids of chia and moringa seeds were (56.97 %) and (79.13 %) respectively.

As for essential amino acids, it could be observed that, Leucine was the dominant acid in chia seeds that recorded (3.97 %), followed by Lysine (3.10 %).Furthermore, Leucine was the dominant acid in moringa seeds that recorded (4.63%) followed by phenylalanine (4.31 %).

Meanwhile, tryptophan was the lowest amino acid in chia and moringa seeds that recorded (0.42 and 0.10 % respectively).These result agreement with Hammad (2018); Mohammed (2019) and Salama (2019) they reported that, Leucine was the dominant acid in chia and moringa seeds. Concerning non-essential amino acids reflected that, glutamic acid was the highest amino acid among all of the other acids in chia and moringa seeds that recorded (11.56 and 16.46 % respectively). Arginine was the second level in both chia and moringa seeds that recorded (6.76 and 11.78 % respectively), followed by aspartic acid and alanine in both chia and moringa seeds. These results agreement with Hammad (2018); Mohammed (2019) and Salama (2019) they found that, glutamic acid was the highest concentration in chia and moringa seeds. Furthermore, glutamic acid considered an important amino acid in the diet. It is able to modulate imm-regulatory response and enhances athletic performance. Also, arginine plays role in preventing heart diseases (Timilsena *et al*., 2017).

The quality of proteins as source of amino acids can usually be adequately assessed by comparison with the recommended pattern of essential amino acids. Moringa and chia seed flours had lower total essential amino acids than the FAO/WHO (1973) reference pattern. Meanwhile, Metheonine + Cycteine were higher amino acid in chia and moringa seeds comparison with the FAO/WHO (1973) recommended pattern. Furthermore, Moringa seeds reported higher Phenylalanine+ Tyrosine content, than the FAO/WHO (1973) reference pattern. These results showed that Moringa and chia seeds could be used to complement cereal proteins (MuneMune, *et al*., 2011). Apparent also from the same Table that, the lower (C-PER) and (B.V.) values were recorded in chia seeds compared with moringa seeds. Our findings are coincided with those of (Hammad 2018).

Table 3: Amino acids profile (%) of chia and moringaseeds.

Amino acids	Chia seeds	Moringa seeds	FAO/WHO (1973)
	A-Essential amino acid		
Valine	2.23	3.49	5.0
Leucine	3.97	4.63	7.0
Isoleucine	2.01	2.28	4.0
Metheonine	1.86	1.92	
Cycteine	1.89	2.89	
Metheonine+Cycteine	3.75	4.81	3.5
Therionine	2.13	2.83	4.0
Phenylalanine	2.89	4.31	
Tyrosine	2.11	1.93	
Phenylalanine+ Tyrosine	5.0	6.24	6.0
Lysine	3.10	2.25	5.50
Tryptophan	0.42	0.10	1.0
Total Essential amino acid	22.61	26.63	36.0
	B-Non-essential amino acid		
Glycine	2.34	4.13	
Alanine	2.68	4.32	
Serine	2.24	3.96	
Aspartic acid	4.89	4.86	
Glutamic acid	11.56	16.46	
Proline	2.13	4.67	
Arginine	6.76	11.78	
Histidine	1.76	2.32	
Total Non-essential amino acid	34.36	52.5	
Total amino acid	56.97	79.13	
C-PER	1.61	1.84	
BV	66.85	69.27	

Tryptophan was determined colorimetrically.
C-PER: The computed protein efficiency ratio..
B.V: Biological value

3.4. Effect of using different solvents on total polyphenol extracted from chia and moringa seeds:

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

Total polyphenols extracted from chia and moringa seeds are givenin Table (4). The data indicate that, ethanol was the best solvent for extracting polyphenols from chia and moringa seeds. High amounts of extracted polyphenolic compounds by ethanol from chia and moringa seeds were 1.63and 1.34 (mg gallic acid/g of DW), respectively, comparing with other solvents. These results are similar with that obtained by Su *et al.*(2007) They revealed that, methanol and ethanol were better ($P < 0.05$) than the ethyl acetate and acetone for extracting phenolic compounds owing to their higher polarity and good solubility for phenolic components from plant materials. The data in the same Table indicated that, chia seed contained highest amounts of polyphenolic compounds with all using solvents comparing with moringa seeds. These result agreement with Marineli *et al*. (2014) and Mohammed (2019).

Antioxidants are compounds that act against free radicals in human bodies. High levels of free radicals cause oxidative stress, which may contribute to chronic diseases like heart disease and type 2 diabetes (Rodrigo *et al*., 2011).Apparent also from this Table that, ethanol was the best solvent for antioxidant activity from chia and moringa seeds. High percentage of DPPH inhibition of polyphenolic compounds by ethanol from chia and moringa seeds were 94.63% and 91.34% respectively,comparing with other solvents due to these extract may contain many phenolic compounds that contributed of antioxidant activity. Meanwhile, diethyl ether recorded the lowest value of antioxidant activity (DPPH) in chia and moringa seeds comparing with other solvents. These findings are in close agreement with previous findings of Fitriana *et al*., (2016) and Reyes *et al.,* (2008).

Table 4: Effect of using different solvents on total polyphenol contents and antioxidant activity by (DPPH) extracted from chia and moringa seeds.

Extraction solvent	Total polyphenols (mg gallic acid/g of DW)		DPPH (%)	
	Chia seed	Moringa seed	Chia seeds	Moringa seeds
Ethanol	1.63 ±0.34^{Aa}	1.34 ±0.25^{Ab}	94.63±0.26^{Aa}	91.34±0.66^{Ab}
Acetone	0.91 ±0.09^{Ca}	0.79 ±0.03^{Cb}	71.43±0.09^{Ca}	66.84±0.78^{Cb}
Ethyl acetate	1.16 ±0.11^{Ba}	1.08 ±0.11^{Bb}	90.00±0.71^{Ba}	85.82±0.59^{Bb}
Diethyl ether	0.69 ±0.17^{Da}	0.55 ±0.07^{Db}	63.32±0.94^{Da}	53.89±0.76^{Db}

Each value is an average of three determinations ± standard deviation.
In column means with the same capital superscript letters are not significantly different at $p \leq 0.05$.
In row means with the same small superscript letters are not significantly different at $p \leq 0.05$.
DPPH = 1,1-diphenyl-2-picrylhydrazyl.

3.5. Some Physical and chemical characteristics of oils extracted from chia and moringa seeds:

Results in Table(5) indicated that, the refraction index, relative density, acid value, peroxide value, iodine index, saponification value and unsaponifiable matter in chia were 1.476 at 25°C, 0.830 at 20°C, 0.70mg KOH/g oil, 0.54 meqo^2/kg oil, 194.28 gI$_2$/100g oil, 186.08 mg KOH/g oil, 0.883 g/kg. while moringa oil were 1.462 at 25°C, 0.860 at 20°C, 1.93 mg KOH/g oil, 0.71 meqo^2/kg oil, 68.03 gI$_2$/100g oil, 180.25 mgKOH/g oil and 0.863 g/kg of the same characteristics respectively. From the obtained results, it could be noticed that, the chia oil had the highest saponification value, Unsaponifiable matter and iodine index, while moringa seed oil contained higher acid value, peroxide value. Because chia and moringa seed oil contains high amount of unsaturated fatty acids its relative density was high. The relative density of chia and moringa seed oil was similar with that of sunflower (0.91), safflower (0.92) and soy (0.919) oils at 20°C (Segura-Campose *et al*., 2014).

The saponification value of chia and moringa seeds oil was also similar with that of sunflower (188-194 mg KOH/g oil), soybean oil (189-195 mg KOHg oil). It means also that, chia and moringa seed oil contains lower proportion of short chain fatty acids like such oils. It is also expected the rise of iodine value of chia seed oil due to its content of unsaturated fatty acids particularly linolenic one, up to 60%. Therefore, this type of oil has higher iodine value than linseed or flaxseed oil (189), sunflower oil (141) (Timilsena *et al*., 2017).

Table 5: Some Physical and chemical properties of chia and moringa oils.

Property	Chia oil	Moringa oil
Refraction index (25°c)	1.476±0.03^{a}	1.462±0.01^{b}
Relative density at (20°c)	0.830±0.02^{b}	0.860±0.01^{a}
Acid value (mgKOH/g oil)	0.70±0.01^{b}	1.93±0.02^{a}
Peroxide value(meq o^2/Kg oil	0.54±0.02^{b}	0.71±0.02 ^{a}
Iodine index(g of I$_2$/100g oil)	194.28±0.15^{a}	68.03±0.06^{b}
Saponification value(mg KOH/g oil	186.08±0.26^{a}	180.25±0.31^{b}
Unsaponifiable matter(g/kg)	0.883±0.01^{a}	0.863±0.01^{b}

Each value is an average of three determinations ± standard deviation.
Values followed by the same letter in rows are not significantly different at $P<0.05$.

Refractive index is one of important physical parameter which used the identification of fats and oil because it could be used for the estimation the degree of saturation of oils.The refraction index value in chia and moringa seed oil was similar that reported by Codex Stan 210 (2003) in sunflower (1.461), safflower (1.467) and soy (1.466) oils at 40°C. According to Alvarado and Aguilera (2001), the refraction index is dependent on the analysis temperature and unsaturation contents of the fatty acids. They establish that high analysis temperatures showed lower refraction index values, and high unsaturation content is related to high refraction index values. Generally chia and moringa seed oils were similar refraction index, unsaponifiable matter and relative density. These results confirmed with (Segura-Campose*et al*., 2014, Timilsena *et al*., 2017 and Mohammed, 2019). They found that, crude chia seed oil contained 0.70-2.54 mg KOH/g oil acid value, 1.64-4.33 meq o^2/kg oil peroxide value, 1.47-1.48 refraction index at 25ºC, 189.1-222.66 mg KOH/g oil saponification value, 0.68-1.27g/kg unsaponifiable matter, 194.7-215 gI_2/100g oil iodine index and 0.923-0.924 relative density at 20ºC. Also,(Vibhute*et al*., 2015;Saad 2015 and Pereira *et al*.,2016).They found that, moringa seed oil contained 1.35-2.73 mg KOH/g oil acid value , 0.59-3.10 meq o^2/kg oil peroxide value , 1.45-1.47 refraction index at 25ºC, 164.09-191.2 mg KOH/g oil saponification value, 0.59-0.84 g/kg unsaponifiable matter, 65.6-86.7 gI_2/100g oil iodine index and 0.88-1.05 relative density at 20ºC.

3.6. Fatty acids composition of chia and moringa seeds oil:

Fatty acids composition of chia and moringa seed oils are presented in Table (6). Data showed that, moringa seeds oil had stearic acid, lignoceric acid and behenic acid more than that of chia seeds oil, but palmitic acid in chia seeds oil was higher than of moringa seeds oil. Generally, total saturated fatty acid of moringa seeds oil were higher (23.43%) than that of Chia seeds oil (9.53%).

Among the saturated fatty acids, the highest concentration was Palmitic acid (7.23%) in chia seeds oil meanwhile, behenic acid (6.77%) was the highest in moringa seeds oil. As for unsaturated fatty acids, it could be cleared that, chia seeds oil had higher linoleic acid and linolenic acid than those of moringa seeds oil, but moringa seeds oil had higher amount of oleic acid and vaccenic acid than that of chia seeds oil. Generally, chia seeds oil contained higher amounts of unsaturated fatty acids (89.72%) than that of moringa seeds oil (75.68%). This is due to that,linolenic acid (63.89%) and linoleic acid (18.89%) were the most abundant fatty acids in chia seeds oil, whereas oleic (66.66%) acid was the major components of moringa seed oil. These results agreement with Salama, (2019) reported that, the major saturated fatty acids in moringa seeds oil was behenic acid (6.49%) followed by palmitic acid (5.90%), while the major components of unsaturated fatty acid was oleic acid (65.68%) followed by vaccenic acid (6.15%).Furthermore, Ixtaina *et al*. (2012)and Mohammed, (2019) they found that,linolenic acid (64.56 – 62.93%), linoleic acid (16.77 - 20.67%), palmitic acid (7.15 – 6.3%) and stearic (1.78 – 1.31%) of chia seeds oil.

Table 6: Fatty acids composition of chia and moringa seeds oil.

` Constituent Fatty acid%	Chia seeds oil	Moringa seeds oil
LauricC12:0	0.22	-
Myristic C14:0	0.03	0.09
Palmitic C16:0	7.23	6.45
Margaric C17:0	0.06	0.09
Stearic C18:0	1.76	5.31
ArachidicC20:0	-	3.43
Behenic C22:0	0.09	6.77
LignocericC24:0	0.14	1.29
Total saturated fatty Acids(SFA)	9.53	23.43
Tetradecanoic C14:1	0.6	-
Palmitoleic C16:1	0.09	1.36
Oliec C18:1 n9	6.22	66.66
Elaidic C18:1 n9	0.03	0.16
Vaccenic C18:1 n7	-	5.43
Linoleic C18:2 n6	18.89	0.49
Linolenic C18:3 n3	63.89	0.15
Gondoic C20:1	-	1.34
Erucic C22:1	-	0.09
Total unsaturated fatty acids (USFA)	89.72	75.68
Saturated to unsaturated FA ratio	1: 9.4	1: 3.2
Total(FA)	99.25	99.11

3.7. Chemical composition of cookies:

The results of chemical composition of cookies made from different levels of defatted chia seeds flour (DCSF) and defatted moringa seeds flour (DMSF) were recorded in Table (7). The obtained results manifested that, the Moisture, crude protein, ash and crude fiber contents increased. while, carbohydrates value decreased gradually with increasing the substitution levels of DCSF and DMSF. These results are in a harmony with the findings of Peiretti and Gai, (2009) and Olosunde *et al*. (2014) who reported that, supplementation with moringa seed flour could also improve the protein contents of kunu-zaki as it has been found to contain essential nutrients needed the body. Also, Chinma *et al*. (2013) mentioned that, the high protein and mineral contents of moringa seeds and its lower carbohydrate content compared with wheat flour, this food product will be beneficial to the nutrition. Furthermore, Barrientos *et al.* (2012); Divyashree *et al*. (2016) and Mesias *et al*. (2016) found that, adding chia seed flour in wheat based biscuit formulation enhanced the nutritional quality of this product, increasing protein, dietary fiber, antioxidants and polyunsaturated fatty acids. The latter component accelerates lipid oxidation in biscuits enriched with high amount of chia seed and that reducing its shelf life. From the same Table, there were significant differences between control and substitution cookies at different levels of DCSF and DMSF in moisture content. The moisture contents ranged between 6.51% in control to 8.70 and 7.80% in substitution cookies with 15% DCSF and DMSF. The increased moisture content can be explained by the higher content of protein which also increases the water binding capacity of dough with higher levels of DCSF and DMSF. It is also reported that, moisture content of cookies increased resulted in addition of defatted maize germ cookies flour (DMGCF) (Farahat *et al*., 2020). Apparent also from the same Table that, substitution of DCSF and DMSF to wheat flour lead to increased significantly protein content from 11.60% in control to 14.71 and 15.91% in cookies substitution with 15% DCSF and DMSF respectively. The protein content of the substitution cookies was increased by increasing the concentrations of substitution DCSF and DMSF. This increment may be due to the DCSF and DMSF were high protein content as compared to the wheat flour. Data of the present study are in agreement with those found by Anwar *et al*. (2006); Ogunsina *et al*. (2010); Abiodun *et al*. (2012); Vuksan *et al*. (2017) and Goyat *et al*. (2018). Therefore, defatted chia and moringa seeds had tendency to improve the protein contents cookies.

Table 7: Chemical composition (% on dry weight basis) of cookies made of different substitution levels of defatted chia and moringa seeds flour.

Parameter / Cookies	Moisture %	Dry matter %	Protein %	Ether Extract %	Ash %	Fiber %	T.C %	A.C %	Total Energy kcal/100g
Contol	6.51 ±0.25^{e}	93.49 ±0.25^{a}	11.60 ±0.29^{f}	8.10 ±0.22^{a}	0.99 ±0.19^{c}	1.90 ±0.12^{f}	77.41 ±0.82^{a}	75.51 ±0.94^{a}	428.94 ±0.14^{a}
5% DCSF	7.21 ±0.17^{cd}	92.79 ±0.17^{bc}	12.71 ±0.11^{e}	8.11 ±0.12^{a}	1.37 ±0.15^{bc}	3.85 ±0.15^{d}	73.96 ±0.53^{b}	70.11 ±0.68^{b}	419.67 ±0.60^{c}
10% DCSF	7.89 ±0.15^{b}	92.11 ±0.15^{d}	13.51 ±0.26^{c}	8.12 ±0.15^{a}	1.75 ±0.31^{ab}	5.79 ±0.28^{b}	70.83 ±1.00^{c}	65.04 ±1.28^{d}	410.44 ±1.61^{f}
15% DCSF	8.70 ±0.23^{a}	91.30 ±0.23^{e}	14.71 ±0.13^{b}	8.14 ±0.27^{a}	2.13 ±0.13^{a}	7.75 ±0.32^{a}	67.27 ±0.85^{e}	59.52 ±1.17^{e}	401.18 ±0.45^{g}
5% DMSF	6.93 ±0.12^{d}	93.07 ±0.12^{b}	13.11 ±0.15^{d}	8.12 ±0.19^{a}	1.35 ±0.28^{bc}	2.86 ±0.25^{e}	74.56 ±0.87^{b}	71.70 ±1.12^{b}	423.76 ±1.17^{b}
10% DMSF	7.35 ±0.21^{c}	92.65 ±0.21^{c}	14.80 ±0.17^{b}	8.09 ±0.30^{a}	1.74 ±0.15^{ab}	3.81 ±0.17^{d}	71.56 ±0.79^{c}	67.75 ±0.96^{c}	418.25 ±0.22^{d}
15% DMSF	7.80 ±0.27^{b}	92.20 ±0.27^{d}	15.91 ±0.30^{a}	8.13 ±0.25^{a}	2.07 ±0.21^{a}	4.85 ±0.11^{c}	69.04 ±0.87^{d}	64.19 ±0.98^{d}	412.97 ±0.03^{e}

Each value was an average of three determinations.
Values followed by the same letter in columns are not significantly different at $p \leq 0.05$.
Control: Wheat flour cookies, DCSF: defatted chia seeds flour DMSF: defatted moringa seeds flour.
T.C: Total carbohydrate A.C: Available Carbohydrates

On the other hand, it could be noticed that, there were no significant differences in ether extract contents of the control and substitution supplemented cookies. Ash content of cookies was ranged between 0.99% in control cookies to 2.13% in cookies substitution with 15% DCSF. There were no significant differences with higher value in the ash contents of the cookies substitution with 15% DCSF and DMSF. Ash contents was decreased in the control but ash contents was increased significantly in sample which substitution with DCSF and DMSF. This increment may be due to incorporation of defatted chia and moringa seeds. Data of the present study are in agreement with those found by Ogunsina *et al*. (2011) and Mohammed (2019).

The data in the same Table revealed that, crude fiber contents of cookies substitution with different levels of DCSF and DMSF were higher than that of control sample. This might be due to the high fiber contents in DCSF and DMSF as compared to wheat flour. Fiber content increased from 1.90 % in control to 7.75% in cookies with 15% DCSF. The variation in crude fiber content between control sample and substitution cookies with 5, 10 and 15% DCSF is high different significant. This is due to DCSF contained up to 40% crud fiber.

On the other hand, the Total carbohydrate content in cookies samples was significantly decreasing by increasing DCSF and DMSF. It was decreased from 77.41% in control to 67.27% and 69.04% in cookies substitution with 15% DCSF and DMSF. This is may be due to DCSF and DMSF are rich in protein and crude fiber. Furthermore, energy value of cookies samples was significantly decreasing by increasing DCSF and DMSF. It was decreased from 428.94 kcal/100g in control to 401.18kcal/100g in cookies substitution with 15% defatted chia seeds. These results were in agreement with Ogunsina *et al*. (2011) who found that, cookies supplemented with moringa seeds powder were more nutritious than the control. The cookies supplemented with moringa seeds had more protein, fat, iron and calcium than control.

3.8. Sensory characteristics of cookies substitution with different levels of defatted chia and moringa seeds flour:

The data pertaining to sensory evaluation of cookies substitution with DCSF and DCMF were presented in Table (8). The higher score of taste was recorded in control cookies (9) followed by substitution cookies with 5% DCSF and DCMF. The lowest score of taste was found in cookies substitution with 15% DCMF (7.3). This may able due to the tasted bitter of defatted moringa seeds and rated as decrease acceptable by panelists (Ogunsina *et al*., 2011).

The color of any kind of food is an important parameter which gives the first sight impression on consumers and effects on its acceptability (Caudillo *et al*., 2008). From the results in this Table, it could be noticed that, significant differences were noticed in color score among control sample and substitution cookies with DCSF and DMSF. The lowest score of color was (7.5) and (7.6) in supplemented cookies with 15% DMSF and DCSF, respectively. Apparent also from the same Table that, there were significant differences were noticed in odor score among control sample and substitution cookies with DCSF and DCMF. The highest score for odor was recorded in control cookies (9.5) and cookies supplemented with 5% DCSF (9.3) and moringa seeds (8.9). Meanwhile, the lowest score of odor was found in cookies substitution with 15% of defatted chia (7.2) and moringa seeds (7.8).

Table 8: sensory characteristics of cookies supplemented with different levels of defatted chia and moringa seeds flour.

Parameters / Cookies	Taste	Color	Odor	Texture	Overall acceptability
Control	9.0±0.21^{a}	9.0±0.23 ^{a}	9.5±0.25^{a}	9.8±0.29^{a}	9.5±0.26^{a}
5% DCSF	8.3±0.32^{b}	8.5±0.35^{abc}	9.3±0.30^{a}	9.3±0.44^{ab}	8.9±0.36^{b}
10% DCSF	8.1±0.35^{bc}	7.9±0.43^{cde}	8.4±0.34^{b}	8.5±0.37^{cd}	8.6±0.30^{b}
15% DCSF	7.7±0.29^{cd}	7.5±0.37^{e}	7.2±0.25^{d}	7.2±0.42^{e}	7.5±0.41^{cd}
5% DMSF	8.4±0.35^{b}	8.7±0.39^{ab}	8.9±0.41^{ab}	9.1±0.33^{bc}	8.8±0.25^{b}
10% DMSF	7.9±0.40^{bc}	8.2±0.31^{bcd}	8.6±0.37^{b}	8.3±0.31^{d}	8.0±0.25^{c}
15% DMSF	7.3±0.25^{d}	7.6±0.25^{e}	7.8±0.35^{c}	7.0±0.40^{e}	7.3±0.21^{d}

Values followed by the same letter in columns are not significantly different at $p \leq 0.05$.
Control: Wheat flour cookies, DCSF: defatted chia seeds flour DMSF: defatted moringa seeds flour.

From the same Table, it could be observed that, there was no significant difference in texture between control sample and supplemented cookies with 5% of defatted chia. Meanwhile, significant differences were noticed in texture score among substitution cookies with defatted moringa and chia seeds. Also, from the same Table that, significant differences were noticed in overall acceptability score among control sample and substitution cookies DCSF and DMSF. The lowest overall acceptability score was found in cookies substitution with 15% of DCSF and DMSF. All samples were acceptability. These results were in agreement with Ogunsina *et al*. (2011); Divyashree *et al*. (2016); Mesias *et* *al*.(2016) ; Goyat *et al*. (2018)and Chelladurai1*et al* .(2019) they reported that, the incorporation of wheat flour with increasing levels of debittered *Moringa oleifera* seed powder meant the surface cracking pattern, crumb color, texture, mouth feel and flavor reduced.

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