

Received:

16 April 2018

Revised:

8 October 2018

Accepted:

18 December 2018

Cite as: O. Olubi,  
J. V. Felix-Minnaar,  
V. A. Jideani.

Physicochemical and fatty  
acid profile of egusi oil from  
supercritical carbon dioxide  
extraction.

Heliyon 5 (2019) e01083.

doi: [10.1016/j.heliyon.2018.e01083](https://doi.org/10.1016/j.heliyon.2018.e01083)



# Physicochemical and fatty acid profile of egusi oil from supercritical carbon dioxide extraction

O. Olubi, J. V. Felix-Minnaar, V. A. Jideani\*

*Department of Food Science and Technology, Cape Peninsula University of Technology, P. O. Box 1906, Bellville, 7535, South Africa*

\* Corresponding author.

E-mail address: [jideaniv@cput.ac.za](mailto:jideaniv@cput.ac.za) (V.A. Jideani).

## Abstract

Oil extraction from egusi seeds using supercritical CO<sub>2</sub> extraction method was performed using series of operational parameters, temperature (55, 60, 75 °C), flow rate (30 g/h) and pressure (450, 600 bar). Egusi oil (EO) extracted at 60 °C, 30 g/h and 450 bar (EO1); 55 °C, 30 g/h and 600 bar (EO2) and 75 °C, 30 g/h and 600 bar (EO3) were investigated in a plant scale supercritical equipment. The fatty acid composition of egusi oil was analysed using gas chromatography, with result showing a high linoleic acid approximately (53%) and oleic acids (19%). The index of atherogenicity (IA%) and thrombogenicity index (IT %) were significantly low for the three oil samples, indicating its health benefits. Oxidative stability of egusi oil was analysed by Methrohm 743 Rancimat, confirming a less oxidised oil. Hence, egusi oil can be used as a raw material in dietary supplements and as a functional oil in the food industry.

Keywords: Food technology, Food analysis, Food science

## 1. Introduction

Egusi seed (*Citrullus lanatus sub Mucosospermus*), a family of *Benincaseae* of the subfamily *Cucurbitoideae*, can be grown in any part of the world with long summer (Mali and Chavan, 2016) and is more prominent in Africa. The flesh of egusi has a bitter taste and a spongy feel and is not considered edible in raw form in communities that consume egusi seed. Egusi is well adapted to extremely divergent agro-ecosystems and can survive extreme weather conditions (Efavi et al., 2018). The meal is high in protein and oil with literature indicating approximately 28.4% w/w protein (60% in defatted flour), 52% w/w fat, 3.6% w/w ash, 2.7% w/w fibre and 8.2% w/w carbohydrate (Akobundu, 1989; Akusu, 2014). Akubor and Ogbadu (2003) reported that the major fatty acids in egusi oil are 59–63% w/w linoleic acid and 16% w/w oleic acid. Egusi oil, with no cholesterol and high omega-3 and 6 fatty acids have never been introduced into the food industry (Jarret and Levy, 2012; Mali and Chavan, 2016).

The conventional oil extraction process is often laborious, and sometimes involves the use of harmful and toxic hydrocarbons for defatting. A more effective method of defatting using the supercritical fluid extraction method is necessary. Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids (Akanda et al., 2012).

A supercritical fluid (SCF) is a substance prevailing at a temperature and pressure above the critical point. It is neither a gas nor a liquid and is best described as an intermediate to these two extremes. The supercritical phase has solvent strengths close to those of liquid properties. Carbon dioxide is the logical choice since it has properties most ideal for extraction with a relatively low critical temperature (31 °C) and critical pressure (73 atm). Carbon dioxide is non-toxic, non-flammable, relatively cheap and commercially readily available.

Carbon dioxide is regarded as an environmentally friendly gas, replacing the hazardous organic solvents and results in extracts free from solvent residue. Chemical substances that have also been used successfully as supercritical fluids include ammonia, argon, propane, xenon, and water. Supercritical CO<sub>2</sub> fluid extraction (SCF-CO<sub>2</sub>) of a wide range of oil seed species including wheat germ, oats, cottonseed, soybean, rice bran, evening primrose, jojoba, rapeseed; peanut and grape seed have been studied by several authors from the processing point of view (Akanda et al., 2012).

SFE has attracted considerable attention in recent years as a promising alternative to conventional solvent extraction and mechanical pressing for extracting oils and other materials as it offers some advantages. These advantages include non-solvent residues and better retention of aromatic compounds (Gouda et al., 2017); chemical,

environmental, health, and safety benefits (Akanda et al., 2012). The environmental benefits of most SCF in industrial processes result from their replacement of the widely known environmentally damaging conventional organic solvents (Wang et al., 2011).

There is an increasing public health awareness of the environmental hazard associated with solvent contamination in the use of organic chemicals to separate components. Also, the high cost of organic solvents is also to be considered during extraction ( Jokić, 2012). The application of supercritical extraction in Green Food Processing (GPE) has been ongoing since the 1970s, and a great number of applications using SCF has been explored and developed in laboratories. In the food development and value adding, supercritical carbon dioxide extraction techniques can be achieved as it relates to particle formation, precipitation and separation (Chemat et al., 2017). The Recycling of by-products in a supercritical experiment is a GPE application, as the residue obtained after extraction retains its nutritional properties and is reusable as a functional food ingredient (Santos et al., 2017).

To date, the nutritional and functional profiles of egusi seed have been studied (Onuora and King, 1984; Kouebou et al., 2013), but not the nutritional and physical properties of its oil extracted using supercritical CO<sub>2</sub> extraction method. Hence, the objective of this study was to characterise the proximate, fatty acid profile and physicochemical properties of egusi seed oil extracted using SCF-CO<sub>2</sub> to drawing the attention of the consumer and the food industries to the potential of egusi oil.

## 2. Materials and methods

### 2.1. Source of egusi seed, reagents and equipment

Dehulled egusi seeds (*Citrullus lanatus subsp Muscospermus*) were purchased from a seed store in Capetown, South Africa. All chemical reagents were obtained from Merck Pty, South Africa. Deionized water was used all through the experiment. The supercritical fluid extraction equipment (Swiss Nova), a plant extractor, was operated manually at the Process Engineering Department of Northwest University, Potchefstroom Campus, South Africa. Egusi oil was tested for proximate, physicochemical and fatty acid profile.

### 2.2. Egusi seed preparation

Dehulled egusi seeds were sorted for chaff and damaged seeds as well as stones and pebbles, and all extraneous matter removed and discarded. The seeds were stored in a perforated sack bag inside a refrigerator before use.

### 2.3. Supercritical carbon dioxide extraction of egusi oil

Supercritical CO<sub>2</sub> extraction of egusi oil was performed using a plant scale unit. An extraction run was initiated by weighing a thimble (sample holder) and inserting the weighed 2 kg egusi seeds into the thimble and weighing again. The mass of the sample of egusi seed in each thimble was obtained by subtracting the weight of thimble from the mass of egusi seed. A collection vial was weighed and positioned for oil collection. After each extraction run, the collection vial was weighed again. The extraction run was initiated by clicking “analyse” from the “samples” menu after entering the extraction parameters required for the egusi seed. Firstly, the system was pressurised (at intervals of approximately 400–600 bars) until the desired pressure of 450 bar, and 600 bar was reached. The temperature at the extractor outlet was varied between 55, 60 and 75 °C by a thermoregulatory device to allow the separation between the extract and the solvent during depressurisation.

After column temperature and the pressure was stabilised, the system was kept in contact with the egusi seed for at least 15 minutes to allow system stabilisation. Then, the supercritical CO<sub>2</sub> was pumped into the bed of egusi seed at 30 g/hr. The volumetric flow rate of the solvent was regulated using the expansion valve at the outlet of the extractor. The pump pressure at 450 bar was regulated at the set value by high variable restrictors (HVRs), electronic flow meters and solenoid valves.

Solenoid valves lowered the thimbles into the chamber and automatically shut-off the sample compartment. A pump was turned on to compress the CO<sub>2</sub> to the set pressure. A pump cooler was also turned on, which cools the pump and CO<sub>2</sub> to approximately 0 °C to enhance compression and increase the pump flow capacity. Pump pressure (CO<sub>2</sub>) continues to increase until the default pressure of 600 bars (9 000 psi) was reached. Three experimental runs were conducted using (1) a low temperature, low pressure (450 bar, 60 °C), (2) high pressure, low temperature (600 bar, 55 °C), and (3) high pressure, high temperature (600 bar, 75 °C) at constant flow rate of 30 g/h.

As the run continues, oil was extracted from egusi seed and collected once ambient conditions were restored at the end of the extraction run (Tenllado et al., 2011). The extracted egusi oil (EO) was collected via an amber glass container and weighed every 10–20 minutes until constant weight.

### 2.4. Determination of egusi oil yield

The oil yield was calculated by taking into account the mass of the extracted oil and the mass of the seeds used for oil extraction by using Eq. (1) (Rebolleda et al., 2012).

$$\text{Oil yield (\%)} = \frac{\text{Mass of extracted oil}}{\text{Mass of seed}} \times 100 \quad (1)$$

## 2.5. Total fat and fatty acid determination of egusi oil

The total fat and fatty acid of egusi oil were determined according to the standard AOAC official method (AOAC, 2005). Samples were digested and analysed with Gas Chromatography Agilent technology (7890A) GC system as discussed in the following paragraph.

### 2.5.1. Egusi oil digestion

Homogenized egusi oil (100 mg), was weighed accurately into 70 ml tube in triplicate. Pyrogallic acids (100 mg), internal standard (2 ml) and ethanol (2 ml) were added and mixed well using a vortex mixer until the product was dispersed. An aliquot (10 ml) of hydrochloric acid (32% concentration) was added to the mixture. The tubes were placed in the water bath at 70–80 °C and shaken gently for 40 minutes. The content of the tubes was gently swirled at every 12 minutes. After digestion, the tubes were removed and cooled to room temperature. Extraction of the sample was carried out by adding 25 ml of diethyl ether, and the mixture gently swirled for 5 minutes. Petroleum ether was also added and swirled for 5 minutes. After the separation of two layers, the clear upper layer was removed and placed in a 150 ml beaker. Ether fumes were evaporated in the fume hood till dryness.

### 2.5.2. Gas chromatography and gas chromatography-mass spectrometry analysis

Derivatization/methylation of the sample was achieved by reconstituting the residue with 3 ml of chloroform and 3 ml of diethyl ether. The solution was transferred into 10 ml tube to evaporate under nitrogen streams. Two milliliters of hydrogen sulfate (H<sub>2</sub>SO<sub>4</sub>) in methanol (1:2) and 1 ml toluene were added; the tube was closed and placed in the oven at 100 °C to prevent dryness. After 45 minutes the tube was removed and cooled to room temperature. Five milliliters of water and 1 ml of hexane was added, capped and shook for 1 minute. After separation of layers, the clear upper layer was carefully transferred to another tube. Anhydrous sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>) was added to the mixture until the solution was clear. The clear sample was transferred into a vial and capped. The vial was placed in the auto sampler and the content analysed by Gas chromatography (GC) analysis. The load method for the gas chromatography (GC) was set using fatty acid methyl esters (FAME); suitable and individual fatty acids were run to confirm retention time. After testing the purity of egusi oil samples, and separating it into different components. The sample was transferred to a gas chromatography-mass spectrometry for identification and confirmation of the type of substance present in the oil sample.

## 2.6. Determination of atherogenicity and thrombogenicity index of egusi oil

To establish the genetic effect of egusi oil on human health the index of atherogenicity (IA) and index of thrombogenicity (IT) (Siano et al., 2016) evaluated. Index of atherogenicity of egusi oil was estimated using Eq. (2).

$$IA = \frac{[(4 \times C14 : 0) + C16 : 0 + C18 : 0]}{[\sum MUFA + \sum PUFA - n6 + \sum PUFA - n3]} \quad (2)$$

where IA = Index of atherogenicity; C14:0 = Myristic, C16:0 = Palmitic, C18:0 = Stearic; MUFA = Monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n6 = omega 6 fatty acid; n3 = omega 3 fatty acid.

The index of thrombogenicity (IT) was estimated using Eq. (3)

$$IT = \frac{C14 : 0 + C16 : 0 + C18 : 0}{0.5 \times MUFA + 0.5 \times PUFA - n3 + PUFA - n3 \div PUFA - n6} \quad (3)$$

where IT = Index of Thrombogenicity; C14:0 = Myristic; C16:0 = Palmitic; C18:0 = Stearic; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; n6 = Omega 6 fatty acid and n3 = Omega 3 fatty acid.

## 2.7. Physicochemical properties of egusi oil

Standard AOAC methods were used to determine the physicochemical properties of egusi oil, checking the iodine value and oxidative stability (AOAC, 2005).

## 2.8. Iodine value of egusi oil

Egusi oil was weighed in triplicate into a 500 ml conical flask containing 20 ml chloroform. Iodine (25 ml) was pipette into the flask, swirled and allowed to stand for 30 minutes. Potassium iodide (20 ml) with a concentration of 15% and 100 ml freshly boiled water were added to the sample. The mixture was titrated against sodium thio-sulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) of 0.1 N until the yellow colour has almost disappeared using a magnetic stirrer. Few drops of starch indicator were added to the mixture and titrated until blue color disappears. The conical flask was stirred continuously to remove iodine from the chloroform phase until a pale blue color was observed and final titer recorded. The iodine number calculated using Eq. (4).

$$\text{Iodine Number} = [(B - S) \times N \times 12.69] / g \text{ sample} \quad (4)$$

where B = Blank titre value; S = Titre value; N = Number of mole of solvent; g = Weight of samples in gram.

## 2.9. Oxidative stability index (OSI) of egusi oil

The oxidative stability of egusi oil was carried out using Methrohm 743 Rancimat. The Rancimat was assembled by placing the air tube below the connection of the reaction vessel. The O-ring was placed over the end of each air tube. Thread adapter (M8/M6) was gently screwed into the connection, and the air tube was simultaneously pressed against the thread adapter. Polytetrafluoroethylene (PTFE) tube was inserted into the opening of the measuring vessel cover that has been filled with the double distilled water. The vessel was covered and placed into the rancimat, and simultaneously the connection plugs were inserted into the electrode.

To prepare the rancimat equipment, a heating block was heated up to the desired temperature; the reaction vessel was also prepared by filling the measuring vessel with 60 ml of deionised water. Approximately 4 g of egusi oil was weighed in duplicate directly into the reaction vessel. The filled vessels were placed on the rancimat together, while the lids were placed tightly until the required temperature of 80 °C was reached. After that, the start button was initiated and left until the induction time was reached. Once the process stops, the peak curve on the induction graph is recorded as the maximum induction time (h).

## 2.10. Data analyses

All data were collected in triplicate and results expressed as the mean  $\pm$  standard deviation. The data were subjected to multivariate analysis of variance to establish mean differences between treatments. Duncan multiple range tests were used to separate means where differences existed. All data analyses were carried out using IBM SPSS software (2016).

## 3. Results and discussion

### 3.1. Effect of temperature and pressure on the SC-CO<sub>2</sub> extraction of egusi oil

Egusi oil was produced using three different extraction parameter (1) 60 °C, 30 g/h, 450 bar (EO1), (2) 55 °C, 30 g/h, 600 bar (EO2) and (3) 75 °C 30, 600 bar (EO3). Increasing the temperature from 55 to 75 °C increased the oil yield from 46 to 50 and 53% w/w, for EO1, EO2 and EO3 respectively. EO1 (low temperature and pressure) has the lowest yield compared to EO2 and EO3 which was extracted at high temperature and pressure. Özkal and Yener (2016) had a similar report for flaxseed particles, which contained 32.74% w/w oil. Also, a similar report was given by Opoku-Boahe et al. (2013) on *Castor Ricinus communis* L, a very ancient oilseed crop cultivated because of the high oil content of the seeds, which ranges between 42 and 58% extracted by cold pressing. An oil seed gives more than 50% oil yield

during any method of extraction. The only disadvantage to cold press method is the discolored meal obtained after extraction.

In a supercritical extraction process, the effect of temperature on the extraction rate, at constant pressure, is due to two mechanisms (1) the increase in process temperature, which increases the solubility due to solute vapor pressure enhancement and (2) reduction in the solubility due to the decrease in solvent density. The solvent power is described regarding the supercritical carbon dioxide (SC-CO<sub>2</sub>) density under the operating conditions and is influenced by the temperature (Gouda et al., 2017).

The high pressure and temperature increased the solubility and diffusivity of the egusi oil so that the mass transfer resistance decreased (Martins et al., 2015). A steady high carbon dioxide flow rate of 30 g/hr in this study increased convection, such that the mass transfer resistance decreased (Eckert et al., 1995; Huang et al., 2011). This could also be due to the high solubility of the fluid phase which is due to a high driving force exerted on the solid material (Santos et al., 2017). The general rule in the SC-CO<sub>2</sub> extraction is that the higher the pressure, the larger the solvent power and the larger the extracted yield. At high temperature and pressure of 75 °C and 600 bar (EO3), egusi oil yield increased significantly.

### 3.2. Proximate composition of egusi oil from SC-CO<sub>2</sub> extraction

Proximate compositions of egusi oil are shown in Table 1. The moisture content of the oil was 1.1% w/w for EO1, 1.1% w/w for EO2 and 1.0% w/w for EO3, which did not differ significantly. The low moisture content in the three oil sample could be due to the low moisture content (5–8 % w/w) of raw egusi seed and also due to the use of high-grade supercritical CO<sub>2</sub>. High-grade supercritical CO<sub>2</sub> is dry with no trace of the water molecule (Perakis et al., 2005). This finding is not similar to the 4.1% moisture content of egusi oil as reported by Onyeike and Acheru (2002) in melon seeds. Özkal and Yener (2016) also reported the moisture of flaxseed oil after supercritical

**Table 1.** Proximate composition of egusi oil.

Proximate (%)	Egusi oil <sup>1, 2</sup>		
	EO1	EO2	EO3
Moisture	1.1 ± 0.0 <sup>a</sup>	1.1 ± 0.9 <sup>a</sup>	1.0 ± 0.3 <sup>a</sup>
Protein	0.8 ± 0.3 <sup>a</sup>	1.0 ± 0.2 <sup>b</sup>	1.1 ± 0.3 <sup>b</sup>
Ash	0.7 ± 0.1 <sup>a</sup>	0.8 ± 0.3 <sup>a</sup>	0.7 ± 0.2 <sup>a</sup>
Fat	97.4 ± 1.4 <sup>a</sup>	97.1 ± 1.2 <sup>a</sup>	97.2 ± 0.9 <sup>a</sup>

<sup>1</sup> Values are mean ± standard deviation. Means with different superscript in each row differ significantly (p ≤ 0.05).

<sup>2</sup> EO1 = oil extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; EO2 = oil extract at 55 °C, carbon dioxide flow rate 30 g/h and pressure 600 bar; EO3 = at 75 °C, carbon dioxide flow rate 30 g/h and pressure 600 bar.



extraction process to be 3.4% w/w in flaxseed oil, the high moisture of 10–14% increases the propensity of the hydrolytic breakdown of the oil, leading to a higher free fatty acid content and rancid flavour. Hence low moisture below 1.5% in egusi oil, will proffer oil that is less prone to rancidity.

Egusi oil protein was 0.8, 1.0 and 1.1% w/w for EO1, EO2 and EO3, respectively. EO1 was significantly ( $p \leq 0.05$ ) lower in protein than EO2 and EO3. The low protein was due to the non-leaching of the nutrient component of the residue (defatted flour) into the oil. This is one of the green processing impacts of the supercritical extraction procedure, as the residue after extraction retains its nutritional composition and can be made into a functional component (Chemat et al., 2017). High pressure and temperature led to the separation of the solid matrix, releasing more of the liquid component of the solid during extraction.

The ash content of egusi oil was 0.7% w/w (EO1), 0.8% w/w (EO2) and 0.7% w/w (EO3), with no significant difference. The ash content of egusi oil was relatively low, and this could be due to less reaction time of the solute with the extraction solvent. Extraction was done at a pulse speed, allowing minimal contact of egusi seed with the supercritical CO<sub>2</sub>. The separation only allows the release of the liquid component, leaving the solid matrix and its constituents intact.

The total fat content ranged from 97.1–97.4%, EO1 (97.4% w/w), EO2 (97.1% w/w) and EO3 (97.2% w/w), with no significant difference. The high-fat contents could be attributed to the high pressure, temperature and high CO<sub>2</sub> flow rate used. An increase in the temperature increased the oil extraction process by causing less resistance in the solid matrix (Martins et al., 2015). Also at high pressure, there is a high yield of soluble nutrient in the liquid phase. This could be due to the high solubility of the fluid phase which indicates high driving force exerted on the solid material (Özkal et al., 2005). El-Adawy and Taha (2001) reported that using solvent extraction, the fat content of 21.33% was observed in watermelon seeds, while the fat content in a fluted pumpkin was 50%, indicating an effective extraction process.

The total fat content of 97.1 to 97.4% obtained in this study indicates that egusi oil has significantly high-fat content compared to watermelon and fluted pumpkin seeds. The proximate properties of egusi oil (EO) decreased with a rise in pressure, due to the destructive effect of high pressures on a solid structure of the seed. As pressure increased from 450 bar to 600 bar, an increase of 46% to 53% w/w in the oil yield was observed. This result is similar to that obtained by Davarnejad et al. (2008) which shows that  $\beta$ -carotene solubility increased with increasing pressure at a constant temperature of 40 °C. The changes in nutritional composition were possibly due to the additional damages on oil cells affected by pressure (Agu et al., 2018).

The residue obtained after a supercritical carbon dioxide extraction procedure retained its nutritional composition and can be made into more functional food.

This is the shortcoming of any other extraction methods, as the residue obtained is discarded as waste due to its off colour, flavour and low nutritional composition.

### 3.3. Fatty acid composition of egusi oil from SC-CO<sub>2</sub> extraction

The fatty acid composition of egusi oil in Table 2 showed the saturated fatty acids in egusi oil were undecanoic (C11), myristic (C14), palmitic (C16) and stearic (C18). Palmitic and stearic acid were (12, 13, and 13 mg/100 g), (13, 13, and 15 mg/100 g) for EO1, EO2 and EO3, respectively. Egusi oil is a source of undecanoic fatty acid 7.8, 8.0 and 8.3 mg/100 g for EO1, EO2 and EO3, respectively with no significant difference. Undecanoic fatty acid helps with wound healing and can be used as an antifungal agent in the treatment of ringworm. The only monounsaturated fatty acid found in egusi oil was oleic acid (C18:1n9c) 17.3, 18.2 and 19.2 mg/100 g respectively, for EO1, EO2 and EO3, which differed significantly ( $p \leq 0.05$ ).

Monounsaturated fatty acid (MUFA) averagely contributed to 18.2% w/w of the total fatty acids, while polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) contributed to 53% w/w and 21.2% w/w, respectively. Omega-3 fatty acid present in

**Table 2.** Fatty acids composition of egusi oil.

Fatty acids	Egusi oil <sup>1,2</sup>		
	EO1	EO2	EO3
<b>Saturated</b>			
Undecanoic (C11)	7.81 ± 0.76 <sup>a</sup>	8.01 ± 0.70 <sup>a</sup>	8.27 ± 0.20 <sup>b</sup>
Myristic (C14)	0.06 ± 0.00 <sup>a</sup>	0.06 ± 0.00 <sup>a</sup>	0.06 ± 0.00 <sup>a</sup>
Palmitic (C16)	12.15 ± 0.75 <sup>a</sup>	13.35 ± 0.18 <sup>b</sup>	13.33 ± 0.42 <sup>b</sup>
Stearic (C18)	12.62 ± 1.09 <sup>a</sup>	14.12 ± 0.51 <sup>b</sup>	14.99 ± 0.03 <sup>b</sup>
<b>Mono</b>			
Oleic (C18:1n9c)	17.31 ± 0.54 <sup>a</sup>	18.18 ± 0.21 <sup>b</sup>	19.16 ± 0.16 <sup>c</sup>
<b>Poly-n-3</b>			
Alpha-linolenic (C18:3n3)	0.32 ± 0.02 <sup>a</sup>	0.32 ± 0.02 <sup>a</sup>	0.38 ± 0.01 <sup>b</sup>
<b>Poly-n-6</b>			
Linoleic (C18:2n6t)	53.30 ± 0.32 <sup>a</sup>	54.13 ± 0.16 <sup>b</sup>	56.21 ± 0.21 <sup>c</sup>
ΣSFA (Saturated fatty acid)	20.02 ± 1.51 <sup>a</sup>	21.41 ± 0.25	21.66 ± 0.61 <sup>a</sup>
ΣPoly n-3 (omega 3)	0.32 ± 0.02 <sup>a</sup>	0.32 ± 0.02 <sup>a</sup>	0.38 ± 0.01 <sup>b</sup>
ΣPoly n-6 (omega 6)	53.62 ± 0.34 <sup>a</sup>	54.14 0.16 <sup>b</sup>	56.22 ± 0.21 <sup>c</sup>
IA (index atherogenicity)	0.35 ± 0.02 <sup>a</sup>	0.38 ± 0.01 <sup>b</sup>	0.38 ± 0.00 <sup>b</sup>
IT (index thrombogenicity)	0.08 ± 0.01 <sup>a</sup>	0.09 ± 0.01 <sup>a</sup>	0.09 ± 0.05 <sup>a</sup>

<sup>1</sup> Values are mean ± standard deviation. Means with different superscript in each row differ significantly ( $p \leq 0.05$ ).

<sup>2</sup> EO1 = oil extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; EO2 = oil extract at 55 °C, carbon dioxide flow rate 30 g/h and pressure 600 bar; EO3 = at 75 °C, carbon dioxide flow rate 30 g/h and pressure 600 bar.

egusi seed was 0.32, 0.32 and 0.38 mg/100 g for EO1, EO2 and EO3. EO3 was significantly high compared to EO1 and EO2.

The major fatty acid in egusi oil was C18:2n6t [conjugated linoleic acid] was identified using gas chromatography-mass spectrophotometer (GCMS) and was high in the three egusi oil samples, making egusi oil a nutraceutical oil to be explored in the food industries.

Among the three oil samples, EO3 was significantly ( $p \leq 0.05$ ) different from EO1 and EO2, having the highest linoleic acid content. This could be due to different temperature and pressure used during extraction. The concentrations of linoleic, oleic, palmitic acid and stearic acids increased with increase in temperature and pressure. [Giwa et al. \(2010\)](#) reported similar result for egusi oil, high in: C18:1 (15.8 mg/100 g), C18:2 (64.0 mg/100 g) and C16:0 (10.3 mg/100 g). Linoleic acid plays a very important role in nervous cell construction. It is also fundamental to the prevention of cardiovascular diseases ([Santos et al., 2017](#)).

The index of atherogenicity (IA) ([Table 2](#)) was low 0.35, 0.38 and 0.38 mg/100 g for EO1, EO2 and EO3, respectively, with a significant ( $p \leq 0.05$ ) difference, EO1, having the lowest IA value. While the thrombogenicity index (IT) was 0.08, 0.09 and 0.09 mg/100 g for EO1, EO2 and EO3, respectively with no significant difference. These lipid indices were reported and calculated according to [Ghaeni et al. \(2013\)](#).

All IT and IA values were lower than those reported for pomegranate and cherry, where IT of 0.30 and 0.75 mg/100 g, respectively and IA of 0.15 and 0.42 mg/100 g, respectively ([Siano et al., 2016](#)) were reported. However, the values obtained were similar to that of pumpkin seed oil with IA (0.34 mg/100 g) and IT (0.65 mg/100 g) ([Siano et al., 2016](#)). Index of atherogenicity (IA) indicates the relationship between the sum of the main saturated and unsaturated fatty acids. The main saturated being considered pro-atherogenic (favoring the adhesion of lipids to cells of the immunological and circulatory system), and the anti-atherogenic [inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, thereby preventing the appearance of micro and macro coronary diseases ([Ruiz-cara & Garcı, 2007](#); [Akanda et al., 2012](#)).

### 3.4. Physicochemical characteristics of egusi oil from SC-CO<sub>2</sub> extraction

The physicochemical properties of egusi oil are shown in [Table 3](#). Oxidative stability index (OSI) is a measure of overall oxidation level of oil samples, which can give a better estimation of the progressive oxidative deterioration of oil ([Adegoke and Ndife, 1993](#); [Smith et al., 2007](#)). The oxidative stability of oil will be high when polyunsaturated fatty acid is low due to the presence of a high degree of saturation in the bond of the oil. The stability of EO1, EO2 and EO3 at 120 °C was expressed as

**Table 3.** Physicochemical properties of egusi oil.

Parameters	Egusi oil <sup>1,2</sup>		
	EO1	EO2	EO3
Iodide value (g/I <sub>2</sub> /100 g)	98.66 ± 5.77 <sup>a</sup>	95.33 ± 5.77 <sup>a</sup>	129.33 ± 4.62 <sup>b</sup>
Oxidation stability (h)	10.17 ± 0.00 <sup>a</sup>	11.50 ± 1.14 <sup>a</sup>	5.26 ± 0.91 <sup>b</sup>

<sup>1</sup> Values are mean ± standard deviation. Means with different superscript in each column differ significantly ( $p \leq 0.05$ ).

<sup>2</sup> EO1 = oil extracted at 60 °C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; EO2 = oil extract at 55 °C, carbon dioxide flow rate 30 g/h and pressure 600 bar; EO3 = at 75 °C, carbon dioxide flow rate 30 g/h and pressure 600 bar.

induction time of oxidation, which ranged from 5.3 to 10.2 h. These values were greater than those reported for linseed oil (1.1 h) and olive oil (6.1 h) (Geerdts, 2005). The high induction time of egusi oil (EO) could be attributed to the presence of a large number of natural antioxidants such as oleic acid (Raes et al., 2004; Jarret and Levy, 2012).

Oxidation stability of EO1, EO2 and EO3 were 10.2, 11.5 and 5.3 h, respectively (Table 3). EO1 and EO2 (10.2 and 11.5 h, respectively) had the highest oxidation induction time, which was significantly ( $p \leq 0.05$ ) higher from EO3 (5.26 h). The differences in the oil sample can be attributed to the different temperature and pressure used during extraction.

The iodine number of egusi oil was relatively high at 98.7, 95.3 and 129.3, respectively for EO1, EO2 and EO3. EO3 has a significantly ( $p \leq 0.05$ ) higher iodine value when compared to EO1 and EO2. This could be due to the presence of polyunsaturated fatty acids which was highest in sample EO3 in egusi oil. This similar to the value 112.53 g/I<sub>2</sub>/100 g reported by Agu et al. (2018), but lower than the 157.15 g/I<sub>2</sub>/100 g reported by Ogunwole (2015). The iodine number equals the number of mg of iodine required to saturate the fatty acids present in 100 mg of the oil or fat. The iodine numbers are often used to determine the amount of unsaturation in fatty acids. The higher iodine value of the other works in the literature compared to that obtained in this work is an indication of the high level of unsaturation in oils (Herchi et al., 2016).

Oil-rich in saturated fatty acids has low iodine numbers, while oils rich in unsaturated fatty acids have high iodine numbers (Abaelu et al., 1990; Igwenyi and Akubugwo, 2010). Egusi oil extracted using high pressure (600 bar) and temperature (75 °C), had the highest iodine number. Iodine numbers are often used to determine the amount of unsaturation in fatty acids. High temperature and pressure denature the nutrient density of the oil, especially its protein and mineral content, leading to less stable oil (Abaelu et al., 1990; Igwenyi and Akubugwo, 2010). EO1 (60 °C and 450 bar) shows a low peroxide number, high iodine number and high stability at high temperature.

### 3.5. Conclusion

Supercritical CO<sub>2</sub> extraction of egusi seed oil was achieved by experimental runs, which was divided into three. The amount of the oil recovered in the process was high due to the high pressure and temperature used. The oil obtained from the high pressure, a high-temperature process has the lowest nutritional composition. The oil with the best yield in term of nutritional composition was obtained from the low-temperature 60 °C, low pressure 450 bar process, which was conclusively the best process parameter to be explored for future processing of egusi seed using supercritical fluid extraction. Nutritious and functional oil was extracted from *Citrullus lanatus* subsp-*Mucosospermus*. Egusi oil should be introduced into the food industry, due to its high omega-6 fatty acid content (conjugated linoleic acid) which will greatly reduce the risk of coronary heart disease and obesity, and serve as new functional oil for the food industry.

### Declarations

#### Author contribution statement

Olakunbi Olubi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Victoria A Jideani: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Joseline Felix-Minnaar: Analyzed and interpreted the data.

### Funding statement

This work was supported by the University Research Fund (Cape Peninsular University of Technology).

### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

### References

Abaelu, A.M., Olukoya, D.K., Okochi, V.I., Akinrimisi, E.O., 1990. Biochemical changes in fermented melon (egusi) seeds (*Citrullis vulgaris*). *J. Ind. Microbiol.*

- Adegoke, G.O., Ndife, J., 1993. Effect of sample pretreatments on the storability of ground melon 'egusi'-*Colocynthis citrillus* L. *Plant Foods Hum. Nutr.* 43 (1), 77–85.
- Agu, C.M., Kadurumba, C.H., Agulanna, A.C., Aneke, O.O., Agu, I.E., Eneh, J.N., 2018. Industrial crops & products nonlinear kinetics, thermodynamics, and parametric studies of *colocynthis vulgaris* shrad seeds oil extraction. *Ind. Crop. Prod.* 123 (July), 386–400.
- Akanda, M.J.H., Sarker, M.Z.I., Ferdosh, S., Manap, M.Y.A., Ab Rahman, N.N.N., Ab Kadir, M.O., 2012. Applications of supercritical fluid extraction (SFE) of palm oil and oil from natural sources. *Molecules* 17 (2), 1764–1794.
- Akobundu, E., 1989. Effect of full-fat egusi (melon seed) meal on quality of cooked Nigerian sausage. *Food Qual. Prefer.* 109–111.
- Akubor, P.I., Ogbadu, R.L., 2003. Effects of processing methods on the quality and acceptability of melon milk. *Plant Foods Hum. Nutr.* 58 (1), 1–6.
- Akusu, D.B.K.O.M., 2014. Effect of processing on the proximate composition , functional properties and storage stability of water melon ( *Citrullus lanatus* ) seed flour. *Int. J. Biotechnol. Food Sci.* 2 (November), 143–148. ISSN: 2384-7344.
- AOAC, 2005. Official Method of Analysis, eighteenth edition. Association of Official Analytical Chemists, Washington DC, pp. 20877–22417.
- Chemat, F., Rombaut, N., Meullemiestre, A., Turk, M., Chemat, F., Rombaut, N., et al., 2017. Review of Green Food Processing techniques. Preservation, transformation, and extraction to cite this version: HAL Id: hal-01552142. *Innovat. Food Sci. Emerg. Technol.* 41, 357–377.
- Davarnejad, R., Kassim, K.M., Zainal, A., Sata, S.A., 2008. Supercritical fluid extraction of b -carotene from crude palm oil using CO<sub>2</sub>. *J. Food Eng.* 89, 472–478.
- Eckert, C.A., Pouillot, F.L.L., Gurdial, G.S., Knutson, B.L., Scott, L.S., 1995. Selective separation of valerie acid from dibasic acids using supercritical carbon dioxide. *J. Supercrit. Fluid* 8 (1), 1–5. PII: 0896-8446(95)90043-90048.
- Efavi, J.K., Kanbogtah, D., Apalangya, V., Nyankson, E., Tiburu, E.K., Doodoo-Arhin, D., et al., 2018. The effect of NaOH catalyst concentration and extraction time on the yield and properties of *Citrullus vulgaris* seed oil as a potential biodiesel feed stock. *S. Afr. J. Chem. Eng.* 25, 98–102.
- El-Adawy, T.A., Taha, K.M., 2001. Characteristics and composition of watermelon, pumpkin, and paprika seed oils and flours. *J. Agric. Food Chem.* 49 (3), 1253–1259.

Geerdts, I., 2005. Extraction of Olive Oil with Supercritical Carbon Dioxide. Master Thesis North West University, Potchefstroom. [http://health120years.com/cn/pdf/hd\\_SFE\\_Olive.pdf](http://health120years.com/cn/pdf/hd_SFE_Olive.pdf).

Ghaeni, M., Ghahfarokhi, K.N., Zaheri, L., 2013. Fatty acids profile , atherogenic ( IA ) and thrombogenic ( IT ) health Lipid Indices in *Leiognathus bindus* and *Upe-neussulphureus*. *J. Marine Sci. Res. Dev.* 3 (4), 3–5.

Giwa, S., Abdullah, L.C., Adam, N.M., 2010. Investigating “egusi” (*Citrullus colocynthis* L.) seed oil as potential biodiesel feedstock. *Energies* 3 (4), 607–618.

Gouda, N., Singh, R.K., Meher, S.N., Panda, A.K., 2017. Production and characterization of bio oil and bio char from fl ax seed residue obtained from supercritical fluid extraction industry. *J. Energy Inst.* 90 (2), 265–275.

Herchi, W., Ammar, K.B.E.N., Bouali, I., Abdallah, I.B.O.U., Guetet, A., Boukhchina, S., 2016. Heating effects on physicochemical characteristics and antioxidant activity of flaxseed hull oil (*Linum usitatissimum* L). *Food Sci. Technol.*

Huang, Z., Xu, L., Li, J., Kawi, S., Goh, A.H., 2011. Organic template removal from hexagonal mesoporous silica by means of methanol-enhanced CO<sub>2</sub> extraction : effect of temperature , pressure and flow rate. *Separ. Purif. Technol.* 77, 112–119.

Igwenyi, I.O., Akubugwo, E.I., 2010. Analysis of four seeds used as soup thickeners in the south eastern part of Nigeria. In: *Proceedings ICCCE 2010 – 2010 International Conference on Chemistry and Chemical Engineering*, pp. 426–430.

Jarret, R.L., Levy, I.J., 2012. Oil and fatty acid contents in seed of *Citrullus lanatus* Schrad. *J. Agric. Food Chem.* 60 (20), 5199–5204.

Jokić, S., Nagy, B., Zeković, Z., Vidovic, S., Bilić, M., Velić, D., Simándi, B., 2012. Effects of supercritical CO<sub>2</sub> extraction parameters on soybean oil yield. *Food Bioprod. Process* 90 (4), 693–699.

Kouebou, C.P., Achu, M., Nzali, S., Chelea, M., Bonglaisin, J., Kamda, A., et al., 2013. A review of composition studies of Cameroon traditional dishes : macronutrients and minerals. *Food Chem.* 140 (3), 483–494.

Mali, A.M., Chavan, N.S., 2016. In vitro rapid regeneration through direct organogenesis and ex-vitro establishment of *Cucumis trigonus* Roxb.-An underutilized pharmaceutically important cucurbit. *Ind. Crop. Prod.* 83, 48–54.

Martins, P.F., De Melo, M.M.R., Silva, C.M., 2015. Gac oil and carotenes production using supercritical CO<sub>2</sub> : sensitivity analysis and process optimization through a RSM – COM hybrid approach. *J. Supercrit. Fluids* 100, 97–104.

- Ogunwole, O.A., 2015. Production of biodiesel from watermelon (*Citrullus lanatus*) seed oil. *Leonardo J. Sci.* (27), 63–74. Retrieved from. [http://js.academicdirect.org/A27/063\\_074.pdf](http://js.academicdirect.org/A27/063_074.pdf).
- Onuora, J.O., King, R.D., 1984. Thermal transitions of melon seed proteins. *Food Chem.* 13, 309–316.
- Onyeike, E.N., Acheru, G.N., 2002. Chemical composition of selected Nigerian oil seeds and physicochemical properties of the oil extracts. *Food Chem.* 77 (4), 431–437.
- Opoku-Boahen, Y., Novick, B.D., Wubah, D., 2013. Physicochemical characterization of traditional Ghanaian cooking oils derived from seeds of Egusi [*Citrullus colocynthis*] and Werewere [*Cucumeropsis manni*]. *Int. J. Biol. Chem. Sci.* 7 (1), 387–395.
- Özkal, S.G., Yener, M.E., 2016. Supercritical carbon dioxide extraction of flaxseed oil: effect of extraction parameters and mass transfer modeling. *J. Supercrit. Fluids* 112, 76–80.
- Özkal, S.G., Yener, M.E., Bayindirli, L., 2005. Response surfaces of apricot kernel oil yield in supercritical carbon dioxide. *LWT-Food Sci. Technol.* 38 (6), 611–616.
- Perakis, C., Louli, V., Magoulas, K., 2005. Supercritical fluid extraction of black pepper oil. *J. Food Eng.* 71 (4), 386–393.
- Raes, K., De Smet, S., Demeyer, D., 2004. Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. *Anim. Feed Sci. Technol.*
- Rebolleda, S., Rubio, N., Beltrán, S., Sanz, M.T., González-Sanjosé, M.L., 2012. Supercritical fluid extraction of corn germ oil: study of the influence of process parameters on the extraction yield and oil quality. *J. Supercrit. Fluids* 72, 270–277.
- Ruiz-cara, T., Garcá, M., 2007. On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (*Sparus aurata*). *Food Chem.* 101, 298–307.
- Santos, Ê.R.M., Oliveira, H.N.M., Oliveira, E.J., Azevedo, S.H.G., Jesus, A.A., Medeiros, A.M., Dariva, C., Sousa, E.M.B.D., 2017. Supercritical fluid extraction of *Rumex acetosa* L. roots : yield , composition , kinetics , bioactive evaluation and comparison with conventional techniques. *J. Supercrit. Fluid* 122, 1–9.
- Siano, F., Straccia, M.C., Paolucci, M., Fasulo, G., Boscaino, F., Volpe, M.G., 2016. Physico-chemical properties and fatty acid composition of pomegranate, cherry and pumpkin seed oils. *J. Sci. Food Agric.* 96 (5), 1730–1735.



Smith, S.A., King, R.E., Min, D.B., 2007. Oxidative and thermal stabilities of genetically modified high oleic sunflower oil. *Food Chem.* 102 (4), 1208–1213.

Tenllado, D., Reglero, G., Torres, C.F., 2011. A combined procedure of supercritical fluid extraction and molecular distillation for the purification of alkylglycerols from shark liver oil. *Separ. Purif. Technol.* 83 (1), 74–81.

Wang, J.P., Yu, B., Xu, X., Yang, N., Jin, Z., Kim, J.M., 2011. Orthogonal-function spectrophotometry for the measurement of amylose and amylopectin contents. *Food Chem.* 127 (1), 102–108.