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## Physicochemical Properties and Fatty Acid Composition of *Ocimum basilicum* L. Seed Oil

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#### Authors' contributions

This work was done in collaboration among all authors. Authors AAI and AHN designed the study, performed the analysis and wrote the first draft of the manuscript. Authors MMA, IYE, OAOI and AHN supervised the study and analysed the data. All the authors managed the literature search writing of the final manuscript, read and approved the final manuscript.

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#### **ABSTRACT**

Ocimum basilicum has been widely used in traditional medicine. Rural communities have used fixed oils for variety purposes since a long time ago. They used for cosmetic applications, fuel, medicine and food. The aim of this study was to characterize the physicochemical properties and fatty acid composition of *O. basilicum* seed oil. Lipids were determined by continuous extraction in a Soxhlet apparatus for 6 hours using hexane as solvent. The physicochemical properties of the oil were assessed by standard and established methods. The fatty acids composition of the seed oil was determined by GC-MS. The Pale yellow with camphor odor oil extracted from the seed has the following properties: yield, 18.01%; freezing point, -2°C; melting point, 5°C; boiling point, 215°C;

refractive index ( $25^{\circ}$ C), 1.48532; iodine value, 108.6 g/100 g of oil; peroxide value, 4.6 meq.  $O_2$ /kg of oil; free fatty acids, 0.20%; acid value, 4.0 mg of KOH/g of oil; saponification value, 164.2 mg KOH/g of oil; unsaponifiable matter, 1.6; moisture and volatile value, 4.97 (wt%); density, 0.91372 g/cm³; viscosity, 10.29 mm²/s; specific gravity, 0.9210. Fatty acids composition showed that linolenic-(43.92%) was the major fatty acid and followed by linoleic- (32.18%), palmitic- (13.38%), stearic-(6.55%), palmitoleic- (0.78%), arachidic- (0.72%), anteisomargaric- (0.45%), nonadecylic- (0.28%), gondoic- (0.27%), margaric- (0.20%), behenic- (0.17%), heneicosylic- (0.14%), lignoceric- (0.13%) and myristic acid (0.11%). Therefore, recommended that more and advanced investigations should be undertaken for this abundant oil as natural source for many industrial applications, especially, for applications that require acids like linolenic and linoleic.

Keywords: Ocimum basilicum; seed oil; physicochemical properties; chemical composition.

#### 1. INTRODUCTION

About 80% of population of developing countries relies exclusively on plants to meet their health care needs, according to World Health Organization [1]. The medicinal plants have a great importance among human population and play a significant role in traditional herbal system besides a source of income in many developing countries [2].

Fixed oils are esters of glycerol with varying consistency, found in both animals (for example, fish oils) and plants (in nuts and seeds). Fixed oils are used as foods, lubricants, making of soaps, paints and varnishes [3]. According to its characteristic, seed oils' were classified as drying, semi-drying or non-drying. Drying oils are highly unsaturated oils that will polymerize when exposed to the oxygen in air, usually in the presence of a catalyst [4]. Fatty acids are widely occurring in natural fats, dietary oils. They are known to have antibacterial, antifungal and among others biological properties. Ocimum basilicum L. (Family Lamiaceae) exist in wide diversity more than 50 species. The exact taxonomy of basil is uncertain due to the immense number of cultivars, its ready polymorphy, and frequent cross-pollination with other members of the genus Ocimum and within the species [5,6]. Among the species of this genus, the regular basil (O. basilicum) is the most important economical species, planted worldwide [7]. O. basilicum was originated in Asia and Africa [8-11]. Different parts of the plant have been widely used in traditional medicine [12-14]. Rural communities have used fixed oils for variety purposes since a long time ago. They used for cosmetic applications, fuel, medicine and food. Moreover, O. basilicum showed its potential for several biological activities, including antioxidant, anti-aging, anti-inflammatory, anticarcinogenic, anti-microbial, and cardiovascular

agents, among others [15]. Additionally, O. basilicum seeds have been traditionally used as a natural remedy for the treatment of indigestion. ulcer, diarrhoea, sore throats, and kidney disorders [16]. O. basilicun seed gum also used for many purposes, such as a source of fiber, disintegrant. pharmaceutical excipient. suspending agent, anti-diabetic agent, seedling growth of plants, and biodegradable edible film [17]. Seed oils vary substantially in fatty acid composition across different taxonomic levels [18]. The richness of this resource of species provides a wide range of fatty acids in seeds [19]. Seed oil fatty acid profiles are distinctive enouah among species and genotypes. Moreover, the fatty acid compositions of seed oils differ notably at generic and infrageneric levels [18]. The final uses of seed oils depend to a large extent on its properties (physicochemical characteristics) and the nature of its fatty acids. Therefore, this study aimed to determine the physicochemical properties and fatty acids composition of O. basilicum seed oil to increase their economic feasibility of future commercial cultivation and products of this herb.

#### 2. MATERIALS AND METHODS

#### 2.1 Plant Material

Dried seeds of *O. basilicum* were collected on October 2017, from Ministry of Agriculture and Forestry, General Directorate of Horticultural Production, Department of Medicinal and Aromatic Plants, Khartoum, Sudan. Taxonomic identification of *O. basilicum* seed was performed by Dr. Yahya of the National Centre for Research, department of biology, Khartoum, Sudan. Specimens of seeds and herbaceous parts of the plant were deposited (ref. No. NCR/09/8/217). The seeds of *O. basilicun* were cleaned, removed the dirt, ground by mortar and

crushed by electric blender to reduce the particle size.

### 2.2 Solvent Semi-continuous (Soxhlet) Extraction Method

The *O. basilicum* seed oil was obtained by continuous extraction in a Soxhlet apparatus for 6 hours using hexane as solvent according to method described by Motojest et al. [19] and Olowokere et al. [20] with slight modification. The oil was filtered through filter paper (Whatman No.2, 125 mm). The solvent was evaporated using rotary evaporator (model Buchi R-3, Switzerland) under reduced pressure and temperature. Further dried under open air in a dark area. The obtained oil (fixed) was stored in hermetically closed dark bottles and kept at -4°C for further studies. The percentage of oil (w/w%) was calculated according to the following formula:

Lipid Yield (%)
$$= \frac{\text{Weight of } oil (g)}{\text{Weigt of sample } (g)} \cdot 100\%$$

### 2.3 Physical Properties of *O. basilicum* Seed Oil

### 2.3.1 Physical state, color and odor determination of seeds oil

Physical state at room temperature (25°C) and color of the oil were observed through visual observation, whereby odor through sensation through volatilized smell.

### 2.3.2 Freezing, melting and boiling points determination of *O. basilicum* seed oil

The oil filled in a clear glass vial and solidified through the usage of ice blocks and then a thermometer was immersed into the oil. The oil was observed to solidify and the point was recorded as freezing point. The solidified oil was melted over a water bath at the temperature of 29°C and the melting point was recorded. Again, around 10 mL of oil filled in a clear glass vial; a thermometer inserted in the vial and exposed to heat on a heating mantle. The oil observed whereby it starts circulating leading to boiling. The temperature at this point was recorded as the boiling point [21].

### 2.3.3 Density determination of *O. basilicum* seed oil

Density determination was done according to method described by Noureddini et al. [22]. Small empty vial was weighed, and then it filled with known amount of oil up to the brim. The vial was weighed again and the density was calculated according to the following formula;

Density, 
$$\rho$$

$$= \frac{(Weight \ of \ vial + Oil) - Weight \ of \ empty \ vial}{Volume \ of \ oil}$$

### 2.3.4 Refractive index analysis of O. basilicum seed oil

The refractive index of the oil was determined by using standard method AOAC Official Method 921.08 (1990) as described by Jessinta et al. [21]. This index was measured at 25°C via pen Refractometer (Atago, Japan) with resolution and accuracy value of 0.1% and  $\pm$  0.2% at 10-60°C. The measurement was repeated in triplicate and the average value was reported.

### 2.4 Chemical Properties of *O. basilicum*Seed Oil

## 2.4.1 Acid value, free fatty acid, iodine value, peroxide value and unsaponifiable matter analysis

The Acid Value (AV) was determined through direct titration method of oil in an alcoholic medium against standard potassium hydroxide via AOCS Official method Cd 3a-63 (2009). The Free fatty acids were analyzed according to standard titration methods described by AOCS Official Method Ca 5a-40 (2009). Iodine value, peroxide value and unsaponifiable matter were analyzed according to standard methods of AOAC Official Method 993.20 (1999) by Wij's reagent, 965.33 (2002) and Ca 6a-40 (1998), respectively [21]. The calculations according to the following equations;

Acid value = % Fatty acid (as oleic)  $\times$  1.99

Free fatty acids as oleic acid = 28.2 VXN per cent by weight /Weight of the sample oil

where: V is volume of potassium hydroxide; N is normality of the potassium hydroxide solution

lodine value = 12.69 X (TB- TS) XN / Weight of the sample oil

where, N is the normality of sodium thiosulphate and TB and TS are the titer volumes of the blank and sample, respectively.

Peroxide value = ((Titration of standard) X (Molarity of standard) X 100) / Weight of sample

Unsaponifiable matter =  $((W_1 - W_2) \times 100) / W$ 

Where:  $W_1$  is weight of the residue;  $W_2$  is weight of the free fatty acids in the extract; W is the weight of oil

#### 2.4.2 Moisture and volatile matter analysis

Moisture and volatile matters were analyzed according to air-oven method of AOCS Ca 2c-25 with some modification. About 5 g of oil was weighed on a previously dried and tiered dish. The dish covered with loose lid and was heated in the oven at 105±1°C for one hour. The dish was removed from the oven, cooled in a desiccator and was weighed. The plate was reheated for the period of one hour. The cooling and weighing process was repeated until the weight changed between two successive observations does not exceed one mg [21]. The following formula was used to calculate the observations:

% of Moisture and volatile matter  $= \frac{\text{Weight loss of material on drying (g)} \times 100}{\text{Weight of material taken for test (g)}}$ 

#### 2.4.3 Saponification value

An amount of 2.0 g of oil was added to 25 mL 0.5M KOH, refluxed for 1 hour. The sample has cooled; solution of 0.5 mL phenolphthalein indicator was added and titrated by 0.5M HCl until the pink color disappeared [23].

Saponification value (mg KOH/g oil) =  $(56.1*N(V_1-V_2))$  / W

where: N is the normality of HCl; W is the weight of oil; and  $V_1$  and  $V_2$  are the volumes of the blank and sample, respectively.

## 2.4.4 Fatty acid composition, percentage of saturated and unsaturated fatty acid analysis

The crude oil was converted into fatty acid methyl ester, through transesterification reaction

to determine the fatty acids composition. An amount of 100 mg of oil sample was dissolved in 10 mL of hexane in a test tube. An amount of 1 mL of 2 M KOH (methanolic potassium hydroxide) was added into the same test tube and was vortexes. The hexane phase was collected and washed twice with 4 mL of water after 15 minutes, afterward, dried over an anhydrous sodium sulfate. The fatty acid compositions were analyzed by GC-MS. The individual fatty acids compositions were expressed as percentage. The sum percentage of saturated fatty acids was represented as total saturated (mono- and polyunsaturated) was represented as total unsaturated fatty acids (21].

#### 2.5 GC-MS Analysis

Fatty acid composition: Fatty acid methyl esters were prepared and analyzed as described by Jessinta et al. [21] using Gas chromatography (GC) with slight modifications. Briefly, 1 µL of fatty acid methyl ester solution was injected into an Agilent 7890 GC equipped with flame ionization detector (FID) with split ratio of 50. Helium was used as carrier gas. Both injection port and detector temperatures were 250°C. For HP Innowax capillary column (30 m, 0.25 mm, 0.25 µm), helium gas flow was 1.5 mL/min. The column temperature was programmed from 50°C for 1 min, increased to 70°C at 5°C min<sup>-1</sup>, held for 9 min, and further programmed to 200°C at 15 °C min<sup>-1</sup>, held for 10 min. Final temperature was 230°C, held for 4 min. For HP 88 capillary column (100 m, 0.32 mm, 0.25 µm), helium gas flow was 2.0 mL/min. The column temperature was programmed from 120 °C for 1 min, increased to 175°C at 10°C min<sup>-1</sup>, held for 10 min. increased to 210°C at 5°C min<sup>-1</sup>, held for 5 min and further programmed to 230°C at 5°C min<sup>-1</sup>, held for 5 min. Fatty acids were identified by comparison of retention time with authentic standards (C4-C24, Supelco 37 component FAME Mix). The standard mixture consist of Hexanoic acid methyl ester (C6:0), Octanoic acid methyl ester (C8:0), Decanoic acid methyl ester (C10:0), Undecanoic acid methyl ester (C11:0), Dodecanoic acid methyl ester (C12:0),acid methyl Tridecanoic ester (C13:0),Tetradecanoic acid methyl ester (C14:0). Tetradecenoic acid methyl ester (C14:1), Pentadecanoic acid methyl ester (C15:0), Cis-10-Pentadecenoic acid methyl ester (C15:1), Hexadecanoic acid methyl ester (C16:0), Cis-9-Hexadecenoic acid methyl ester (C16:1),

Heptadecanoic acid methyl ester (C17:0), Cis-10-Heptadecenoic acid methyl ester (C17:1), Octadecanoic acid methyl ester (C18:0), Trans-9-Octadecenoic acid methyl ester (C18:1n9t), Cis-9-Octadecenoic acid methyl (C18:1n9c). Trans-9. 12-Octadecadienoic acid (C18:2n6t), ester Cis-9, 12-Octadecadienoic acid methyl ester (C18:2n6c). Eicosanoic acid methyl ester (C20:0), Cis-6, 9, 12-Octadecatrienoic acid methyl ester (C18:3). Cis-11-Eicosenoic acid methyl ester (C20:1), Cis-9,12,15-Octadecatrienoic acid methyl ester (C18:3n3), Heneicosanoic acid methyl ester (C21:0), Cis-11,14-Eicosadienoic acid methyl ester (C20:2), Docosanoic acid methyl ester (C22:0), Cis-8,11,14-Eicosatrienoic acid methyl ester (C20:3n6), Cis-13-Docosenoic acid methyl ester (C22:1n9), Cis-11,14, 17-Eicosatrienoic acid methyl ester (C20:3n3), Cis-5,8,11,14-Eicosatetraenoic acid methyl ester (C20:4n6), Tricosanoic acid methyl ester (C23:0), Cis-11,16-Docosadienoic acid methyl ester (C22:2), Tetracosanoic acid methyl ester (C24:0), Cis-5,6,11,14,17-Eicosapentaenoic acid methyl ester (C20:5n3), Nervonic acid methyl ester (C24:1), Cis-4,7,10,13,16, 19-Docosadienoic acid methyl ester (C22:6n3). Results of fatty acids are given

as relative percentage area of the sum of all identified peaks.

#### 2.6 Statistical Analysis

The analyzes were performed in three repetitions and the results expressed as mean average.

#### 3. RESULTS AND DISCUSSION

### 3.1 Lipid Content, Physical State, Color and Odor of *O. basilicum* Seed Oil

Table 1 represents various physicochemical properties of the O. basilicum seed oil. The yield of the obtained oil was 18.01% (w/w) and this value was represented in terms of lipid content, and the oil was highly unsaturated (77.29%). The oil was liquid at room temperature of 25°C, pale vellow with characteristic odor. The freezing-. melting- boiling and density points were -2°C. 5°C, 215°C and 0.913 g/cm<sup>3</sup>, respectively. Kadam et al. [3] reported that the seed oil of O. basilicum is yellowish with characteristic odor. The oil yield of O. basilicum in this study was within the range

Table 1. Physicochemical properties of *O. basilicum* seed oil

Lipid Content         %         18.01           Physical State at 25 °C         -         Liquid           Color         -         Pale yellow           Odor         -         Characteristic           Freezing pint         °C         -2           Melting point         °C         5           Boiling point         °C         215           Density at 25 °C         g/cm³         0.913           Viscosity         mm²/s         10.29           Specific gravity         0.921         1.485           Refractive index at 25 °C         1.485           Acid value (% FFA as oleic)         mg KOH/g         4.0           FFA**         Linolenic         %         43.95           Linoleic         %         32.18           Palmitic         %         32.18           Palmitic         %         6.55           Iodine value         g l₂/100g         108.6           pH         4           Peroxide value         meq O₂/kg         4.6           Moisture and volatile matter         wt %         4.97           Saponification value         mg KOH/g         164.2           Unsaponifiable matter         wt % <th colspan="2">Parameters</th> <th>Units</th> <th>Experimental values*</th> <th></th>	Parameters		Units	Experimental values*	
Color         -         Pale yellow           Odor         -         Characteristic           Freezing pint         °C         -2           Melting point         °C         5           Boiling point         °C         215           Density at 25 °C         g/cm³         0.913           Viscosity         mm²/s         10.29           Specific gravity         0.921           Refractive index at 25 °C         1.485           Acid value (% FFA as oleic)         mg KOH/g         4.0           FFA**         Linolenic         %         43.95           Linoleic         %         32.18           Palmitic         %         13.38           Stearic         %         6.55           Iodine value         g I₂/100g         108.6           pH         4         4           Peroxide value         meq O₂/kg         4.6           Moisture and volatile matter         wt %         4.97           Saponification value         mg KOH/g         164.2           Unsaponifiable matter         wt %         1.6           Total Saturated Fatty Acids         wt %         22.28	Lipid Conten	t	%	18.01	
Odor         -         Characteristic           Freezing pint         °C         -2           Melting point         °C         5           Boiling point         °C         215           Density at 25 °C         g/cm³         0.913           Viscosity         mm²/s         10.29           Specific gravity         0.921           Refractive index at 25 °C         1.485           Acid value (% FFA as oleic)         mg KOH/g         4.0           FFA**         Linolenic         %         43.95           Linoleic         %         32.18           Palmitic         %         13.38           Stearic         %         6.55           Iodine value         g I <sub>2</sub> /100g         108.6           pH         4         4           Peroxide value         meq O <sub>2</sub> /kg         4.6           Moisture and volatile matter         wt %         4.97           Saponification value         mg KOH/g         164.2           Unsaponifiable matter         wt %         1.6           Total Saturated Fatty Acids         wt %         22.28	Lipid Content Physical State at 25 °C Color Odor Freezing pint Melting point Boiling point Density at 25 °C Viscosity Specific gravity Refractive index at 25 °C Acid value (% FFA as oleic) FFA** Linolenic Linoleic Palmitic Stearic Iodine value		-	Liquid	
Freezing pint         °C         -2           Melting point         °C         5           Boiling point         °C         215           Density at 25 °C         g/cm³         0.913           Viscosity         mm²/s         10.29           Specific gravity         0.921           Refractive index at 25 °C         1.485           Acid value (% FFA as oleic)         mg KOH/g         4.0           FFA**         Linolenic         %         43.95           Linoleic         %         32.18           Palmitic         %         13.38           Stearic         %         6.55           Iodine value         g I₂/100g         108.6           pH         4           Peroxide value         meq O₂/kg         4.6           Moisture and volatile matter         wt %         4.97           Saponification value         mg KOH/g         164.2           Unsaponifiable matter         wt %         1.6           Total Saturated Fatty Acids         wt %         22.28	Color	Physical State at 25 °C Color Color Color Color Color Creezing pint Melting point Boiling point Coensity at 25 °C Viscosity Specific gravity Refractive index at 25 °C Acid value (% FFA as oleic) FFA** Linolenic Linoleic Palmitic		Pale yellow	
Melting point         °C         5           Boiling point         °C         215           Density at 25 °C         g/cm³         0.913           Viscosity         mm²/s         10.29           Specific gravity         0.921           Refractive index at 25 °C         1.485           Acid value (% FFA as oleic)         mg KOH/g         4.0           FFA**         Linolenic         %         43.95           Linoleic         %         32.18           Palmitic         %         13.38           Stearic         %         6.55           Iodine value         g I₂/100g         108.6           pH         4           Peroxide value         meq O₂/kg         4.6           Moisture and volatile matter         wt %         4.97           Saponification value         mg KOH/g         164.2           Unsaponifiable matter         wt %         1.6           Total Saturated Fatty Acids         wt %         22.28	Odor		-	Characteristic	
Boiling point         °C         215           Density at 25 °C         g/cm³         0.913           Viscosity         mm²/s         10.29           Specific gravity         0.921           Refractive index at 25 °C         1.485           Acid value (% FFA as oleic)         mg KOH/g         4.0           FFA**         Linolenic         %         43.95           Linoleic         %         32.18           Palmitic         %         13.38           Stearic         %         6.55           lodine value         g I₂/100g         108.6           pH         4           Peroxide value         meq O₂/kg         4.6           Moisture and volatile matter         wt %         4.97           Saponification value         mg KOH/g         164.2           Unsaponifiable matter         wt %         1.6           Total Saturated Fatty Acids         wt %         22.28	Freezing pint	t	°C	-2	
Density at 25 °C         g/cm³         0.913           Viscosity         nm²/s         10.29           Specific gravity         0.921           Refractive index at 25 °C         1.485           Acid value (% FFA as oleic)         mg KOH/g         4.0           FFA**         Linolenic         %         43.95           Linoleic         %         32.18           Palmitic         %         13.38           Stearic         %         6.55           Iodine value         g I₂/100g         108.6           pH         4           Peroxide value         meq O₂/kg         4.6           Moisture and volatile matter         wt %         4.97           Saponification value         mg KOH/g         164.2           Unsaponifiable matter         wt %         1.6           Total Saturated Fatty Acids         wt %         22.28	Odor Freezing pint Melting point Boiling point Density at 25 °C Viscosity Specific gravity Refractive index at 25 °C Acid value (% FFA as oleic) FFA** Linolenic Linoleic Palmitic		°C	5	
Viscosity         mm²/s         10.29           Specific gravity         0.921           Refractive index at 25 °C         1.485           Acid value (% FFA as oleic)         mg KOH/g         4.0           FFA**         Linolenic         %         43.95           Linoleic         %         32.18           Palmitic         %         13.38           Stearic         %         6.55           Iodine value         g I₂/100g         108.6           pH         4           Peroxide value         meq O₂/kg         4.6           Moisture and volatile matter         wt %         4.97           Saponification value         mg KOH/g         164.2           Unsaponifiable matter         wt %         1.6           Total Saturated Fatty Acids         wt %         22.28	Boiling point		°C	215	
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pH $4$ Peroxide value $meq O_2/kg$ $4.6$ Moisture and volatile matter $wt \%$ $4.97$ Saponification value $mg KOH/g$ $164.2$ Unsaponifiable matter $wt \%$ $1.6$ Total Saturated Fatty Acids $wt \%$ $22.28$	lodine value		g l <sub>2</sub> /100g	108.6	
Moisture and volatile matter wt % 4.97 Saponification value mg KOH/g 164.2 Unsaponifiable matter wt % 1.6 Total Saturated Fatty Acids wt % 22.28	рН			4	
Saponification value mg KOH/g 164.2 Unsaponifiable matter wt % 1.6 Total Saturated Fatty Acids wt % 22.28	Peroxide value		meq O₂/kg	4.6	
Unsaponifiable matter wt % 1.6 Total Saturated Fatty Acids wt % 22.28	Moisture and volatile matter		wt %	4.97	
Total Saturated Fatty Acids wt % 22.28	•		mg KOH/g	164.2	
	Unsaponifiable matter		wt %	1.6	
Total Unsaturated Fatty Acids wt % 77.29	Total Saturated Fatty Acids		wt %	22.28	
	Total Unsatu	rated Fatty Acids	wt %	77.29	

\*Values were recorded as mean average, \*\* FFA >1%

reported by Kakaraparthi et al. [24] (12.4-21.6%) and Nour et al. [4] (8.82-30.01%), less than that reported by Angers et al. [25] (24%), Ghaleshahi et al. [26] (22.04%) and Amini et al. [27] (22%) and slightly higher than amounts reported for Mostafavai et al. [7] (3.1-17.78%).

### 3.2 Viscosity and Specific Gravity O. basilicum Seed Oil

The viscosity of the *O. basilicum* seed oil was 10.29. The viscosity defined as resistance liquid to flow. Viscosity increased with molecular weight, but decreased with increasing unsaturated level and temperature. Viscosity of oil depends upon the density; when density of oil increase, there are chances that its viscosity would be increase. Previously, Kadam et al. [3] found the viscosity of the *O. basilicum* seed oil is 11.51.

Specific gravity (SG) of the *O. basilicum* seed oil was 0.921. The SG is considered as a good index of purity of oils. The increase in chain length of fatty acid present in oil tends to increase the specific gravity of oils. The specific gravity of oil samples increases during frying and this may be interpreted due to the generation of dipoles on heating of oils, which interact with each other and increase the specific gravity of oils. In previous study, the SG of *O. basilicum* seed oil found to be 0.9525 [3].

### 3.3 Refractive Index (RI) of *O. basilicum* Seed Oil

The refractive index (RI) of the *O. basilicum* seed oil was 1.485. The RI varies with temperature and wavelength. RI of oils increases with the increase in unsaturation and also chain length of fatty acids. Researchers reported the RI for *O. basilicum* seed oil in range of 1.460-1.484[3].

### 3.4 Acid Value (AV) of *O. basilicum* Seed Oil

The acid value (AV) is the relative measure of rancidity as FFAs that are formed during decomposition or hydrolysis of oil glycerides because action of moisture, temperature and/or lipolytic enzyme lipase. Thus, AV is a measure of the free fatty acids in oil. Normally, fatty acids are found in the triglyceride form, however, during processing the fatty acids may get hydrolyzed into free fatty acids. The AV obtained

in this study was 4.0 mg KOH/g and this amount was high when compared to the previously reported 0.9525 mg KOH/g [3]. The percentage of unsaturated fatty acids is increasing by some factors, like hydrolysis and oxidation, which leads to rise of AV [28].

#### 3.5 Saponification Value and Unsaponifiable Matter of *O. basilicum* Seed Oil

The numbers of milligrams of potassium hydroxide require converting one gram of the fat into soap and glycerin known as saponification value (SV). SV gives information concerning the character of the fatty acids of the fat [28]. Moreover, the saponification value (SV) used to know the amount of free fatty acids present in the oil. Determination of the quantity of alkali that must be added to the fat to render it neutral will estimate the amount of free fatty acids [3]. Higher SV indicates high proportion of lower fatty acids, since SV is inversely proportional to the average molecular weight or chain length of the fatty acids. The SV for the studied oil was 164.2 mg KOH/g of oil. Previously, Kadam et al.[3] reported that the SV for O. basilicum seed oil as 194.94 mg KOH/g of oil.

Unsaponifiable matter is that fraction of oils and fats which is not saponified by caustic alkali, but is soluble in ordinary fat solvents. Unsaponifiable matters such as hydrocarbon, pigments, waxes, higher molecular weight alcohols, and sterols do not react with bases during formation of soap. The unsaponifiable matter value for O. basilicum seed oil was 1.6 wt%. Previously, unsaponifiable matter for O. basilicum seed oil which is determined by titration with sodium hydroxide solution in alcoholic, found to be 36.6, which is too high [3]. The current studied oil showed lower unsaponifiable matter than reported value. Therefore, due to the small value of unsaponifiable matter (< 2 wt%), O. basilicum could be suitable in the application of biodiesel production [29].

### 3.6 Iodine Value (IV) of *O. basilicum* Seed Oil

The drying quality of the oil can be considered as one of factors of oil classification; it could be non-drying, semi-drying or drying oil through the analysis of the IV [30]. IV represents true unsaturation of fats only when double bonds are

unconjugated and addition of iodine is not interfered by other groups. The higher iodine value, the more unsaturated fatty acid bonds are present in a fat/oil. It is a measure, which indicates the potential of a fat to be oxidized. Previously, researchers reported the IV for some oils as groundnut oil (84 - 99), olive (79 - 90) and castor oil (81 - 91). The oil of the current study in the range, but is higher than some edible oils as reported. The IV for current study was 108.6 q/100 g; and it suggests that it is semi-drying oil and it is comparable to the standard IV of more than 100 gl<sub>2</sub>/100 g in accordance with its physical state of being liquid at room temperature of 25°C under expose air condition [31]. The high IV represents the more amounts of unsaturated bonds and thus the oil has higher tendencies to go through oxidative rancidity [28]. Researchers reported that the IV for O. basilicum ranged from 172 to 200 gl<sub>2</sub>/100 g [3].

### 3.7 Peroxide Value (PV) of *O. basilicum* Seed Oil

The peroxide value (PV) indicates the rancidity process, whereby the higher the PV, the higher is the oxidation level and the deterioration of lipids [32]. Oil that shows a high amount of PV is considered more prone to undergo rancidity, which affects the total quality of the oil [33]. In this study, the obtained PV for *O. basilicum* seed oil is 4.6 mEq/kg and was determined immediately after the extraction of the oil. A low peroxide value increases the suitability of the oil for a long storage due to low level of oxidative and lipolytic activities [33]. In previous study, the PV was found to be 4.5 mEq/kg, which is similar to that in this study.

#### 3.8 Moisture and Volatile Matter of O. basilicum Seed Oil

The moisture and volatile matter recorded for *O. basilicum* seed oil is 4.97%wt%. The presence of water or moisture contributes towards hydrolysis in breaking up of triglycerides into glycerol and FFAs. Orhevba et al. [28] documented that, both oxidation and hydrolysis reduce the amount of unsaturated FFA and thus contributing towards the reducing of IV and increasing in the AV. In this study, the observed moisture content in *O. basilicum* seed oil was 4.97%, thus, the low moisture content of the oil serves as an indication that, the activities of the microorganisms would be reduced and thereby increases the shelf life of the oil.

# 3.9 Free Fatty Acids (FFA), Fatty Acid Composition, Percentage of Saturated and Unsaturated Fatty Acid of *O. basilicum* Seed Oil

Table 2 shows the fatty acids composition of the *O. basilicum* seed oil. In general, fatty acids are main constituents of seed oils and known to be a major parameter that differentiates the physicochemical properties of the seed oils.

In this study, twenty two different fatty acids were identified and they include both saturated and unsaturated acids. The dominant fatty acid was linolenic acid (43.92%), followed by linoleic (32.18%), palmitic (13.38%) and stearic (6.55%). The total percentage of fatty acids chains were 99.57 wt%. All the values are represented as the relative percentage area from the sum of all identified peaks. The overall results of this analysis showed that the unsaturated fatty acid (UFA) makes 77.29 wt% of the compositions, whereby the monounsaturated fatty acids (MUFA) are 1.06 wt%, polyunsaturated fatty acids (PUFA) are 76.23 wt%; and the saturated fatty acids (SFA) were 22.28 wt% as shown in Fig. 1. It is reported that, O. basilicum seed oil contained up to 91.6% UFA; the major constituents were alpha-linolenic (57.4 to 62.5%), linoleic (18.3 to 21.7%), and oleic (8.7 to 11.6%) [25]. Moreover, Mostafavai et al. [7] claimed that (69%), followed by palmitic- $\alpha$ -linolenic-(16.2%) and linoleic acid (9.7%) were the major fatty acids for O. basilicum seed oil grown in

In addition, in this study the dominant SFA were palmitic (13.38%) and stearic (6.55%). Similar results reported previously, the most abundant SFA were palmitic (6.1% to 11.0%) and stearic (2.0% to 4.0%). O. basilicum seeds from Pakistan contain lauric- (0.85%), myristic-(0.36%), palmitic- (9.70%), stearic- (5.45%), oleic- (13.33%), linoleic- (32.18%) and linolenic acid (48.50%) [7]. On the other hand, in our previous study in 2005, we found that the major fatty acids were palmitic- (5-13%), stearic- (2-3%), oleic- (6-10%), linoleic- (12-32%) and linolenic acid (44-75%) for seed oils of O. basilicum grown in Sudan. Linolenic acid which is a desirable for industrial uses as a drying oil is high as (75%) in seeds of the Sudanese wildtype basil [4]. A high linolenic acid oil, such as that found in O. basilicum and O. canum, could be used in the paint, varnish and ink industries. and as a source of linolenic acid, while oils with

lower linolenic acid content, such as those of O. gratissimum and O. sanctum, might be used by the food industry. The differences in the results obtaining by the researchers can be attribute to many factors, like environmental conditions, geographical origin, soil, cultivation climate. harvesting time, maturity and the drying process [21,34]. Fatty acid composition of some O. basilicum seed oil reported in the literature is shown in Table 3. Our results agreed with many previously reported results (Tables 2 and 3). However, there are some differences in some physicochemical properties and fatty acids composition, e.g. Mostafavai et al. [7] investigated 18 basil (O. basilicum) populations and reported that the populations were significantly different in terms of saturated FA ranging from 10.73% to 13.51%, but unsaturated FA (expect linoleic acid) were not significantly different from each other (average=87.27%). The differences may be because of some factors. such as environmental conditions and extraction methods. Seed oils vary to a great or significant extent in fatty acid composition across different taxonomic levels. Many studies have reported

strong phylogenetic patterns in the fatty acid profiles of the seed oils. The relative abundance of common fatty acids in seed oils is more strongly explained by taxonomic affiliation than by climate. The importance of genetics over plastic response to environment as determinants of fatty acid composition [18]. Moreover, Mostafavai et al. [7] claimed that the composition and quantity of fatty acids (phytovariability) in the O. basilicum can be affect by certain parameters such as environmental and the climate; but the most important parameter was plant genotype. The large variation in fatty acid composition indicates a large potential to select ideal plants for specific health, nutritional and industrial usages [18]. In general, oils rich in certain acids like linoleic, linolenic, lauric, myristic and stearic acids are used in many applications such as treatment of acne and skin permeation enhancement effects [35]. Therefore, according to literature and results obtained in this study, we suggest that the current studied O. basilicum seed oil would be suitable for industrial purposes such as cosmetic uses.

Table 2. Fatty acids composition of O. basilicum seed oil

Fatty acid	Formula	Systematic name	Structure	Composition (%)
Saturated				
Palmitic acid C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>		Hexadecanoic acid	C16:0	13.38
Stearic	$C_{18}H_{36}O_2$	Octadecanoic acid	C18:0	6.55
Arachidic	$C_{20}H_{40}O_2$	Eicosanoic acid	C20:0	0.72
Anteisomargaric	$C_{17}H_{34}O_2$	Hexadecanoic acid,	C17:0	0.45
Nonadecylic	$C_{19}H_{38}O_2$	Nonadecanoic acid	C19:0	0.28
Margaric	$C_{17}H_{34}O_2$	Heptadecanoic acid	C17:0	0.20
Behenic	$C_{22}H_{44}O_2$	Docosanoic acid	C22:0	0.17
Heneicosylic	$C_{21}H_{42}O_2$	Heneicosanoic acid	C21:0	0.14
Lignoceric	$C_{24}H_{48}O_2$	Tetracosanoic acid	C24:0	0.13
Myristic	$C_{14}H_{28}O_2$	Tetradecanoic acid	C14:0	0.11
Tricosylic	$C_{23}H_{46}O_2$	Tricosanoic acid	C23:0	0.07
Pentadecylic	$C_{15}H_{30}O_2$	Pentadecanoic acid	C15:0	0.04
Tridecylic acid	$C_{13}H_{26}O_2$	Tridecanoic acid	C13:0	0.02
Pentacosylic	$C_{25}H_{50}O_2$	Pentacosanoic acid	C25:0	0.02
Unsaturated				
Linolenic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	9,12,15-octadecatrienoic acid(Z,Z,Z)	C18:3	43.92
Linoleic acid	$C_{18}H_{32}O_2$	9,12-octadecadienoic acid	C18:2	32.18
Palmitoleic	$C_{16}H_{30}O_2$	9-Hexadecenoic acid	C16:1	0.78
Gondoic	$C_{20}H_{38}O_2$	Cis-11-Eicosenoic acid	C20:1	0.27
Docosatrienoic	$C_{22}H_{38}O_2$	8,11,14-Docosatrienoic acid	C22:3	0.08
Cinnamic	$C_9H_8O_2$	2-Propenoic acid, 3-phenyl	C9:5	0.05
5-Octadecenoic	$C_{18}H_{34}O_2$	5-Octadecenoic acid	C18:1	0.01

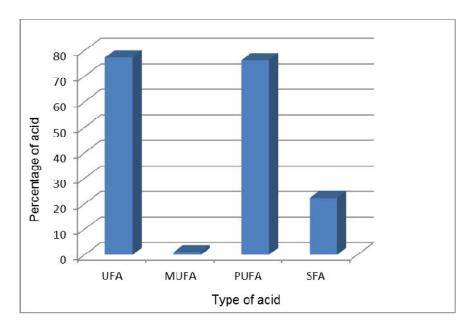


Fig. 1. Different types of fatty acids present in O. basilicum seed oil

Table 3. Fatty acid composition of O. basilicum seed oil reported in the literature

Fatty acids	Composition (%)*						
Palmitic	C16:0	6.8-8.8	4.90	1.6-7.5	8-9.2	6.23-10.16	5-13
Palmitoletic	C16:1	0.2-0.3	0.07	0.1	0.2	na	na
Stearic	C18:0	2.0-2.8	2.50	0.7-3.8	3.6-3.8	2.97-4.88	2-3
Oleic	C18:1	8.7-11.6	7.55	0.9-11.0	10.3-12.3	6.22-19.92	6-10
Linoleic	C18:2	18.3-21.7	20.20	1.8-19.1	23.4-26.0	16.73-24.93	12-32
Linolenic	C18:3	57.4-62.5	63.80	6.1-50.1	49.3-52.4	42.45-61.85	49-62
Arachidic	C20:0	0.2	0.25	0.3	0.2-0.3	na	na
	SFA	9-11.8	7.89	2.6-7.9	11.9-13.3	10.38-14.76	11.5
	MUFA	8.9-11.9	7.86	1-11.1	10.5-12.5	6.22-19.92	8.0
	PUFA	75.7-84.2	84.3	7.9-69.2	72.7-78.4	59.18-86.78	77.5
	UFA	84.6-96.1	92.2	8.9-80.3	85.6-88.1	81.03-93.07	85.5
References		(a)	(b)	(c)	(d)	(e)	(f)

na: Data not avaible, \*some data modified as mean average from origin source (a): Angers et al. [25], (b):
Ghaleshahi et al.[26], (c): Amin et al.[27], (d): Kakaraparthi et al.[24], (e): Mostafavai et al.[7] and
(f): Nour et al.[4]

#### 4. CONCLUSION

This study provides an insight on the physicochemical characteristics and fatty acids composition of *O. basilicum* seed oil. This seed oil demonstrates promising properties that could be a potential source for unlimited applications. The results of fatty acid composition analysis revealed that linolenic acid (43.92%) and linoleic acid (32.18%) are present in significant quantities. This oil could be a good natural source for linolenic and linoleic acids. Linolenic acid is desirable for industrial uses as a drying oil could be used in the paint, varnish, ink and

cosmetic industries. Therefore, this study may suggest that the *O. basilicum* seed oil may be an alternative good source for several industrial applications, especially for applications that require acids such as linolenic and linoleic.

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#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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