# PHYSIOCHEMICAL AND ANTIOXIDANT PROPERTIES OF FENUGREEK AND MORINGA OLEIFERA SEED OILS, A COMPARATIVE STUDY

# FEKRIA A. MOHAMED<sup>1</sup>, YOUSIF M. IDRIS<sup>1</sup>, MAHDI A. SAAD<sup>1</sup> AND ELFADIL E. BABIKER<sup>2\*</sup>

<sup>1</sup>Sudan University of Science and Technology, College of Agricultural Sciences, Khartoum North, Shambat, Sudan <sup>2</sup>Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Khartoum North, Shambat, Sudan \*Corresponding authors: <u>elfadilbabiker@yahoo.com</u> (Elfadil E. Babiker)

Abstract: The current study focused on the extraction, physicochemical and oxidative characterization of fenugreek and *Moringa* seeds oils. The *Moringa* seeds (43.21%) produced significantly (P  $\leq$  0.05) more oil than fenugreek (5.58%). Fenugreek oil had significantly (P  $\leq$ 0.05) higher iodine (g/100 g oil) and saponification (mg KOH/g oil) values than Moringa seed oil (98.35 and 174.78, respectively), while acid and peroxide values were comparable. Furthermore, the viscosity of *Moringa* oil (71.55 Pa·s) was significantly ( $P \le 0.05$ ) higher than that of fenugreek oil (63.18 Pa·s), while other physical properties such as refractive index and density were comparable. Moringa seed oil had significantly ( $P \le 0.05$ ) higher total phenols (39.17 mg GAE/g), total flavonoids (17.24 mg CE/g), and antioxidant activity (37.56%) than fenugreek oil. The phenolic components in both oils were examined using gas chromatography coupled with mass spectrometry (GC-MS). In fenugreek seed oil, 20 chemical components were presumptively identified, such as 9, 12-octadecadienoic acid (Z, Z)-, methyl ester (41.23%), and 9-octadecenoic acid (Z), methyl ester (23.28%). While there were 21 phenolic compounds in Moringa seed oil, the predominant components included 9-octadecenoic acid (Z)-, methyl ester (41.21%) and docosanoic acid, methyl ester (18.45%). The Moringa seed oil outperformed fenugreek seed oil in terms of physico-chemical and oxidative qualities.

Keywords: Fenugreek, Moringa oleifera, seed oil, physiochemical, antioxidants

# Introduction

In Sudan, as in many developing countries, medicinal plants have played an important role in the treatment of diseases and the preservation of food, particularly in rural areas. Herbal plants contain natural antioxidants, which have been used in food since ancient times to improve flavor and organoleptic properties (Srinivasan, 2014). The most often used synthetic antioxidants in food are butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Both are very powerful antioxidants, but their use in high concentrations in food products had a negative effect (Yang et al., 2018). As a result, there is renewed interest in the investigation of natural additives as potential antioxidants. Antioxidants are chemicals that considerably delay or prevent oxidation when present in low quantities in comparison to oxidizable substrates. Antioxidants are added to foods to prevent or delay oxidation, which is caused by free radicals created as a result of exposure to environmental variables such as air, light, and temperature, and to avoid the detrimental influence on food quality (Lorenzo et al., 2018). The majority of antioxidant compounds obtained from plants have a wide range of chemical properties. The ability to scavenge free radicals supports the antioxidant function. Through the chain reaction, free radicals created by oxidation responses can initiate tie responses that lead to more oxidation. Antioxidants terminate these chain reactions by oxidizing free extreme intermediates and inhibiting further oxidation processes (Saha and Tamrakar, 2011).

Fenugreek, (*Trigonella-Foenum Graecum*), is a legume in the *Leguminosae* family that is considered to be one of the most traditional and promising therapeutic plants. Aside from its therapeutic use, it is also utilized as a food stabilizer, glue, and emulsifying agent in the

manufacture of numerous food products (Syed et al., 2020). Previous research has revealed that fenugreek can be introduced into our regular diet due to its nutritional and health benefits (Wani and Kumar, 2018). The consumption of fenugreek seed in various forms is healthy because of its high content of antioxidants and polyphenolic components such as nicotinic acid, alkaloids, salicylate, and flavonoids (Naidu et al., 2011). When fenugreek seed oil was tested to codex standards, it was revealed that with further purifications, it might be considered edible oil (Munshi et al., 2020). According to Wang et al. (2000) study, conserving fenugreek seed oil is considerably easier than maintaining other oils due to the presence of antioxidant activity, and fenugreek seed oil displayed substantially higher stability and decreased auto-oxidation. In addition to its nutritional benefits, fenugreek seed oil has antibacterial capabilities that are of major significance. The principal elements of fenugreek seed oil, notably linoleic acid, palmitic acid, pinene, and other minor components, were discovered to be particularly beneficial in decreasing free radicals due to their inherent antioxidant qualities (Akbari et al., 2019). According to AL Juhaimi et al. (2018), fenugreek is classed as a drying oil since it includes a high percentage of linolenic acid (16.99-25.66%).

The most common *Moringaceae* species is *Moringa oleifera*, a tiny deciduous tree. In India, Ethiopia, the Philippines, and Sudan, *Moringa oleifera* is a prominent crop. *M. oleifera* is a valuable food product that has gained prominence as the "natural nutrition of the tropics" (Anwar and Rashid 2007). Because of the presence of significant quantities of polyphones, *Moringa oleifera* is also well-known for its antioxidant activity (Fahey, 2005). *M. oleifera* produced a significant yield of oil with strong antioxidant capacity and promise for industrial, nutritional, and health applications (Ogbunugafor et al., 2011). Furthermore, *Moringa oleifera* has been identified as a potential new oil source, particularly given the global demand for oleo-chemicals and oil/fat-

derived fuels (Salaheldeen et al., 2014). Other applications include shortening production and hydrogenation (Salaheldeen et al., 2015). *Moringa oleifera* oil may increase the oxidative stability of commercial food oils (Anwar et al., 2010). The purpose of this study is to extract fenugreek and *Moringa* seed oil and investigate its physicochemical properties, antioxidant content, and activity as future potential preservatives for other oils and food products.

## Materials and Methods

#### **Materials**

Fenugreek and *Moringa* seeds were purchased in Sudan at a local market. All chemicals used in this study were reagent grade.

#### **Oil** extraction

*Moringa oleifera* and *Trigonella-Foenum Graecum* seeds were used to extract oil; full seeds were crushed with a mortar and pestle. Using the Soxhlet extraction method, oil was extracted from powdered seeds for 16 hours using 0.5 L of n-hexane (95%). The extraction solvent (n-hexane) was evaporated over a water bath for 12 hours or until it was gone.

# Determination of acid value

Following the AOAC (2011) method, 1 g of oil was weighed in a clean conical flask and dissolved in 50 ml of neutralized ethanol before the mixture was titrated against 0.1N potassium hydroxide solutions. Three drops of the phenolphthalein indicator were also added. Under the same circumstances, a blank titration was also carried out, but without the oil. The following equation was used to compute the acid value:

Acid value = 
$$\frac{(V1-V2) \times 0.1 \times 56.1}{\text{Weight of sample}}$$

V1 = volume of KOH used in the titration of oil, V2 = volume of KOH used to titrate the blank *Determination of peroxide value* 

In accordance with the AOAC (2011) protocol, 25 mL of the solvent mixer which consists of 15 mL glacial acetic acid and 10 mL chloroform was added to 2 g of oil in a 250 mL conical flask. Next, 1 mL of saturated potassium iodide (KI) was added, and the flask was then left in a dark area for 10 minutes. 0.1 M sodium thiosulfate was used to titrate the mixer after adding 1 mL of a starch indicator. With a blank, a similar experiment was conducted.

Peroxide value =  $\frac{(A-B) \times 0.1 \times 1000}{\text{Weight of sample}}$ 

A = volume of 0.1 M sodium thiosulfate solution used in sample titration, B = volume of 0.1 M sodium thiosulfate solution used in sample titration

### Determination of Iodine value

Using the AOAC (2011) method, 1g of oil, 10 mL of carbon tetrachloride, and 20 mL of Wij's solution were weighed into a 250 mL conical flask. After closing the flask and leaving it at room temperature for 30 minutes in the dark, it was filled with 100 mL of distilled water and 15 mL of 10% potassium iodide solution. This was titrated against a 0.1 M sodium thiosulfate solution using starch as an indicator. Similar conditions were used for a blank titration, which was conducted without any oil.

Iodine value = 
$$\frac{(A-B) \times 0.1 \times 12.69}{\text{Weight of sample}}$$

A and B are volumes of 0.1N sodium thiosulfate solutions that were employed for the sample and blank titrations, respectively. 12.69 was a conversion factor.

# Determination of saponification value

The Pearson et al. (1970) method was used to determine the saponification value. About 1g of oil and 25 ml of a 0.5 M ethanolic potassium hydroxide solution were weighed and placed in a 200 ml conical flask. Before allowing the contents to cool, the flask's condensing configuration was set up, and it was heated in a water bath for one hour while being frequently shaken. The solution

was titrated using a 1% phenolphthalein indicator and 0.5 M hydrochloric acid. The corresponding titration for the blank was carried out, and the saponification value was determined using the values that were produced.

Saponification value = 
$$\frac{(A - B) \times 28.05}{\text{Weight of sample}}$$

A and B are volumes of 0.5 M hydrochloric acid employed for the sample and blank titrations, respectively. 28.05 was a conversion factor.

# Density of oil

The specific density was measured in a density bottle at a constant temperature (25 °C) in a water bath according to the AOAC method (2011).

# Oil refractive index

The oil refractive index was measured according to the AOAC method (2011). The prism of Abbe's refractometer was properly cleaned, and two drops of oil were added to it. The knobs on the instrument were set, and the oil was defined by a crisp line splitting the field of view into two halves, which was conceded with the spot marks "X" in the field of view. At this point, the temperature was 25 degrees Celsius, which is considered average.

# Oil viscosity

The oil viscosity was measured using a U-shape viscometer (Bioevopeak Co., Ltd., China, Jinan). The viscometer was filled with water, and the time it took for the water to move down was measured. The viscometer was then emptied of water, dried, and refilled with oil. The time it took for the oil to tick down was then measured, and the viscosity was calculated using the equation below.

$$Viscosity (Pa s.) = \frac{Time (s) of oil to pass}{Time (s) of water to pass}$$

# **Total Phenolics content**

By using the Folin-Ciocalteu reagent and external calibration with gallic acid (0-200 mg/ml), the amount of total phenolic in the oil was quantified. Briefly, 0.2 mL of the oil and 0.2 mL of the Folin-Ciocalteu reagent were added and the mixture was strongly stirred. Thereafter, 1 mL of 15% Na<sub>2</sub>CO<sub>3</sub> was added after 4 minutes of shaking, and the mixture was then left to stand for 2 hours at room temperature. Using an HITACHI, U-2000 spectrophotometer, the absorbance was measured at 750 nm. Three measurements were made. The amount of total phenolics in every 100 grams was expressed as milligrams of gallic acid equivalent (GAE).

# Total flavonoid content

According to Kim et al. (2003), a colorimetric approach to assess the total flavonoid concentration was used. About 1 mL of oil was combined with 0.3 mL of 10% aluminum chloride, 2 mL of 1M sodium hydroxide, and 4 mL of distilled water. With pure water, the reaction amount was instantly increased to 10 ml. After 15 minutes at room temperature, the absorbance at 510 nm was measured. A calibration curve for catechin was produced using the equation Y = 0.004X - 0.0044 with R2 = 0.9553, and the result was expressed as mg of catechin equivalent (mg CE/g of the oil).

# Oil DPPH radical scavenging assay

According to Lee et al. (1998), the DPPH radical scavenging technique was applied. Following methanol dilution, the DPPH reagent (2 mL) and 1 mL of each extract were thoroughly mixed in a vortex mixer. A control test was made of methanol. The absorption of mixtures was measured using a spectrophotometer at 518 nm. The DPPH was calculated using the algorithm shown below:

$$DPPH (\%) = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100$$

Determination of bioactive compounds of oil

According to the instruction manual, 2 mL of sample was well blended with 7 mL of alcoholic sodium hydroxide, which was made by combining 2 g with 100 mL of methanol. It was then followed by the addition of 7 mL of alcoholic sulfuric acid (1 mL  $H_2SO_4$  to 100 mL methanol). The mixture was then shaken for a further five minutes. The contents of the test tube were allowed to stand overnight. The solution was then agitated after adding 1 ml of supersaturated sodium chloride (NaCl). After adding 2 mL of hexane, the mixture was briskly agitated for three minutes. Then, using a disposable syringe, the n-hexane layer (the test tube's top layer) was removed. A volume of 5 mL of n-hexane extract was combined with 5 mL of diethyl ether. Before being injected into the GC/MS instrument, the mixture was filtered through a 0.45 m syringe filter and dried with 1g of anhydrous sodium sulfate as a drying agent. A GM/MS technique model (GC/MS-QP2010-Ultra) from Japan's 'Shimadzu Company, with serial number 020525101565SA and a capillary column (Rtx-5ms-30m0.25 mm0.25m) was used. The sample was injected in split mode with helium as the carrier gas passing at a flow rate of 1.61 mL/min, and the temperature program was initiated from 50 degrees Celsius at a rate of 10 degrees Celsius per minute to 300 degrees Celsius as the final temperature degree with a hold time of 5 minutes. The injection port temperature was 300 degrees Celsius, the ion source temperature was 200 degrees Celsius, and the interface temperature was 250 degrees Celsius. With a total run period of 30 minutes, the material was studied in scan mode in the m/z 40-500 charges to ratio range. The compounds were presumptively identified based on their retention time and the data stipulated in the scientific literature, and also depending on their mass fragmentation patterns which were compared to those from the National Institute of Standards and Technology (NIST) library.

## **Statistical analysis**

Analyses were performed in triplicate for each sample. The results are shown as Mean  $\pm$  SD. At a level of P  $\leq$  0.05, Duncan's Multiple Range Test results with a statistically significant difference were accepted. All data were analyzed with the Statistical Package for the Social Sciences 16.0 for Windows.

#### **Results and discussion**

# Oil yield and physicochemical properties

In this work, the physicochemical and antioxidant characteristics of the oil extracted from Moringa oleifera seeds were compared to those of fenugreek oil. As shown in Table 1, M. oleifera seeds (43.21%) produced significantly higher ( $P \le 0.05$ ) oil than fenugreek seeds (5.58%). Moringa oil was brilliant light yellow, fluid at room temperature, and had a pleasant flavor, whereas fenugreek oil was yellowish. In terms of oil yield, the present result showed that *Moringa* seed grown in Sudan yielded high oil compared to that collected from Nigeria which gave oil yield of 41.47% and 38% as shown by Ogbunugafor et al. (2011) and Adegbe et al. (2016), respectively and that grown in India gave yield range from 32 to 40% (Lalas and Tsaknis, 2002). The Fenugreek seed oil yield shown in the current investigation was higher than that shown by Yang et al. (2012) who obtained 4.08% oil yield from fenugreek seed, and Akbari et al. (2019) obtained 5.55% oil yield from the seed, and Gu et al. (2017a) reported 3.80%. Generally, the variation in oil yield depends on several factors including location and extraction conditions (temperature and time) as reported by Akbari et al. (2019) and Yang et al. (2012). Temperature, extraction time, particle size, and liquid/solid ratio have all been reported to influence oil yield.

Table 1. Oil yield and physico-chemical properties of Fenugreek and Moringa seeds oils

Parameters	Source of oil		
T arameters	Fenugreek	Moringa	
Oil yield (%)	5.58±0.71 <sup>b</sup>	43.21±1.26 <sup>a</sup>	
Acid value (mg KOH/gm oil)	$5.15 \pm 0.42^{a}$	$4.85\pm0.23^{a}$	
Free fatty acid value (as % oleic acid)	$2.41{\pm}0.37^{a}$	$2.39{\pm}0.51^a$	
Peroxide value (meq O <sub>2</sub> /Kg oil)	3.58±0.021ª	$2.61 \pm 0.41^{b}$	
Saponification value (mg KOH/g oil)	186.28±0.59 <sup>a</sup>	174.78±0.72 <sup>b</sup>	
Iodine value (g/100 g oil)	103.62±0.82 <sup>a</sup>	98.35±0.72 <sup>b</sup>	
Viscosity (Pa.s) at 25°C	63.18±0.23 <sup>b</sup>	71.55±0.53 <sup>a</sup>	
Refractive index at 37°C	1.468±0.31ª	1.468±0.44 <sup>a</sup>	
Specific density at 25°C	0.918±0.28 <sup>a</sup>	0.921±0.12 <sup>a</sup>	

Values are means of triplicate samples ( $\pm$  SD). Means not sharing a common superscript (s) a, b, or c in a row are significantly different at  $p \le 0.05$  as assessed by Duncan's Multiple Range Test.

The oil yield increased continuously as the temperature rose; however, it remained constant as the temperature increased further. The oil yield increased as the extraction time increased, peaking at 30 minutes before declining further as the extraction time increased. Similar patterns were discovered in other oil extraction processes (Gu et al., 2017b). The oil yield grew considerably as the particle size of the material reduced from 0.9 to 0.3 mm, but it stayed constant below 0.3 mm. The results can be explained by mass transfer resistances (Rai et al., 2016). Oil yield increased significantly when the liquid/solid ratio was less than 30:1. The effect was not significant when the liquid/solid ratio was greater than 30:1. The increase in the contact area between the material powder and the butane fluid would explain these observations (Gu et al., 2017b).

*Moringa oleifera* seed oil had acid, peroxide, saponification, free fatty acid, and iodine values of 4.85 mg KOH/kg oil, 2.61meq O2/kg oil, 174.78 mg KOH/kg oil, 2.39 as% oleic acid, and 98.35

gm/100 gm oil, respectively. At room temperature, the refractive index, viscosity, and specific density of the seed oil were 1.468, 71.55 mPa.s, and 0.921, respectively. Fenugreek seed oil, on the other hand, had acid, peroxide, saponification, free fatty acid, and iodine values of 5.15 mg KOH/kg oil, 3.58 meq O2/kg oil, 186.28 mg KOH/kg oil, 2.41 as% oleic acid, and 103.62 gm/100 gm oil, respectively. At ambient temperature, the refractive index, viscosity, and specific density of Moringa oleifera seed oil were 1.468, 63.18 mPa.s, and 0.918, respectively. As shown in Table 1, the physicochemical properties of the oils revealed a significant ( $P \le 0.05$ ) difference in saponification, peroxide, iodine, and viscosity between M. oleifera seed oil and fenugreek oil. The viscosity of M. oleifera oil was higher, while the saponification, peroxide, and iodine values of fenugreek oil were higher. Their acid value, free fatty acid value, refractive index, and specific density, however, did not differ significantly. The physicochemical properties of M. oleifera seed oil agree with those shown by Fu et al. (2021), Lalas and Tsaknis (2002), and Ogbunugafor et al. (2011). Munshi, et al. (2020) reported similar values for fenugreek seed oil.

Fenugreek and Moringa seed oil acid levels were within the FAO/WHO (2009) standards for edible oils. The oil's iodine value shows the degree of unsaturation. A higher iodine value suggests more unsaturated fats and oils. The iodine value of M. Oleifera and fenugreek oil, on the other hand, was higher than the FAO/WHO (2009) criterion for edible oil, indicating that the bulk of its fatty acids is unsaturated. Peroxide levels were lower than the FAO/WHO (2009) limit but greater than Adegbe et al (2016) reported. Because of lower levels of oxidative and lipolytic activities, the lower peroxide value shown in our study boosts the oil's appropriateness for lengthy storage. The saponification value of the oils demonstrates compliance with the FAO/WHO (2009) standard. Oils, on the other hand, have free fatty acids that are within the FAO/WHO (2009) range. Because of the large percentage of fatty acids, soap is formed during the decomposition of alcohol from its

derivatives. Furthermore, the physical qualities of the oil produced from M. Oleifera seeds met the FAO/WHO (2009) criterion.

# Oxidative characteristics of the extracted oil

Figure 1 depicts the total phenolic, flavonoid, and antioxidant activities of fenugreek and *Moringa* seed oil. The total phenolic, flavonoid, and antioxidant activity of *M. oleifera* oil and fenugreek oil revealed a significant ( $P \le 0.05$ ) difference, with *M. oleifera* oil having higher values. The phenolic and flavonoid contents of fenugreek seed oil obtained in this investigation are consistent with those reported by Akbari et al. (2019), while those of *Moringa* are consistent with those reported by Ogbunugafor et al. (2011). Phenolic substances are a form of antioxidant



Figure 1. Oxidative characteristics of Fenugreek and Moringa seeds oils.

that acts as a free radical terminator while also slowing lipid oxidation (Souri et al., 2022). *Moringa* oil contains more phenols than fenugreek oil, which is evident in its vivid bright color, which is contributed by phenols, which are believed to be responsible for many plant colors (Bose et al., 2018). Furthermore, Viol et al. (2013) discovered a robust link between phenolic content and antioxidant activity in a wide range of fruits and vegetables. As a result, the presence of phenolic

compounds in *Moringa* seed oil enhances its nutritional and physiological advantages. Furthermore, the inclusion of flavonoids, which are also phenolic chemicals, in *Moringa* oil boosts the oil's economic and health potential. This is consistent with recent results that flavonoids, which have been associated with a lower risk of cancer and heart disease (Perez-Vizcaino and Fraga, 2018), execute antioxidant action through scavenging or chelating mechanisms. As a result, Moringa oil's antioxidant contents are becoming increasingly significant in health maintenance, as the future trend is toward employing foods as a potential therapeutical agent in the management of many chronic diseases. The antioxidant ability of *Moringa* oil was further shown by the quenching of DPPH free radicals, a proton free radical that is frequently used to assess antioxidants' capacity to scavenge free radicals (Adesegun et al., 2008). Additionally, the ability of a chemical to transfer electrons influences its reducing power, which may result in the neutralization of free radicals (Anissi et al., 2014). The reducing power of the oils was shown to grow concentration-dependently, indicating that they are effective electron donors. According to several other studies, the reduced power capacity of a chemical can be a reliable predictor of its potential antioxidant activity (Sofidiya et al., 2006, Adesegun et al., 2008). Plant antioxidant activity was found to be favorably associated with phenolic and flavonoid content (Noreen et al., 2017). The current investigation supported this idea by discovering that Moringa oil with a greater phenolic content has more antioxidant components and activity than fenugreek.

## GC-MS characterization of fenugreek and Moringa seed oil

The extracted samples were characterized through a GC-MS analysis. According to the scientific literature and their retention time (min), a total number of 20 chemical components were presumptively identified in the fenugreek oil (Table 2, Figure 2) while in the *Moringa* oil, 21 compounds were detected (Table 3, Figure 3). Also, a percentual quantification was achieved

based on their area percentage. The main chemical components of fenugreek seed oil were identified as 9, 12-octadecadienoic acid (Z, Z)-methyl ester with a common name linoleic acid (41.23%), 9-octadecenoic acid (Z)-methyl ester with a common name oleic acid (23.28%), methyl stearate (16.79%) is a fatty acid methyl ester and docosanoic acid, methyl ester with a common name behenic acid (5.23%). Furthermore, the Moringa seed oil's major compounds were 9octadecenoic acid (Z)-methyl ester which is a synonym for oleic acid (41.21%), docosanoic acid, methyl ester with a common name behenic acid (18.45%), the methyl ester of eicosanoic acid, commonly known as arachidic acid (11.93%) and methyl stearate (9.43%). Several variations were observed between fenugreek and Moringa in terms of chemical components. According to the data obtained, fenugreek was rich in linoleic acid followed by oleic acid while Moringa was rich in oleic acid followed by behenic acid. In nature, linoleic acid typically exists as a triglyceride rather than a free fatty acid. It is a fatty acid that contains two types of cis-alkenes. Since these types of compounds induce an antibacterial effect, longer-chain unsaturated fatty acids, such as linoleic acid, are the primary ingredients of natural antimicrobial foods and several antibacterial herbs (Zheng et al., 2005). The bioactive oxidized linoleic acid metabolites have linoleic acid as a direct precursor. It is also a precursor to arachidonic acid, which creates endocannabinoids and eicosanoids that promote inflammation (Choque et al., 2014). Oleic acid, a mono-saturated omega-9 fatty acid, has various health advantages and it is rather safe to be used in cosmetics in safe quantities (Burnett et al., 2017). According to De Silver et al. (2014), oleic acid decreases blood pressure, boosts fat burning, protects cells from free radical damage, and prevents ulcerative colitis (Lim et al., 2013). Moreover, behenic acid (22:0), a saturated, extremely long-chain fatty acid, has been discovered to be a natural inhibitor of pancreatic lipase (Kojima et al., 2010). According to Özcan (2020), behenic acid was discovered in the seeds of *M. oleifera* and is used to provide

smoothing properties to hair conditioners and moisturizers. It is also well-known as an antifoaming agent in the production of detergents.

SN	Name	RT	Area	Area%
1	Hexadecanoic acid (Palmitic acid)	15.090	9996750	4.63
2	9,12-Octadecadienoic acid (Linoleic acid)	16.848	89056315	41.23
3	9-Octadecenoic acid (Oleic acid)	16.893	50292767	23.28
4	Methyl stearate (Methyl octadecanoate)	17.022	36266521	16.79
5	11,14-Eicosadienoic acid	18.383	927118	0.43
6	cis-11-Eicosenoic acid (Gondoic acid)	18.524	2685917	1.24
7	Eicosanoic acid (Arachidic acid)	18.729	4647959	2.15
8	8,11,14-Docosatrienoic acid, methyl ester	18.938	610269	0.28
9	Methyl (11R,12R,13S)-(Z)-12,13-epoxy-11-	19.059	286338	0.13
	methoxy-9-octadecenoate			
10	Naphthalene, decahydro-2,6-dimethyl ( $C_{12}H_{22}$ )	19.256	741574	0.34
11	Docosanoic acid (Behenic acid)	20.344	11295530	5.23
12	Tricosanoic acid, methyl ester	21.107	473556	0.22
13	Tetracosanoic acid (Lignoceric acid)	21.841	4445608	2.06
14	Squalene (C30H50)	22.558	305135	0.14
15	Tetratetracontane (n-Tetratetracontane)	22.976	101680	0.05
16	Hexacosanoic acid, methyl ester	23.241	128357	0.06
17	Vitamin E	24.791	126106	0.06
18	Stigmasterol	25.991	112277	0.05
19	β-sitosterol	26.553	1067474	0.49
20	9, 19-Cyclolanostan-3-ol, 24-methylene-,	28.123	2457819	1.14
	(3.beta.)-			

Table 2. GC-MS	analysis of	chemical com	ponents of	fenugreek oil.
10010 21 00 1110	unuryono or	enemieur com	ponento or	i tomagi oon on.

SN = Serial number, RT = Retention time



Figure 2. GC-MS separation profile of the fenugreek seed oil

SN	Name	RT	Area	Area%
1	9-Hexadecenoic acid, methyl ester, (Z)-	14.871	1214122	0.67
2	Hexadecanoic acid, (Palmitic acid)	15.079	6231374	3.43
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.725	307882	0.17
4	9-Octadecenoic acid (Z)-, (Oleic acid)	16.859	74917268	41.21
5	Octadecanoic acid, methyl ester (Methyl	17.006	17124755	9.43
	stearate)			
6	10-Nonadecenoic acid, methyl ester	17.664	105803	0.06
7	Octadecanoic acid, 17-methyl-, methyl ester	17.880	34498	0.02
8	9,12,15-Octadecatrienoic acid, methyl ester	18.030	1641198	0.90
9	cis-11-Eicosenoic acid, methyl ester	18.531	14284838	7.86
10	Eicosanoic acid (Arachidic acid)	18.738	21666828	11.93
11	Heneicosanoic acid, methyl ester	19.561	176565	0.10
12	13 ((Z)-Docosenoic acid, methyl ester	20.167	466171	0.26
13	Docosanoic acid, (Behenic acid)	20.365	33520513	18.45
14	Tricosanoic acid, methyl ester	21.115	255171	0.14

Table 3. GC-MS analysis of chemical components of Moringa seed oil

15	Tetracosanoic acid (Lignoceric acid)	21.846	8081359	4.45
16	Pentacosanoic acid, methyl ester	22.561	89802	0.05
17	Hexacosanoic acid, methyl ester	23.247	264772	0.15
18	Campesterol	25.721	94951	0.05
19	Stigmasterol	25.993	341487	0.19
20	β-Sitosterol	26.553	775569	0.43
21	Fucosterol	26.732	85079	0.05

SN = Serial number, RT = Retention time



Figure 3. GC-MS separation profile of Moringa seed oil

# Conclusions

Despite differences between fenugreek and Moring oil, the physiochemical properties of both oils are equivalent to those of other edible oils, implying that oil from fenugreek and M. Oleifera seeds could be utilized to enhance other meals. Moringa was also discovered to have more antioxidant chemicals and activity than fenugreek. The oil is high in omega-6 essential fatty acids (linoleic

acid) according to GC-MS analysis of fenugreek and Moringa seed oil. Furthermore, the primary components of the oil include oleic acid and behenic acid. Based on the findings, it is possible that fenugreek and Moringa seed oil could be useful natural additives to the food industry as potential antioxidants.

## References

- Adegbe A.A., Larayetan R.A. and Omojuwa T.J. (2016) Proximate analysis, physicochemical properties and chemical constituents' characterization of *Moringa oleifera* (Moringaceae) seed oil using GC-MS analysis. American Journal of Chemistry, 6(2), 23-28. https://doi.org/10.5923/j.chemistry.20160602.01
- Adesegun S.A., Elechi N.A. and Coker H.A. (2008) Antioxidant activities of methanolic extract of Sapium ellipticum. Pakistan Journal of Biological Science, 11(3), 453-457. https://doi.org/10.3923/pjbs.2008.453.457
- Akbari S., Abdurahman H.N., Yunus M.R., Alara R.O. and Abayomi, O.O. (2019) Extraction, characterization and antioxidant activity of fenugreek (*Trigonella-Foenum Graecum*) seed oil. Materials Science for Energy Technologies, 2(2), 349–355. <u>https://doi.org/10.1016/j.mset.2018.12.001</u>
- 4. AL Juhaimi F., Şimşek Ş., Babiker E.E., Ghafoor K. and Özcan M.M. (2018) Effect of location on chemical properties, amino acid and fatty acid compositions of fenugreek (Trigonella foenum-graecum L.) seed and oils. J. Food Processing and Preservation, 2018, e13569. https://doi.org/10.1111/jfpp.13569
- Anissi J., El Hassouni M., Ouardaoui A. and Sendide K. (2014) A comparative study of the antioxidant scavenging activity of green tea, black tea and coffee extracts: A kinetic approach. Food Chemistry, 150, 438-447. <u>https://doi.org/10.1016/j.foodchem.2013.11.009</u>
- Anwar F., Qayyum H.M.A., Hussain A.I. and Iqbal S. (2010) Antioxidant activity of 100 and 80% methanol extracts from barley seeds (*Hordeum vulgare* L.): stabilization of sunflower oil. Grasas Y Aceites,, 61, 237–243. <u>https://doi.org/10.3989/gya.087409</u>

- 7. Anwar F. and Rashid, U. (2007) Physico-chemical characteristics of Moringa oleifera seeds and seed oil from a wild provenance of Pakistan. Pakistan Journal of Botany, 39, 1443-1453.
- AOAC (2011) Official Method of Analysis. 18<sup>th</sup> Edn. Association of Official Agricultural Chemistrys Official Methods of Analysis, Washington, DC.
- Bose S., Sarkar D., Bose A. and Mandal S.C. (2018) Natural flavonoids and its pharmaceutical importance. The Pharma Review, 94, 61-75.
- Burnett C.L., Fiume M.M., Bergfeld W.F., Belsito D.V., Hill R.A. Klaassen C.D. and Alan Andersen F. (2017) Safety assessment of plant-derived fatty acid oils. International Journal of Toxicology, 36, 51S-129S. <u>https://doi.org/10.1177/1091581817740569</u>
- Choque B., Catheline D., Rioux V. and Legrand P. (2014) Linoleic acid: between doubts and certainties. Biochimie, 96, 14-21. <u>https://doi.org/10.1016/j.biochi.2013.07.012</u>
- De Silver P.S., Luben R., Shrestha S.S., Khaw K.T. and Hart A.R. (2014) Dietary arachidonic and oleic acid intake in ulcerative colitis etiology: A prospective cohort study using 7-days food diaries. European Journal of Gastroenterology & Hepatology, 26(1): 11-8. <u>https://doi.org/10.1097/MEG.0b013e328365c372</u>
- Fahey J. W. (2005) *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for Life Journal, 1, 1-33. <u>http://www.tfljournal.org/article.php/20051201124931586</u>
- FAO/WHO (2009) Report on the 21st session of the Codex Alimentarius Committee on fats and oils. Kola Kinabalu, Malaysia.
- 15. Fu X., Su J., Hou L., Zhu P., Hou Y., Zhang K. and Xu J. (2021) Physicochemical and thermal characteristics of Moringa oleifera seed oil. Advanced Composites and Hybrid Materials, 4, 685-695. <u>https://doi.org/10.1007/s42114-021-00302-4</u>
- Gu L.B., Liu X.N., Liu H.M., Pang H.L. and Qin G.Y. (2017a) Extraction of fenugreek (Trigonella foenum-graceum L.) seed oil using subcritical butane: Characterization and process optimization. Molecules, 22(2), 228. <u>https://doi.org/10.3390/molecules22020228</u>

- 17. Gu L.B., Pang H.L., Lu K.K., Liu H.M., Wang X.D. and Qin G.Y. (2017b) Process optimization and characterization of fragrant oil from red pepper (*Capsicum annuum* L.) seed extracted by subcritical butane extraction. Journal of the Science of Food and Agriculture, 97(6), 1894-1903. https://doi.org/10.1002/jsfa.7992
- Kim D.O., Chun O.K., Kim Y.J., Moon H.Y. and Lee C.Y. (2003) Quantification of polyphenolics and their antioxidant capacity in fresh plums. Journal of Agricultural and Food Chemistry, 51(22), 6509-6515. https://doi.org/10.1021/jf0343074
- Kojima M., Tachibana N., Yamahira T., Seino S., Izumisawa A., Sagi N. and Ikeda I. (2010) Structured triacylglycerol containing behenic and oleic acids suppresses triacylglycerol absorption and prevents obesity in rats. Lipids in Health and Disease, 9(1): 1-6. <u>https://doi.org/10.1186/1476-511X-9-77</u>
- Lalas S. and Tsaknis J. (2002) Characterization of *Moringa oleifera* seed oil variety "Periyakulam 1". Journal of Food Composition and Analysis, 15(1), 65-77. <u>https://doi.org/10.1006/jfca.2001.1042</u>
- Lee S.K., Mbwambo Z.H., Chung H., Luyengi L., Gamez E.J., Mehta R.G. and Pezzuto J. M. (1998) Evaluation of the antioxidant potential of natural products. Combination Chemistry High Throughput Screen, 1(1), 35-46.
- Lim J.H., Gerhart-Hines Z., Dominy J. E., Lee Y., Kim S., Tabata M. and Puigserver P. (2013) Oleic acid stimulates complete oxidation of fatty acids through protein kinase A-dependent activation of SIRT1-PGC1α complex. Journal of Biological Chemistry, 288(10): 7117-7126. https://doi.org/10.1074/jbc.M112.415729
- 23. Lorenzo J.M., Munekata P.E.S., Sant'Ana A.S., Baptista R.C., Barba F.J., Toldrá F., Mora L. and Trindade M.A. (2018) Main characteristics of peanut skin and its role for the preservation of meat products. Trends in Food Science and Technology, 77, 1–10. <u>https://doi.org/10.1016/j.tifs.2018.04.007</u>
- Munshi M., Arya P. and Kumar P. (2020) Physico-Chemical analysis and fatty acid profiling of fenugreek (*Trigonella foenum-graecum*) seed oil using different solvents. Journal of Oleo Science, 69(11), 1349-1358. <u>https://doi.org/10.5650/jos.ess20137</u>

- 25. Naidu M.M., Shyamala B.N., Naik J.P., Sulochanamma G. and Srinivas P. (2011) Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds. LWT- Food Science and Technology, 44, 451-456. <u>https://doi.org/10.1016/j.lwt.2010.08.013</u>
- Ogbunugafor H.A., Eneh F.U., Ozumba A.N., Igwo-Ezikpe M.N., Okpuzor J., Igwilo I.O., Adenekan S.O. and Onyekwelu O.A. (2011) Physico-chemical and Antioxidant Properties of Moringa Oleifera Seed Oil. Pakistan Journal of Nutrition, 10 (5), 409-414.
- Özcan M.M. (2020) Moringa spp: Composition and bioactive properties. South African Journal of Botany, 129, 25-31. <u>https://doi.org/10.1016/j.sajb.2018.11.017</u>
- Pearson D., Surry J. and Chuchill A. (1970) "The Chemical Analysis of Food National College of Food Technology. "University of Reading, Wey bridge Surry T and of A Churchill.
- Perez-Vizcaino F. and Fraga, C.G. (2018) Research trends in flavonoids and health. Archives of Biochemistry and Biophysics, 646, 107-112. <u>https://doi.org/10.1016/j.abb.2018.03.022</u>
- 30. Rai A., Mohanty B. and Bhargava R. (2016) Supercritical extraction of sunflower oil: A central composite design for extraction variables. Food chemistry, 192, 647-659. https://doi.org/10.1016/j.foodchem.2015.07.070
- 31. Saha D. and Tamrakar A. (2011) Xenobiotics, oxidative stress, free radicals vs. antioxidants: dance of death to heaven's life. Asian Journal of Research in Pharmaceutical Science, 1, 36.
- 32. Salaheldeen M., Aroua M.K., Mariod A.A., Cheng S.F. and Abdelrahman M.A. (2014) An evaluation of Moringa peregrina seeds as a source for bio-fuel. Industrial Crops and Products. 61, 49–61. https://doi.org/10.1016/j.indcrop.2014.06.027
- 33. Salaheldeen M., Aroua M.K., Mariod A.A., Cheng S.F., Abdelrahman M.A. and Atabani A.E. (2015) Physicochemical characterization and thermal behavior of biodiesel and biodiesel-diesel blends derived from crude Moringa peregrina seed oil. Energy Conversion and Management. 92, 535–542. <u>https://doi.org/10.1016/j.enconman.2014.12.087</u>

- 34. Noreen H., Semmar N., Farman M. and Mccullagh J.S.O. (2017) Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*, Asian Pacific Journal of Tropical Medicine. 10 (2017), 792–801. https://doi.org/10.1016/j.apjtm.2017.07.024
- 35. Sofidiya M.O., Odukoya O.A., Familoni O.B. and Inya-Agha S.I. (2006) Free radical scavenging activity of some Nigerian medicinal plants. Planta Medica, 72(11), 171. <u>https://doi.org/10.1055/s-2006-949971</u>
- 36. Souri E., Amin G., Farsam H., Jalalizadeh H. and Barezi S. (2022) Screening of thirteen medicinal plants extracts for antioxidant activity. Iranian Journal of Pharmaceutical Research, 7(2), 149-154. <u>https://doi.org/10.22037/ijpr.2010.758</u>
- Srinivasan K. (2014) Antioxidant potential of spices and their active constituents. Critical Reviews in Food Science and Nutrition, 54, 352-372. <u>https://doi.org/10.1080/10408398.2011.585525</u>
- 38. Syed Q. A., Rashid Z., Ahmad M. H., Shukat R., Ishaq A., Muhammad N. and Rahman H. U.U. (2020) Nutritional and therapeutic properties of fenugreek (*Trigonella foenum-graecum*): A review. International Journal of Food Properties, 23(1), 1777-1791. <u>https://doi.org/10.1080/10942912.2020.1825482</u>
- Viol D. I., Chagonda L. S., Munodawafa T., Muchuweti M., Moyo S.R. and Mericli A.H. (2013) Antioxidant activity and total phenolic contents of some traditional medicinal plants from Zimbabwe. Journal of Biologically Active Products from Nature, 3(5-6), 345-352. https://doi.org/10.1080/22311866.2013.782753
- 40. Wang W., Weng X. and Cheng D. (2000) Antioxidant activities of natural phenolic components from Dalbergia odorifera T. Chen. Food Chemistry, 71, 45-49. <u>https://doi.org/10.1016/S0308-8146(00)00123-0</u>
- 41. Wani S.A. and Kumar P. (2018) Fenugreek: A review on its nutraceutical properties and utilization in various food products. Journal of the Saudi Society of Agricultural Sciences, 17(2), 97-106. https://doi.org/10.1016/j.jssas.2016.01.007

- 42. Yang X., Sun Z., Wang W., Zhou Q., Shi G., Wei F., Jiang G. (2018) Developmental toxicity of synthetic phenolic antioxidants to the early life stage of zebrafish. Science of the Total Environment, 643, 559–568. <u>https://doi.org/10.1016/j.scitotenv.2018.06.213</u>
- Yang R., Wang H., Jing N., Ding C., Suo Y. and You, J. (2012) Trigonella foenum-graecum L. seed oil obtained by supercritical CO<sub>2</sub> extraction. Journal of the American Oil Chemists' Society, 89(12), 2269-2278. <u>https://doi.org/10.1007/s11746-012-2123-x</u>
- 44. Yoo K.M., Lee K.W., Park J.B., Lee H.J. and Hwang I.K. (2004) Variation in major antioxidants and total antioxidant activity of Yuzu (Citrus junos Sieb ex Tanaka) during maturation and between cultivars. Journal of Agricultural and Food Chemistry, 52(19), 5907-5913. <u>https://doi.org/10.1021/jf0498158</u>
- 45. Zheng H.C., Liu J.X., Yao J.H., Yuan Q., Ye H.W., Ye J.A. and Wu Y.M. (2005) Effects of dietary sources of vegetable oils on performance of high-yielding lactating cows and conjugated linoleic acids in milk. Journal of Dairy Science, 88(6), 2037-2042. <u>https://doi.org/10.3168/jds.S0022-0302(05)72880-0</u>