Chemical and Nutritional Evaluation of Bitter Melon Seeds and their Use in the Preparation of Tahini

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ABSTRACT: This work was designed to study the chemical and nutritional properties of bitter melon seeds, where the traditional method used by the Tabou tribes in Southern Libya, Northern Chad and Niger was used to remove the compounds responsible for bitterness and to compare untreated bitter melon seeds (UTBMS) with conventionally treated bitter melon seeds (TBMS) to benefit from their application in food technology. The TBMS contains higher levels of protein, fat, minerals and micronutrients than the UTBMS. TBMS showed a higher concentration of essential amino acids than UTBMS. The fatty acid composition of both UTBMS and TBMS contained a mixture of saturated and unsaturated fatty acids, with linoleic acid being the most abundant fatty acid in both. The content of the total phenolics and flavonoids, as well as antioxidant activity was decreased in the TBMS compared to UTBMS. The study also found that the phenolic profile of both extracts contains phenolics and flavonoids. Sensory evaluation results showed that tahini made entirely from sesame seeds had higher acceptability scores compared to tahini made entirely from TBMS. However, sensory evaluation results showed no significant differences between tahini with added 40% and 20% TBMS. There were no significant differences also between them and 100% sesame. Thus we can prepare healthy tahini with 60% sesame seeds and 40% TBMS.

Keywords *Citrullus colocynthis seeds, Amino acids, Fatty acid composition, Phenolic compounds Antioxidant activity, Minerals, Sensory evaluation and Tahini.*

INTRODUCTION

The *Citrullus colocynthis* a species of the Cucurbitaceae family, is a bitter melon. The Sahara and Arabian deserts, Sudan, and Southern Asia, including Pakistan, India, and the Southern Islands, are all places where the plant is extensively distributed. It contains one of the finest collections of genetically diverse organisms in the plant kingdom. This family consists of approximately 965 species of plants; the most famous of which is a large number of drought-tolerant plants, susceptible to floods, and cold seasons, and resistant to the harsh effects of poor soils. The fruit of bitter melon is known for its phytochemical and pharmacological properties (Paris, 1996; Maatooq *et al.*, 1997; Christenhusz and Byng, 2016; Hameed *et al.*, 2020).

Bitter melon *(Citrullus colocynthis)* is a perennial plant, containing an abundance of nutrients and a source of several bioactive compounds such as essential oils, glycosides, flavonoids, alkaloids, and fatty acids that play a key role in the improvement of well-being. The dried unripe fruit pulp and leaves are used medicinally for their drastic purgative and hydragogue cathartic action on the intestinal tract and are a folk remedy for cancerous tumours as well. Roots may also be used as purgative against ascites, jaundice, urinary diseases, rheumatism, and for snake-poison (Kumar *et al.*, 2008; Sharma *et al.*, 2020).

Bitter melon has a variety of biological properties, including antioxidative, hypoglycemic, antimicrobial, anti-cancerous, analgesic, anti-inflammatory, antidiabetic, hypolipidemic, antineoplastic, pesticidal, and immune-stimulatory properties. It also affects the reproductive system and fertility (Li *et al.*, 2021).

The proximate analysis showed that bitter melon seed contains oil, moisture, protein, ash and fiber. They are an excellent source of different amino acids like arginine, methionine, and tryptophan and the biological indices of its protein quality have been described as lower than soybean but comparable to or higher than most oilseeds. Nutritionally, the amino acids that are limited are lysine and threonine and the bitter melon seeds contain essential amino acids (Sabo *et al.*, 2015; Abudayeh *et al.*, 2016; Ambrose, 2019).

The oil of bitter melon seeds is liquid at room temperature with good Physicochemical characteristics. The main fatty acids which comprise approximately 90% of oil content are linoleic, stearic, oleic and palmitic acid, with 50% of that counted as linoleic acid content (Riaz *et al.*, 2015; Kapoor *et al.*, 2021).

The seeds contain many micronutrients, vitamins and minerals such as Ca, K, Mg, P, Na, Fe, Cu and Zn. and vitamins B1, B2, and niacin, which can contribute significantly to the diet (Hussain *et al.,* 2014; Kapoor *et al.,* 2021).

The seeds are available in West African markets shelled or unshelled and are widely utilized in West African cuisine. The roasting shelled seeds can be ground or milled and used in soups and as a soup garnish. Ogiri is a fermented, high-protein Nigerian soup seasoning. The undefatted meal has been used in a variety of nutritional preparations that vary according to an individual's diet habits. Whole *Citrullus colocynthis* seeds are also consumed as a snack after dry roast (Hussain *et al.,* 2014).

For over a decade, the scientists of the United States Department of Agriculture (USDA), have investigated the nutritional and functional properties of the seeds of this plant and concluded that they have the potential to find a place in the food and industry (Zaini *et al.,* 2011; Sabo *et al.,* 2015). Therefore, the present study aims to evaluate the chemical composition and bioactive components found in bitter melon seeds before and after the technological treatment for bitter taste elimination and application them in food products and a functional food ingredient for the production of healthy good products.

MATERIALS AND METHODS

Materials:

Bitter melon seeds:

Bitter melon (*Citrllus colocynthis*) seeds (10 kg) were obtained from some areas in the South of Libya (Kufra and Rabiana) and Southwest (Sabha, Murzuq, Umm al-Aranib, Qatrun and Tajerhi) during the spring, of 2022. The study area is located between longitudes (13° 00′ 0″ - 25° 00′ 0″ E), and latitudes (23° 00′ 0″ - 26° 00′ 0″ N), in Libyan desert areas located in the South of Libya.

Chemicals:

All chemicals used in this study were of analytical grade. Hexane, benzene, methanol, aluminium chloride, sulphuric acid, acetonitrile, sodium sulphate, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and -tocopherol were purchased from Sigma-Aldrich Co., Germany and Algomhorya Co. for Chemicals and Medical Instruments, Egypt.

Methods:

Preparation of bitter melon seeds:

Bitter melon seeds were processed using the traditional method used by the Tabou tribes in Southern Libya, Northern Chad and Niger. This method includes separating the seeds manually and mixing them in quantities with palm bark ashes (ampula), and boiling them in water in special pots to extract the bitter compounds. Then the seeds were sun-dried ranging at 42-47 0C and then these seeds were dehulled by a fraction between two surfaces to remove the husks, then boiled in the same pots by changing the water until all the bitter compounds were completely removed for 2-3 hours.

Proximate chemical composition:

The proximate chemical composition including moisture, crude protein (N X 6.25), crude fat, crude fiber, and total ash were determined according to AOAC (1990) methods unless otherwise stated. Nitrogen-free extract (NFE) was calculated by difference. Energy value was calculated using the universally acceptable conversion factors as described by Kanu *et al.* (2009) by multiplying, protein and carbohydrate by 4.0 and fat by 9.0 kcal/g.

Minerals:

The elements Ca, K, Mg, P, Na, Fe, Cu and Zn were measured after the total ash determination, the residue was dissolved in 10 ml of 50% nitric acid solution and diluted with distilled water making the final volume of 25 ml. The mineral contents were measured using iCAP Triple Quadrupole Inductively Coupled Plasma 56 Mass Spectrometer (iCAP TQ ICP-MS) Thermo Scientific, Inc. and the Thermo Scientific Qtegra 57 Intelligent Scientific Data Solution Software. The instrument's operating conditions such as RF 58 power, nebulization gas flow rate, and the sampling position within the plasma were optimized for 59 the maximum signal-to-noise ratio described by Rigas (2012).

Determination of total phenolics and flavonoid contents:

The total phenolics content of bitter melon defatted flour extracts was determined using a modified Folin–Ciocalteu colorimetric method as described by Sakanaka *et al.* (2005). The total phenolics content was expressed as gallic acid equivalent from the calibration curve of gallic acid. Total flavonoid content was determined using a modified method by Sakanaka *et al.* (2005). Briefly, the measurement was compared to a preset standard curve of prepared catechin standard solution and expressed as mg of catechin equivalents per 100 g of defatted flour.

Estimated quantitative phenolic compounds by HPLC-UV:

Analyses of phenolic and flavonoid compounds were performed using HPLC (Agilent Series 1100, USA) composed of an auto-sampling injector, solvent degasser, two LC- pumps (series 1100), with ChemStation software, and UV/Vis detector (set at 250 nm for phenolic acids and 360 nm for flavonoids). The analysis achieved C18 column (125 mm × 4.60 mm, 5 µm particle size). Phenolic acids were separated by employing a gradient mobile phase of two solvents, Solvent A (Methanol) and Solvent B (Acetic acid in water

(1:25). The gradient program was started at 100 % B and was held at this concentration for the first 3 minutes. This was followed by 50 % eluent A for the next 5 minutes after which concentration was increased to 80 % for the next 2 minutes and then reduced to 50 % again for the following 5 minutes with detection wavelength at 250 nm. Flavonoids were separated by employing the mobile phase of two solvents used- acetonitrile (A) and 0.2% (v/v) aqueous formic acid (B) with an isocratic elution (70: 30) program. The solvent flow rate was 1 ml/min, and separation was performed at 25°C (Goupy *et al.,* 1999, Mattila *et al.,* 2000).

Antioxidant activity:

DPPH radical assay was carried out to measure the free radical scavenging activity as described by Ahmad *et al.* (2015) Briefly, a solution of DPPH radical (2.9 ml, 0.1 mm) in 96% ethanol was added to 0.1 ml of *C. colocynthis* extract solution at different concentrations. After 30 min incubation period at room temperature, the absorbance was measured at 517 nm (A517 nm). Ethanol (96%) was used as a blank. The control solution consisted of 0.1 ml of 96% ethanol and 2.9 ml of DPPH· solution. The percent scavenging was calculated by the following formula:

Scavenging (%) = 100 × (A blank – A sample / A blank).

The IC_{50} value is defined as the amount of sample necessary to decrease the initial DPPH radical concentration by 50% and was calculated from the plot of inhibition percentage against concentration. All measurements have been performed in triplicates.

Amino acid composition:

The amino acid profile in the defatted samples was determined using a method described by Spackman *et al.* (1958). The samples were dried to constant weight, defatted, and hydrolyzed by 6N HCL containing 0.1% mercaptoethanol at 100ºC for 24h. the solvent evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-sample Amino Acid Analyzer (TSM). using the Technico Sequential Multisample (TSM) Amino Acid Analyzer (Technicon Instruments Corporation, New York). Nor–leucine was the internal standard. Tryptophan was determined after alkali (NaOH) hydrolysis by the colourimetric method described by Spies (1967).

Fatty acid composition:

Total fat content was extracted from better melon seeds according to Folch *et al.* (1957). Fatty acid methyl esters are prepared according to the procedure of Radwan (1978). using 1% sulphuric acid in absolute methanol. The fatty acid methyl esters obtained were injected into the GC column ACME model 6100 GC split injector with FID detector (Young LIN Instrument Co., Ltd.). The column is initially maintained at 140ºC for 5 minutes and then raised to 240ºC at a rate of 4ºC / min.

Preparation of tahini from treated bitter melon seeds:

The following steps were performed to prepare bitter melon tahini, the processes listed below were followed:

Treated bitter melon seeds that had been peeled and dried were then cleaned by sieving to remove impurities and foreign objects, soaked in salt water for three hours, roasted at low temperatures, and allowed to cool before being transferred to a home blender and blended for about five min while adding amounts of water to achieve the desired tahini consistency. Before evaluation, the tahini paste is then partially replaced with sesame (0, 20, 40, 60, 80, and 100%).

Sensory evaluation:

A panel of 22 judges comprising students, staff and faculty members from the Department of Food Science and Technology at Omar Al-Mukhtar University, with a wide age distribution in the department and recruited by advertisements evaluated the organoleptic characteristics of bitter melon tahini and sesame tahini. They assessed (colour - taste - odour - appearance - texture - and overall acceptability). The samples (control and 0, 20, 40, 60, and 80%) were served in dishes labelled randomly numbers for all panelists. Each panelist was given a rating form scored on a 1–9 hedonic scale (9 being considered excellent; 5, acceptable; and 1, extremely poor), as suggested by Austin and Ram (1971).

Statistical analysis:

The results are expressed as mean±standard deviation of triplicate analysis. One Way Analysis of Variance (ANOVA) test based on the completely randomized design (CRD) as described by Bewick *et al*. (2004) and the Least Significance Difference (LSD) was carried out the significance level was set at $p \leq 0.05$. Means were separated using Duncan (1955).

RESULTS AND DISCUSSION

Proximate chemical composition of untreated and treated bitter melon seeds.

The proximate composition of bitter melon seeds, (*Colocynthis citrullus*), was compared before and after the seeds were treated by traditional processes. The results in Table (1) showed the proximate chemical composition of untreated raw bitter melon seeds (UTBMS) and treated bitter melon seeds (TBMS) ($p \leq 0.05$), where the untreated seeds contained significantly higher moisture content (6.80%), compared to the

treated seeds (6.44%), which was different moisture content value of bitter melon seeds obtained in this work is very similar to that of Ogundele *et al.* (2012) and Gado *et al.* (2019). (UTBMS) moisture ranged between (1.90 - 8.37%). Increasing moisture levels in seeds can encourage the growth of microbes that cause spoilage. The results recorded also showed that the crude protein content of TBMS was significantly higher than UTBMS being 37.15% and 19.33%, respectively and these results are less than what was found by Ogundele *et al.* (2012) and Gado *et al*. (2019) who founds that the crude protein content of untreated seeds (24.37%) also, Adam *et al.* (2011) found that the crude protein content of the untreated was (27.67%) and Li *et al.* (2021) (25.37%). The (TBMS) crude protein value analyzed in this work was generally high than that of (UTBMS) this is corroborated by the report of Gado *et al.* (2019) who found the UTBMS to be 5.37%. This may be a result of the conventional technological processes used to prepare the seeds to extract the bitter compounds accordingly, the high protein content consuming TBMS may help vulnerable people, especially children, achieve their daily nutritional requirements. It can also be used to augment low-protein animal diets. The results also revealed that the crude fat content of (TBMS) was significantly higher than (UTBMS) being 54.19% and 27.54%, respectively. and these results are higher than those reported by Alzarah *et al.* (2021) (17.9%). In the same trend, the ash content of (TBMS) was seen to be nearly double that of (TBMS) being (4.90%) and (2.70%), respectively. These results match up with the data found by Alzarah *et al.* (2021). Meanwhile, in the present study the seeds processed traditionally (TBMS) increased the ash content to 4.90 %, and this result is higher than most studies, and this may be due to the use of palm bark ash in extracting bitter compounds. It was clear from the results of crude fiber that (UTBMS) was significantly higher than (TBMS) being (38.23%) and (1.06%), respectively. These results are not in close agreement with the findings of Alzarah *et al.* (2021) and Riaz *et al.* (2015). This indicates that the treatments carried out on the seeds by temperature and dehulling to get rid of the bitter compounds reduced the proportion of fiber to a large extent. The study's findings demonstrated that the nitrogen-free extract (NFE), also was significantly reduced in (TBMS) as compared to (UTBMS) being (2.70%) and (11.80%), respectively. The findings of (NFE) were in agreement with those of Ogundele *et al.* (2012), who reported that the nitrogen-free extract was 10.88% in (UTBMS) and that this percentage in our work significantly dropped in the (TBMS) due to the treatments used on the seeds during the bitterness extraction. The energy (kcal /100 g) content of (TBMS) logically increased as both crude fat and protein content were increased as a result of seed dehulling.

Table (1) Proximate chemical composition of untreated and treated bitter melon seeds on a dry weight basis.

Component %	UTBMS	TBMS
Crude protein	19.33 ± 0.17^{b}	37.15 ± 0.67^{a}
Crude fat	27.54 ± 0.38^{b}	54.19 ± 0.73^{a}
Crude Fiber	38.23 ± 0.61^{a}	1.06 ± 0.28^{b}
Total ash	3.10 ± 0.11^{b}	4.90 ± 0.27^{a}
Nitrogen-free extract (NFE)*	11.80 ± 0.86^{a}	2.70 ± 121^{b}
Energy (kcal/100 g)	372.58	647.11

- The results are expressed as mean±standard deviation of triplicate analysis.
- Mean values in a row having different superscript are significantly different at $p\leq 0.05$.
- UTBMS= Untreated bitter melon seeds.
- TBMS =treated bitter melon seeds.
- * Calculated by difference.

The mineral content of untreated and treated bitter melon seeds.

The mineral composition of UTBMS and TBMS, achieved in this work is presented in Table (2) Results indicated that the levels of the elements are high in TBMS extract compared to UTBMS. Macronutrient minerals in UTBMS and TBMS were as follows Na, K, Ca, Mg and P being (26.47,42.88), (947.15,916.05), (71.75,447.70), (37.87,41.28), (361.55,1283.98), respectively. Whereas, the micronutrient minerals Zn, Cu, Fe and Mn were (2.91,8.59), (8.60, 15.12), (0.75, 1.50), and (1.28, 3.40), respectively. The mineral composition in TBMS, especially phosphorus, calcium, iron and zinc, was found to be higher than that mentioned by Riaz *et al.* (2015) and Kapoor *et al.* (2021). It is important to note that minerals play important roles in many physiological processes in the body, and the presence of high levels of certain minerals in food products can have both positive and negative health effects. Macronutrient minerals, such as sodium, potassium, calcium, magnesium, and phosphorus, are essential nutrients that the body needs in massive amounts. These minerals play important roles in various bodily functions, such as maintaining fluid

balance, regulating blood pressure, and building strong bones. Micronutrient minerals, such as zinc, iron, copper, and manganese, are also essential nutrients required in smaller amounts by the body. These minerals are necessary for a variety of body activities such as energy production, immune function, and wound healing. depending on the amount consumed and other factors (Rahman *et al.,* 2014).

Table (2) Mineral contents of untreated and treated bitter melon seeds.

Element (mg/100 g)	UTBMS	TBMS
Macronutrient minerals		
Sodium	26.47±0.15[b]	42.88±0.15[a]
Potassium	947.15±1.13[a]	916.07±1.14[b]
Calcium	71.75± 0.16[b]	447.71±0.52[a]
Magnesium	37.87±0.03[b]	41.28±0.06[a]
Phosphorus	361.55±1.11[b]	1283.98±1.23[a]
Micronutrient minerals		
Zinc	2.91±0.04[b]	8.59±0.06[a]
Iron	8.60±0.07[a]	15.12±0.07[b]
Copper	0.75±0.02[b]	1.50±0.09[a]
Manganese	1.28±0.02[b]	3.40±0.02[a]

- The results are expressed as mean±standard deviation of triplicate analysis.
- Mean values in a row having different superscript are significantly different at p≤ 0.05.
- UTBMS= Untreated bitter melon seeds.
- TBMS =treated bitter melon seeds.

Total phenolic (TP) and total flavonoid(TF) compounds of untreated and treated bitter melon seeds.

The results Table (3) showed that the total phenolics (TP) of the UTBMS was significantly higher than TBMS being (175.34%) and (47.09%), respectively. thus the later lost 73% of the original TP. Meanwhile UTBMS recorded a significantly high amount of total flavonoids (TF) compared to TBMS being (48.29%) and (15.49%), respectively. Thus the later lost above 68% of the original TF. These results are higher than the study done by Tannin-Spitz et al. (2007) and Hsouna and Alayed (2012) and they found that the total phenolic and total flavonoid contents of the extracts can vary depending on the polarity of the solvent used in the extraction. In the previous study, the highest total phenolic content was found in the ethyl acetate extract, followed by methanol, while the hexane and water extracts had significantly lower total phenolic contents. The total flavonoid content also varied with solvent polarity, with the highest levels observed in ethyl acetate and water extracts. This indicates that the choice of solvent for extraction can have a significant impact on the phytochemical composition of the extract. Another explanation may be due to the harsh treatment of eliminating the bitterness of seeds during preparation. The antioxidant activity as measured by DPPH Table (3) showed that the UTBMS extract exhibited a higher radical scavenging activity compared to TBMS compared to extract being (76.53%) and (32.30%), respectively. The IC50 values, which represent the concentration of the extract required to inhibit 50% of the DPPH radicals, were (13.51 mg/ml) for UTBMS compared to (30.53 mg/ml) for TBMS. These results indicate that UTBMS had a more potent antioxidant activity than TBMS. A previous study (Tannin-Spitz et al., 2007) on the aqueous extract of Citrullus colocynthis seeds from Tunisia reported a very potent DPPH radical scavenging activity with an IC50 of 0.021 mg/ml, which is significantly lower than the values obtained for both UTBMS and TBMS extracts. Inspite of the decreased activity of TBMS after harsh treatment, still it processes a valuable antioxidant activity compared to many extracts of fruits and vegetables (Lee et al., 2021).

Table (3) Total phenolics (TP), total flavonoids (TF) and antioxidant activity of UTBMS and TBMS

Component	UTBMS	TBMS
Total phenolics (mg GAE/100 g)	175.34±0.57[a]	47.09±0.09[b]
Total flavonoids (mg QE/100 g)	48.29±0.19[a]	15.49±0.45[b]
DPPH radical scavenging activity%	76.53±0.25[a]	32.30±0.50[b]
IC_{50} (mg/ml)	13.51±0.66[b]	30.53±0.54[a]

- The results are expressed as mean±standard deviation of triplicate analysis.
- Mean values in a row having different superscript are significantly different at p≤ 0.05.
- UTBMS= Untreated bitter melon seeds.
- TBMS =treated bitter melon seeds.

Phenolic profile of untreated and treated bitter melon seeds.

The results of the study Table (4) showed the number of the phenolic profile of both (mg/ml) UTBMS and TBMS, where chlorogenic acid (0.41, 0.34), Syringic acid (0.57, 0.22), cinnamic acid (0.21, 1.43), caffeic acid (1.44%, ND), gallic acid (0.13, ND), salicylic acid (0.23, ND), pyrogallol (ND, 1.12), ferulic acid (ND, 1.34), and benzoic acid (1.07, 0.11) mg/ml, respectively. As can be seen both phenolic acids in UTBMS and TBMS contain chlorogenic acid, Syringic acid, cinnamic acid, and benzoic acid, with higher amounts of chlorogenic acid, Syringic acid and benzoic acid in UTBMS and higher amounts of cinnamic acid. pyrogallol and ferulic acid were only found in TBMS, with no detectable levels in UTBMS. Gallic acid, salicylic acid, and caffeic acid were only present in UTBMS, and no detectable levels in TBMS. The decrease in phenolic compounds such as chlorogenic acid, Syringic acid and benzoic acid in TBMS can be attributed to the treatments carried out during the processing of the seeds to extract the bitterness from bitter melon seeds, especially the high temperatures and the addition of palm bark ash, which may cause degradation or changes in the structure of these compounds. On the other hand, the appearance of pyrogallol and ferulic acid in TBMS could be due to the same processing trend, which probably led to the formation of these compounds from precursors in bitter melon seeds. The disappearance of some compounds such as caffeic acid, gallic acid, and salicylic acid in TBMS can also be attributed to the seed's harsh processing process, which probably caused these compounds to degrade or transform into other compounds. The increase in cinnamic acid in TBMS could be due to the conversion of other compounds (Samec et al., 2021). The results also showed a significant impact concerning flavonoid profile, with a decrease in rutin content from 15.15±0.14 mg/ml in TBMS to 10.57±0.06 mg/ml in TBMS. and an increase in quercetin, kaempferol, luteolin, and catechin content in (TBMS) compared to (UTBMS) were (14.09, 10.26), (4.19, 1.16), (5.16, 3.46), (3.90, 2.50) mg/ml, respectively. This may be a result of the conventional technological processes used to prepare the seeds to extract the bitter compounds. The seeds of bitter melon contain flavonoids such as rutin, quercetin, kaempferol, luteolin, and catechin, which have antioxidant, anti-inflammatory, and anti-cancer properties. These flavonoids may support an increased risk of free radical-mediated diseases such as coronary heart disease and cardiovascular health also protect against neurodegenerative diseases, reduce the risk of developing certain types of cancer, promote weight loss and can also protect cells from oxidative stress (Hussain et al., 2014).

Table(4). Phenolic and flavonoid profile of untreated and treated bitter melon seeds.

Compound mg/ml	UTBMS	TBMS
Phenolic profile		
Chlorogenic acid	0.41 ± 0.01^{a}	0.34 ± 0.02^{b}
Syringic acid	0.57 ± 0.56^{a}	0.22 ± 0.01^{b}
Cinnamic acid	0.21 ± 0.01^{b}	1.43 ± 0.02^{a}
Caffeic acid	1.44 ± 0.02^{a}	0.00 ± 0.00^{b}
Gallic acid	0.13 ± 0.02^{a}	0.00 ± 0.00^{b}
Salicylic acid	0.23 ± 0.02^{a}	0.00 ± 0.00^{b}
Pyrogallol	0.00 ± 0.00^{b}	1.12 ± 0.00^{a}
Ferulic acid	0.00 ± 0.00^{b}	1.34 ± 0.00^{a}
Benzoic acid	1.07 ± 0.02^{a}	0.11 ± 0.02^{b}
flavonoids profile		
Rutin	15.15 ± 0.14^{a}	10.57 ± 0.06^{b}
Quercetin	10.26 ± 0.04^{b}	14.09 ± 0.06^{a}
Kaempferol	1.16 ± 0.05^{b}	4.19 ± 0.02^{a}
Luteolin	3.46 ± 0.03^{b}	5.16 ± 0.05^{a}
Catechin	2.50 ± 0.05^{b}	3.90 ± 0.01^{a}

- The results are expressed as mean±standard deviation of triplicate analysis.
- Mean values in a row having different superscript are significantly different at $p\leq 0.05$.
- UTBMS= Untreated bitter melon seeds.
- TBMS =treated bitter melon seeds.

Amino acids profile of untreated and treated bitter melon seeds

Table (5) shows that bitter melon seeds treated in the traditional way TBMS were distinguished by the presence of higher essential amino acids (g/100 g protein) than untreated seeds (UTBMS), such as Tryptophan (2.14%, 1.35%), Threonine (1.84%, 0.31%), valine (3.52%, 1.11%), Methionine (1.77%, 0.18%), Isoleucine (4.08%, 1.03%) phenylalanine (4.12%, 0.43%), %) Histidine (3.38%, 0.73%), and Lysine (3.50%, 0.41%), then leucine (0.64%, 0.81%), respectively. The results were consistent with Ogundele *et al.* (2012) but the results of these amino acids in UTBMS disagree with what was found by Sabo *et al.* (2015). It was also clear from the results presented in the same Table that the content of non-essential amino acids of bitter melon seeds treated in the traditional way TBMS, was significantly higher than that of the untreated ones. In general, the total essential and non-essential amino acids in the TBMS protein were higher than (UTBMS) being 64.41 and 26.54 (g/100 g protein), respectively. These results agreed with those of Abudayeh *et al.* (2016). Amino acids are the basic components of proteins and are involved in a variety of physiological activities. Essential amino acids are unable to be produced by the body and have to be received from the diet, whereas non-essential amino acids can be synthesised by the body. Therefore, the higher essential amino acid content of TBMS seeds may make them a more nutritionally valuable food source (FAO, 2007).

Table (5) Amino acid profile of untreated and treated bitter melon seeds.

Amino acids (g/100 g protein)	UTBMS	TBMS	*FAO/WHO/UNU (2007) pre-school.
Essential amino acids			
Tryptophan (TRP)	1.35±0.02^{b}	2.14±0.03^{a}	
Threonine (THR)	0.31±0.09^{b}	1.84±0.65^{a}	2.7
Valine (VAL)	1.11±0.05^{b}	3.52±1.75^{a}	4.2
****Methionine (MET)**	0.18±0.02^{b}	1.77±0.03^{a}	2.6
Isoleucine	1.03±0.41^{b}	4.08±0.31^{a}	3.1
Leucine (LEU)	0.81±0.05^{a}	0.64±0.09^{b}	6.3
*****Phenylalanine (PHE)**	0.43±0.01^{b}	4.12±0.01^{a}	4.6
Histidine (HIS)	0.73±0.02^{b}	3.38±0.02^{a}	
Lysine (LYS)	0.41±0.01^{b}	3.50±0.18^{a}	5.2
Non- Essential amino acids			
Aspartic acid(ASP)	0.80±010^{b}	6.47±0.13^{a}	
Serine (SER)	0.40±0.02^{b}	2.82±0.10^{a}	
Glutamic acid (GLU)	0.16±0.01^{b}	14.31±0.20^{a}	
Proline (PRO)	0.76±0.30^{b}	5.60±0.18^{a}	
Glycine (GLY)	0.54±0.01^{b}	5.27±0.01^{a}	
Alanine (ALA)	3.94±0.06^{a}	0.60±005^{b}	
****Cystine (CYS)**	0.23±0.30^{a}	1.40±0.06^{b}	
*****Tyrosine (TYR)**	0.11±0.03^{b}	1.72±1.14^{a}	
Arginine (Arg)	13.15±0.16^{a}	1.23±0.03^{b}	
Total	26.54	64.41	

- The results are expressed as mean±standard deviation of triplicate analysis.
- Mean values in a row having different superscript are significantly different at p≤ 0.05.
- UTBMS= Untreated bitter melon seeds.
- TBMS =treated bitter melon seeds.
- *FAO: FAO/WHO/UNU (2007) pre-school.
- ** Cytsine + Methionine.
- ***Tyrosine+ Phenylalanine.

Fatty acid composition of untreated and treated bitter melon seeds

The fatty acid composition of bitter melon seed oil (UTBMS) and TBMS are summarized in Table (6) The results showed that the most abundant fatty acid in UTBMS and TBMS was found to be linoleic acid (59.93% and 67.01%), respectively followed by oleic acid (13.05% and 12.55%), palmitic acid (19.43% and 11.01%), and stearic acid (7.59% and 9.43%). These results were found to be similar to those reported by Kapoor *et al*. (2021) for untreated *Citrullus colocynthis* seed oil containing palmitic acid (8.1–17.3%) and stearic acid (6.1–10.5%), as well as linoleic acid (50.6–60.1%) as the major monounsaturated fat. However, these results were not in close agreement with those of Sawaya *et al*. (1983) who recorded different proportions in UTBMS of linoleic acid (50.60%), oleic acid (25%), palmitic acid (13.50%) and stearic acid (10.50%). T discrepancy in our study may be attributed to differences in the methods of processing and extracting bitterness from seeds using traditional methods.

Table (6) Fatty acid composition of untreated and treated bitter melon seeds.

Fatty acid %	UTBMS	TBMS
Palmitic acid C16:0	19.43	11.01
Stearic acid C18:0	7.59	9.43
Oleic acid C18:1n-9	13.05	12.55
Linoleic Acid C18:2n-6	59.93	67.01
Total Saturate	27.02	20.44
Total unsaturated	72.98	79.56
Unsaturated/ Saturate	2.7 :1	3.9 :1

- UTBMS= Untreated bitter melon seeds.
- TBMS =treated bitter melon seeds.

Sensory evaluation of treated bitter melon seeds tahini partially replaced with sesame seed tahini.

Table (7) presents the sensory evaluation results for various tahini samples, including the control samples (tahini made entirely from sesame seeds) and tahini made entirely from treated bitter melon seeds (TBMS), and four samples containing different proportions of sesame seeds and (TBMS) tahini. The samples were evaluated based on appearance, colour, odour, taste, texture, and overall acceptance. Tahini made entirely from sesame seeds had higher rating scores in appearance, taste, texture, and overall acceptance compared to tahini made entirely from bitter melon seeds. As the percentage of TBMS tahini decreased and sesame seeds tahini increased, the sensory evaluation scores generally improved. On the other hand, according to our findings, the 20% sesame seeds tahini and 80% TBMS tahini sample had lower scores compared to the 80% sesame seeds tahini and 20% TBMS tahini sample which recorded the highest scores in all categories. But generally speaking, there were no significant differences among mixed tahini's 80%, 60%,40%, TBMS tahini and they were scored acceptable. As another fact, there were no significant differences when applying 40% and 20% TBMS tahini also there were no significant differences between them and whole sesame seeds tahini. Thus we can apply as much as 40% TBMS with sesame seeds tahini when preparing new healthy nutritional products with high acceptance.

Table (7). Sensory evaluation of treated bitter melon seeds tahini partially replaced with sesame seed tahini.

Samples	Appearance	Colour	Odour	Taste	Texture	Overall acceptance
A	7.32 ± 1.67^{ab}	7.36 ± 1.56^{ab}	7.41 ± 1.47^{a}	7.41 ± 1.76^{ab}	7.50 ± 1.37^{ab}	7.68 ± 1.52^{ab}
B	6.18 ± 1.40^{b}	6.32 ± 1.84^{b}	5.86 ± 1.67^{c}	5.90 ± 1.74^{c}	6.27 ± 1.70^{c}	6.36 ± 1.33^{c}
C	6.46 ± 1.47^{ab}	6.46 ± 1.63^{b}	6.18 ± 1.56^{bc}	6.23 ± 1.45^{bc}	6.41 ± 1.65^{bc}	6.68 ± 1.29^{bc}
D	6.87 ± 1.42^{ab}	7.05 ± 1.25^{ab}	6.77 ± 1.41^{abc}	6.64 ± 1.36^{abc}	7.00 ± 1.19^{abc}	6.82 ± 1.14^{bc}
E	7.32 ± 1.49^{ab}	7.46 ± 1.26^{ab}	7.32 ± 1.32^{ab}	7.27 ± 1.26^{ab}	7.46 ± 1.10^{ab}	7.59 ± 1.10^{ab}
F	7.46 ± 0.80^{a}	7.77 ± 1.07^{a}	7.91 ± 0.81^{a}	7.54 ± 0.80^{a}	7.86 ± 0.64^{a}	8.00 ± 0.76^{a}

- **The results are expressed as mean±standard deviation of triplicate analysis.**
- **Mean values in a column having different superscript are significantly different at $p\leq 0.05$.**
- **A =Sesame Seeds Tahini (control).**
- **B =TBMS Tahini.**
- **C =20% sesame seeds tahini: 80% TBMS.**
- **D =40% sesame seeds tahini: 60% TBMS.**
- **E =60% sesame seeds tahini: 40% TBMS.**
- **F =80% sesame seeds tahini: 20% TBMS.**

CONCLUSION

This study investigated the chemical and nutritional properties of bitter melon seeds using the Tabou tribe's traditional methods of treatment. The results showed that conventionally treated bitter melon seeds (TBMS) had higher levels of protein, fat, minerals, and micronutrients compared to untreated bitter melon seeds (UTBMS), and also contained a higher concentration of essential amino acids. However, the content of total phenolics and flavonoids, as well as antioxidant activity, was lower in TBMS compared to UTBMS. The study also found that tahini made entirely from sesame seeds had higher acceptability scores than tahini made entirely from TBMS, but there were no significant differences in sensory evaluation results between tahini with added 40% and 20% TBMS and 100% sesame. Therefore, it is possible to prepare healthy tahini with 60% sesame seeds and 40% TBMS.

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الملخص العربي

تقييم كيميائي وغذائي لبذور الحنظل واستخدامها في تحضير الطحينة

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صمم هذا العمل لدراسة الخصائص الكيميائية والغذائية لبذور الحنظل، حيث استخدمت الطريقة التقليدية المستخدمة لدى قبائل التبو في الجنوب الليبي وشمال تشاد والنيجر لإزالة المركبات المسؤولة عن المرارة ومقارنة هذه البذور غير المعاملة مع بذور الحنظل المعاملة بالطريقة التقليدية للاستفادة من تطبيقاتها في تكنولوجيا الأغذية. احتوي البذور المعاملة على مستويات عالية من البروتين الخام ، الزيت ، المعادن والعناصر الغذائية مقارنة بالبذور غير المعاملة وأظهرت البذور المعاملة تركيزًا أعلى من الأحماض الأمينية الأساسية، بما في ذلك التريبتوفان، الثريونين، الفالين، الميثيونين والإيزولوسين والفينيل ألانين، الهستيدين واللايسين، مقارنة بالبذور غير المعاملة .احتوت تركيبة الأحماض الدهنية لكل من البذور غير المعاملة و البذور المعاملة على مزيج من الأحماض الدهنية المشبعة وغير المشبعة، حيث يعد حمض اللينوليك هو الحمض الدهني الأكثر وفرة في كليهما. كما وجدت الدراسة أن كلا المستخلصين يحتويان على مركبات فينولية وفلافونويدات هامة. أظهرت نتائج التقييم الحسي أن الطحينة المصنعة بالكامل من بذور السمسم لديها درجات قبول أعلى مقارنة بالطحينة المصنوعة بالكامل من بذور الحنظل المعاملة الا أنه لم يظهر أي فروق معنوية بين كل من الطحينة التي تحتوي على 20، 40 % من البذور المعاملة وان كلاهما لا يوجد فروق معنوية بينهما وبين الطحينة المصنوعة من السمسم فقط. ولذا يمكن عمل طحينة صحية من60% سمسم و40% بذور الحنظل المعاملة.

الكلمات المفتاحية: بذور الحنظل، الأحماض الأمينية، الأحماض الدهنية، المركبات الفينولية، مضادات الأكسدة، المعادن، الطحينة والتقييم الحسي.