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Extraction and Physicochemical Properties of Pumpkin (*Cucurbita moschata*) Seed Oil as a Renewable Source for Biodiesel Production

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Abstract

In this study, oil was experimentally extracted from pumpkin seeds (*Cucurbita moschata*) using a chemical Soxhlet extraction, cold solvent extraction and mechanical pressing. The Percentage yields were found to be 39.5%, 30.2%, 26% respectively. The physicochemical properties were determined according to AOAC, AOCS and ASTM standards. Free fatty acids content of the oil was found to be 1.2% as oleic acid, acid value (2.4 mg KOH/g), peroxide value (6.77 meq O_2/kg oil), saponification value (191.09 mg KOH/g oil), iodine value (104.81 g I_2/100 g), density (0.931 g/ml) at 25°C, kinematic viscosity (48.05 cst)at 40°C. The color of pumpkin seed oil was greenish brown, pH(6), cloud point (-5 °C), refractive index (1.471), water content (1.95%). Additionally, the elements in the oil were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES). The fatty acid composition of the oil was analyzed utilizing gas chromatography-mass spectrometry (GC-MS). The results showed that the oil contains about 22.57% saturated fatty acids and 73.21% unsaturated fatty acids. Furthermore, Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups present in the oil.

Keywords: Oil content, Extraction, pumpkin seeds, physicochemical properties, Free fatty acid profile. FTIR.

INTRODUCTION

Pumpkin oil is one of the vegetable oils extracted from pumpkin seeds. Pumpkin is a plant from the Cucurbitaceae family, which is widely cultivated around the world. There are three common and different types of pumpkin**:** Cucurbita pepo, Cucurbita moschata, and Cucurbita maxima. All of these are commonly referred to as pumpkin (Kukeera *et al.,* 2015). Among the most widely cultivated species of Cucurbita in Sudan is Cucurbita moschata. It is an annual dicotyledonous plant, with creeping or climbing stems (growing up to 5 m) bearing tendrils. The stems are strong, cylindrical or pentangular. The fruits are round, oblate, oval, oblong, or pear-shaped and contain seeds inside. The seeds differ greatly in size based on variety and type (Ezin *et al.,* 2022). Cultivars of C. moschata are generally more tolerant of hot, humid weather than squash of other domesticated species and exhibit a greater resistance to certain diseases and insects (Men *et al.,* 2021).

Pumpkin is valued as a functional food due to its rich content of nutrients, including carbohydrates, lipids, fiber, protein, along with the phytochemical compounds like carotenoids, tocopherols, and β-sitosterol (Kim *et al.,* 2012). Different parts of the pumpkin can be used in diverse applications. Pumpkin seeds are rich in protein, lipids, starch, minerals, sugars, and bioactive compounds (Hussain *et al.,* 2022). They are chewable, naturally sweet, and have a nutty flavor. In addition, they are considered a by-product of pumpkin fruit consumption.

Pumpkin seeds contain over 30.0% lipids in their composition. The exact content in seeds varies depending on several factors, such as environmental conditions, soil type, and storage conditions. The oil is a liquid at room temperature, non-volatile and water-insoluble, but it dissolves in organic solvents. It can be obtained from pumpkin seeds using different extraction methods, including solvent extraction and mechanical pressing. The extraction of pumpkin seed oil is an essential step in identifying its composition and evaluating its quality and suitability for industrial applications, particularly biodiesel production (Hu *et al.,* 2023). Pumpkin seed oil (PSO) has gained attention in recent years due to its potential health benefits, which are attributed to the content of unsaturated fatty acids, micronutrients, and antioxidant compounds (Hu *et al.,* 2023). The major fatty acids identified in pumpkin seed oil include oleic acid, palmitic acid, stearic acid, and linoleic acid. Among these, oleic acid and linoleic acid are the most abundant (Rahman *et al.,*2015).

The purpose of this study is to determine the physicochemical properties and chemical composition of pumpkin seed oil.

MATERIALS AND METHODS

Materials

Pumpkin seeds were collected from Kordofan state in Sudan and cleaned of impurities. They were sun-dried for three days, kept in a plastic container, and stored at room temperature. The chemicals used in all experiments were of analytical grade.

Methods of Analysis

Oil Extraction

Cucurbita moschata seeds were coarsely ground using a mortar and pestle. Oil was extracted using two techniques: chemical extraction and mechanical pressing. The chemical extraction involved cold solvent extraction and Soxhlet extraction. N-hexane was used as a solvent in both techniques. The yield percentage from each method was calculated as follows:

$$\text{Oil yield} = \frac{\text{weight of oil}}{\text{weight of seed}} \text{ X } 100$$

Soxhlet Extraction Method

100g of seeds were placed in a thimble and inserted into a Soxhlet extractor connected to a round-bottom flask containing n-hexane as the solvent. The extraction was continued for approximately six hours. The solvent was evaporated under reduced pressure using a rotary evaporator (Handa *et al.*, 2008).

Cold Solvent Extraction Method

200 g of seeds were placed in a 1000 ml beaker, and 400 ml of n-hexane was added to the seeds. The mixture was kept for 36 hours at room temperature under stirring at 1000 rpm using a magnetic stirrer. The solvent was evaporated using a rotary evaporator with a water bath at 70 °C. Finally, the oil extracted was kept in the oven at 70 °C for one hour to ensure the removal of any residual solvent (Hassan *et al., 2016).*

Mechanical Pressing

In this technique, the oil extraction process was conducted using an oil presser machine (WEG CFW500-vector inverter), with specifications (power: 1.5 KW), (voltage:230 V), made in Germany. 1kg of pumpkin seeds was introduced into the presser to clean it and avoid cross-contamination, and the oil extracted from this cleaning step was discarded. Then, 7kg of pumpkin seeds were introduced again to the presser for extraction. The collected oil was filtered to eliminate residual particles and stored in a dark, clean plastic container.

Physical and Chemical Properties of Pumpkin Seed Oil

The physicochemical properties of oil were determined according to ASTM, AOAC and AOCS standard methods.

Free Fatty Acid

7 g of pumpkin seed oil was placed in a conical flask, 100 ml of 95 % ethanol was added to the sample and mixed well, then 2 ml of phenolphthalein indicator (1% in 95% alcohol) was added. The mixture was titrated against a standardized sodium hydroxide (0.1 M) solution until a faint pink color appeared (AOCS Ca-5a-40).

The percentage of free fatty acid was expressed as oleic acid as per the equation below:

$$\text{Free Fatty acid as Oleic acid, \%} = \frac{\text{A X M X 28.2}}{\text{W}}$$

Where:
A: Volume of NaOH required for the titration of the sample.
M: Molarity of NaOH.
W: Weight of the sample.

Saponification Value

The saponification value of pumpkin seed oil was determined according to ASTM D 5558.

2.0g of pumpkin seed oil was weighed into a clean, dried Erlenmeyer flask, and 25ml of 0.5N ethanolic KOH was added to the flask containing the oil and mixed thoroughly. The mixture was heated in a reflux condenser for 60 min at 60-70 °C and then cooled at room temperature. 2drops of 1% phenolphthalein solution as an indicator were added to the mixture and titrated against 0.5N HCl until the color of the solution changed from pink to colorless. The same procedure was repeated for the blank. The saponification value was calculated as follows:

$$\text{Saponification Number} = \frac{\text{A} - \text{B x N x 56.1}}{\text{W}}$$

Where:
A = HCl(mL) for blank
B = HCl (mL) for sample used
N = normality of HCl
W = weight of sample oil (g)

Water Content

The water content of pumpkin seed oil was determined using the standard oven-dry method (AOAC, 2000). 5 g of oil was weighed in a dried crucible with a lid and heated at 105 °C for 6 hours to ensure stabilization of weight through successive observation. The crucible with the oil sample was cooled at room temperature in a desiccator. The weight was recorded again, and the water content was calculated using the following formula:

$$\text{Water content }\% = \frac{initial\ weight - final\ weight}{initial\ weight} * 100$$

Refractive Index (RI)

The refractive index (RI) of the oil sample was measured utilizing a precision Abbe refractometer according to the AOAC official method, with a measuring range of refractive index of 1.300-1.700 and an accuracy within ±0.0002 (Ghani *et al.,* 2018). One drop of the oil sample was placed between the illuminating and refracting prisms of the Abbe refractometer using a syringe. The sample was then illuminated with monochromatic light from a sodium vapor lamp and left to stand for 5 minutes before recording the reading displayed on the screen.

Elemental analysis

Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to determine the elements in the sample. Analysis was performed using an Agilent ICP-OES 5110 equipped with an Agilent SPS 4 autosampler. High-purity argon gas was utilized as a source of plasma. Plasma flow rate was set at 15 L/min, Auxiliary was set at 1.0 L/min, nebulizer was set at 0.5 L/min, RF power generator was set at 1300 watts, and the sample pump was set at 12 RPM. Multi-element standards (S-21+K+Li) and Boron Standards from (Conostan, Canada) were used for external calibration with concentrations of 5, 10, 20 and 50 ppm. The samples and standards were prepared and then positioned in the auto sampler for injection.

Gas Chromatography-Mass Spectrometry (GC-MS)

The fatty acid composition (%) of pumpkin seed oil was determined using a gas chromatography technique after derivatization to fatty acid methyl esters (FAMEs). GC-MS analysis was performed using GC/MS-QP2010SE (Shimadzu Japan) equipped with a capillary column (Rtx-5MS-30m×0.25mm i.d.×0.25μm). Injector temperature was 300°C, and the injector was operated in split mode. The oven temperature was programmed from 60°C to 300°C at 10 °C/min. The carrier gas was helium at a flow rate (1.6 mL/min). The volume of injection was 1μL, and the total run time was 34 minutes. The spectra of the components were compared with the database of spectra of known components stored in the GC-MS library (NIST).

Fourier Transform Infrared Spectroscopy (FTIR)

The principal functional groups present in pumpkin seed oil were detected and identified using Fourier Transform Infrared Spectroscopy (FTIR). The FT-IR spectra were recorded using FTIR-8400S instrument (Shimadzu, Japan).

RESULTS AND DISCUSSION

Oil Extraction

The oil yields from pumpkin seeds using different methods of extraction are shown in Table 1

Table 1: Oil yields of pumpkin seeds

No	Extraction method	Oil yield%
1	Soxhlet solvent extraction	39.5
2	Cold solvent extraction	30.2
3	mechanical pressing	26

The highest yield of oil (39.5%) was obtained using the Soxhlet Solvent Extraction method. This method requires more time and energy, but it is more efficient, especially when the oil content in the seeds is low. Using the cold solvent extraction method, the percentage of oil was found to be 30.2%, which was lower than that obtained by Soxhlet extraction due to the absence of continuous solvent circulation. The lowest yield among the three methods (26%) was obtained by using mechanical pressing. This method depends on force and pressure and is therefore considered eco-friendly. The oil yield of pumpkin seed (30.2%) in the present study was lower than the yields from seeds of three different pumpkin fruit shapes (32.3%, 36.9%, and 31.7%) reported by Ali *et al.* (2022), which were achieved using the same technique.

Physical and Chemical Properties of Pumpkin Seed Oil

Table 2 shows the physicochemical properties of pumpkin seed oil. The FFA value of pumpkin seed oil was found to be 1.2% as oleic acid, which is lower than the 15.88 % reported by Galander *et al*. (2017). High free fatty acid levels are unfavorable in crude vegetable oils because they cause significant losses of oil during the refining process. A low FFA value indicates that the conversion of pumpkin seed oil to biodiesel can be achieved in a single-step process through direct transesterification using an appropriate catalyst without requiring complex pre-treatment stages.

Table 2: physicochemical properties of pumpkin seed oil

Parameter	Unit	Pumpkin Seed Oil
free fatty acid (as oleic acid) FFA	%	1.2
Acid value	mg KOH/g	2.4
Saponification value	mg KOH/g	191.09
Peroxide value	meq/kg	6.778
Iodine value	g I_2/100g	104.81
Water content	WT%	1.95

Parameter	Unit	Pumpkin Seed Oil
Kinematic viscosity at 40 °C	cSt	48.05
Density at 25 °C,	g/ml	0.931
Cloud point	°C	-5
PH	-	6
Refractive index	-	1.471
Color	-	Greenish brown

Acid value is an important measure of oil quality (Igile *et al.,* 2023). A low acid value indicates that the oil remains stable over a long period (Metrohm AG, 2019). In this study, the acid value of the pumpkin seed oil was found to be 2.4 mg KOH/g, which is higher than (0.78 mg KOH/g) of pumpkin seed oil reported by Gohari Ardaili *et al.* (2010).

The saponification value of pumpkin seed oil was found to be 191.09 mg KOH/g, which is very close to (190.69 mg KOH/g) reported by Gohari Ardaili *et al.* (2010).

The peroxide value (PV) of oil was found to be 6.77 meq/kg, which is lower than the peroxide value (10.3 meq/kg) of pumpkin seed oil reported by Ibrahim *et al.* (2017). PV reflects the amount of primary oxidation products generated during the initial stages of fat degradation. Additionally, it is considered the most common indicator of lipid oxidation and is also used to evaluate the quality and age of oils.

The iodine value measures the degree of unsaturation in oils and fats (Dymińska *et al.,* 2017). The iodine value of pumpkin seed oil was found to be 104.81 gI_2/100g, which is close to (104.36 gI_2/100g) of pumpkin seed oil studied by Gohari Ardaili *et al.* (2010) and lower than the iodine values (107.9 and 109.4 gI_2/100g) reported by Ali *et al.* (2022).

Water content is an important factor in assessing oil quality because it affects oil properties such as color, odor, and promotes oxidation and microbial degradation. The water content of pumpkin seed oil was found to be 1.95% using the oven-dry method according to AOAC.

The analysis showed that the viscosity value of pumpkin seed oil was found to be 48.05 cSt, which is much higher than (29.3 cSt) of palm kernel oil reported by Charity and George (2023).

One of the most important factors affecting viscosity is temperature; it decreases at high temperatures (the oil flows more easily) and with increasing levels of unsaturation. Viscosity is commonly used to evaluate the quality of oils; old or long-stored oils usually have higher viscosity (Nichols and Sanderson, 2003).

The density of the oil was found to be 0.931 g/ml, using a density bottle, which is higher than the density of neem oil (0.88g/ml) reported by Kale *et al.* (2020). Density is considered an essential physical property to assess the purity of oils. The density of seed/vegetable oils is dependent on their fatty acid composition, minor components, and temperature (Fakhri and Qadir, 2011).

The cloud point value of pumpkin seed oil was found to be -5 °C. The cloud point is the temperature at which the oil starts to become cloudy due to crystallization (Bezergianni and Dimitriadis, 2013). It is one of the important cold-flow properties that ensures the oil performs well under different temperature conditions.

The refractive index of pumpkin seed oil was found to be 1.471 using a refractometer according to AOAC. This value is close to the refractive index of un-hulled sesame oil (1.465) reported by Amini *et al.* (2023). The refractive index of oils depends on their molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation (Nichols and Sanderson, 2003).

The oil exhibits a greenish-brown color, and it remains in a liquid state at room temperature. Its pH was found to be 6.

Elemental Content

Elemental analysis showed that the oil extracted from pumpkin seeds contains boron (0.27 ppm), calcium (1.38 ppm), sodium (0.68 ppm), silicon (0.27 ppm) and phosphorus (4.40 ppm) as presented in Table 3. These values may vary depending on the type of pumpkin, geographical location, climatic conditions, soil type and extraction method of oil. The measured concentrations of Ca and Na differ from the concentrations of Ca (0.57 ppm) and Na (1.57 ppm) in groundnut oil reported by Ichu and Nwakanma (2019), while the silicon content observed was lower than (0.97 ppm) in karaya seed oil reported by Galander *et al.* (2017). Furthermore, the analysis revealed a significant difference in the phosphorus concentration in the oil in this study compared with that in seeds from three pumpkin genotypes (9108.84 ppm, 5846.76 ppm and 8060.10 ppm) reported by Seymen *et al.* (2016). This reflects the natural variation in element content between the seeds and their extracts, as well as among the different parts of the fruits.

Table 3: Elements content of pumpkin seed oil

Elements	Concentration, ppm
Boron B	0.27
Calcium, ca	1.38
Sodium, Na	0.68
Silicon, Si	0.27
Phosphorus, P	4.40

Fatty Acid Profile of Pumpkin Seed Oil

Table 4 shows the major fatty acids present in pumpkin seed oil. The composition of fatty acids varies depending on some factors such as variety, climate conditions, cultivation region, and ripeness (Murkovic *et al.,* 1999). Pumpkin seed oil consists of 73.21% unsaturated fatty acids. The highest proportion is attributed to linoleic acid (37.01%), which is significantly lower than (50.7%) reported by Hagos *et al.,* (2023), followed by oleic acid (36.11%), which is higher than (18.8%) reported by Hagos *et al.* (2023). A trace amount of palmitoleic acid (0.09%) was also detected. In addition, pumpkin seed oil consists of 22.57% saturated fatty acids, with the major one being palmitic acid (14.29%), which is lower than (17.9%) reported by Hagos *et al*. (2023).

Table 4: Fatty acid composition of pumpkin seed oil

Free fatty acid	Formula	Structure	Area%
Myristic acid	$C_{14}H_{28}O_2$	C14:0	0.11%
Palmitoleic acid	$C_{16}H_{30}O_2$	C16:1	0.09%
Palmitic acid	$C_{16}H_{32}O_2$	C16:0	14.29%
Margaric acid	$C_{17}H_{34}O_2$	C17:0	0.13%
Linoleic acid	$C_{18}H_{32}O_2$	C18:2	37.01%
Oleic acid	$C_{18}H_{34}O_2$	C18:1	36.11%
Stearic acid	$C_{18}H_{36}O_2$	C18:0	7.53%
Arachidic acid	$C_{20}H_{40}O_2$	C20:0	0.51%

Fourier Transform Infrared Spectroscopy (FTIR) of Pumpkin Seed Oil

The FTIR analysis of pumpkin seed oil reveals distinct functional groups indicative of its molecular structure, as shown in Fig.1. The spectrum exhibits absorbance peaks at 2925.51 cm^{-1} and 2856.61 cm^{-1} due to the stretching vibration of (C-H) in the CH2, CH3 groups, which generally occur in fats and oils. The band located at 1745.77cm^{-1}corresponds to the stretching vibration of the carbonyl group (C=O) of esters. The peak at 1162.14 cm^{-1} is attributed to the C-O vibration.

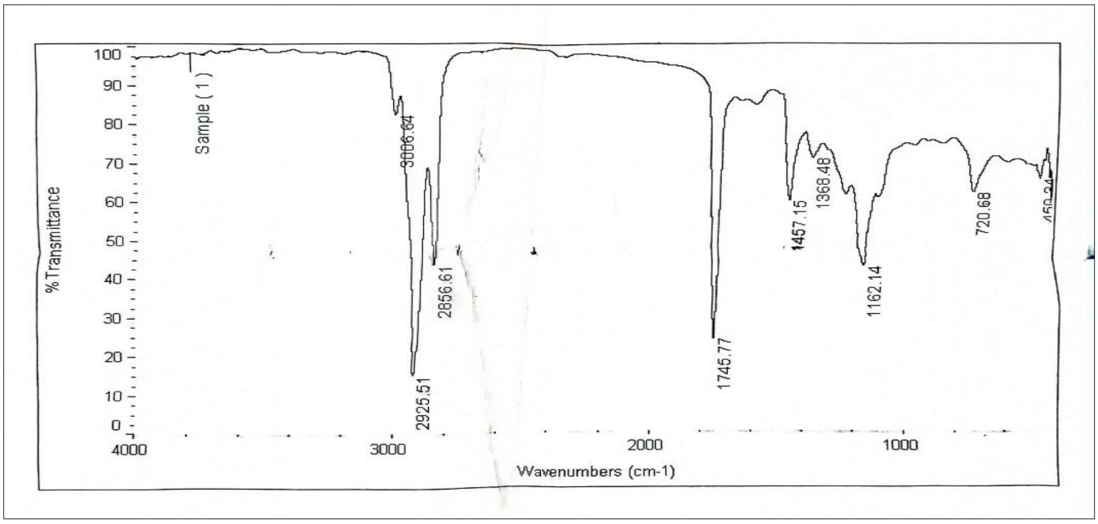

Fig. 1: FTIR spectrum of pumpkin seed oil

CONCLUSION

According to the results of this study, it can be concluded that pumpkin seeds are considered a valuable source of oil, and the highest oil yield was obtained using the Soxhlet extraction technique. The physicochemical properties of pumpkin seed oil, along with its fatty acid composition, indicate its suitability as a feedstock for biodiesel production and other applications.

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