Bull. Chem. Soc. Ethiop. **2023**, 37(3), 565-577.
DOI: https://dx.doi.org/10.4314/bcse.v37i3.3

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

DETERMINATION OF FATTY ACIDS COMPOSITION BY GC-MS AND PHYSICOCHEMICAL PARAMETERS OF PUMPKIN (*CUCURBITA MAXIMA*) SEED OIL CULTIVATED IN ETHIOPIA

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(Received December 7, 2022; Revised January 16, 2023; Accepted January 16, 2023)

ABSTRACT. Pumpkin (*Cucurbita maxima*) seed oil was extracted using petroleum ether and analyzed for fatty acid compositions and physicochemical parameters of the oil. The seeds were found to contain 43.6% (%w/w) oil. Linoleic (50.7%), oleic (18.8%), palmitic (17.9%) and stearic (12.4%) were found as the primary fatty acids in pumpkin seed oil. Physicochemical parameters of pumpkin seed oil were found to be acid value (1.32 mg KOH/g oil), saponification value (191 mg KOH/g oil), iodine value (114 g I_2/100 g), peroxide value (3.6 meq/kg), specific gravity (0.91 g/mL), refractive index (1.47) and viscosity (24.7). According to the study's findings, 50% of the oil contains the essential fatty acid omega-6 (linoleic acid). The high concentration of essential linoleic acid in pumpkin seed oil implies that it is a nutrient-dense food. The physicochemical study of the pumpkin seed oil samples also showed that they were suitable as industrial ingredients for making soap, cosmetics, medications, and food additives, among other things.

KEY WORDS: Pumpkin seed oil, Fatty acids, Oil content, Physicochemical parameters, Gas chromatography-mass spectrometry, Ethiopia

INTRODUCTION

Pumpkin (*Cucurbita*) is a vegetable that is grown around the world. It belongs to the *Cucurbitaceae* family. There are three varieties of pumpkins: *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata* [1]. Pumpkin seeds have nutritional and medicinal benefits, and they are also eaten as a delicacy in many parts of the world. The majority of the nutrients are located in the seed. They are nutrient-dense foods that are rich in vitamin E, zinc, β-carotene, vitamin C and important omega (omega-3, omega-6 and omega-9), commonly known as essential fatty acids [2-9]. Proteins, triterpenes, lignans, phytosterols, antioxidative phenolic substances, carotenoids, tocopherol, and minerals are all found naturally in them [8, 10-12]. Antidiabetic, antitumor, antibacterial, anticancer, antimutagenic, and antioxidant properties have been found in the pumpkin seed extract [10, 13-15].

There is a growing interest in the unique formation of vegetable oil, and pumpkin seed oil is a promising contender. Vegetable oils are one of the most often derived compounds from plants. Vegetable oils are insoluble in water (hydrophobic) and belong to the lipids chemical class, which is the most abundant in nature. Lipids are made up of a variety of chemical compounds, the most important of which are fatty acids and their derivatives [16, 17]. Vegetable oils are nutritionally important and are used in a variety of dietary and commercial purposes [18-20].

Pumpkin seed oil is a high-quality vegetable oil extracted from the seeds of the pumpkin by crushing the seeds. At the same time, pumpkin seed oil contains two colors: green and red. Carotene is responsible for the red color, while chlorophyll is responsible for the green hue. The pumpkin seed oil is used in cooking, marinades, and salad dressings. It can be found in a variety of foods, including chocolates, gourmet treats, cereal bars, bread, cakes, soups, pesto, muffins, pasta garnishes, and stew garnishes [7].

To determine the quality of vegetable oil, it is essential to conduct research on the physicochemical characteristics and composition of vegetable oils. The determination of physicochemical parameters, such as the iodine value (degree of unsaturation), peroxide value (formation of primary oxidation products), density (purity), and acid value (formation of free fatty acids as a result of rancidity), is

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crucial because these factors affect the shelf-life quality and consequently the economic value of oils [21].

Various methods of oil extraction from pumpkin cultivars have been mentioned in the literature. These methods include organic solvent extraction [22], cold pressing [22], mechanical pressing [23], supercritical fluid extraction [24], aqueous enzymatic extraction assisted by microwave [25], and microwave-assisted extraction [26, 27]. However, the most common and efficient extraction method for vegetable oils is extraction with an organic solvent [1, 2, 19]. To produce methyl ester from pumpkin seed oil, the trans-esterification process was commonly used. The content of pumpkin seeds oil and the composition of fatty acids is affected by a most of parameters (extraction condition, growing region, variety, climate, ripeness, etc.). In addition, the proportion of saturated, monounsaturated, and polyunsaturated fatty acids, as well as the quantity of essential fatty acids like, linoleic and oleic acids (omega-3, omega-6, and omega-9), differs significantly amongst oils [6, 10, 28, 29].

Several reports on pumpkin seed cultivars from various countries have been published, analyzing the oil's quantitative and qualitative qualities. The chemical composition and oil characteristics of seeds from a Tunisian pumpkin cultivar, were investigated by Rezig *et al.* [22]. The principal fatty acids discovered were oleic, linoleic, and palmitic acids. According to Siano *et al.* [30], saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) content of *C. maxima* grown in southern Italy were similar (25.20 and 25.54%, respectively), whereas polyunsaturated FA (PUFA) content was 48.14%. The proximate composition of powdered seed and the lipid composition of *C. maxima* oil obtained in Bangladesh were determined by Habib *et al.* [31]. The high degree of unsaturation makes the oil appropriate for use as a valuable drying agent, and the low free fatty acid concentration suggests that the oil is suitable for human consumption. The fatty acid composition and physicochemical properties of pumpkin grown in northeast Nigeria were examined by Uba and Muhammad [19]. Linoleic acid (53.42%), linolenic acid (20.92%), palmitic acid (17.53%), and stearic acid (8.13%) were discovered in the oil. Unsaturated fatty acids were found to be the most plentiful in pumpkin seed oil. The fatty acid profile of several cultivars of pumpkin seed oil derived from diverse pumpkin sources shows substantial variances [32].

There have been numerous studies on pumpkin seed oil, but there have only been two reports on Ethiopian pumpkin seed oil. Ethiopia and Dessalegn [33] and Redrouthu *et al.* [34] determined the fatty acids compositions and physicochemical behavior, particularly in the pumpkin (*Cucurbita pepo*) seed oil. However, neither the clear extraction solvent nor the fatty acid extraction procedure were disclosed by the authors. Furthermore, they did not compare their findings with the other published data from other countries.

There are three pumpkin species namely *Cucurbita pepo*, *Cucurbita maxima*, and *Cucurbita moschata*, which are widely cultivated around the world, including Ethiopia. In this study, pumpkin species (*Cucurbita maxima*) seed oil was used. Furthermore, an effort was made to clearly and understandably present the extraction, analysis, and characterization of pumpkin (*Cucurbita maxima*) seed oil as well as other physicochemical characteristics and to compare the results with that of previously published data from other countries. In this work, attempts were made to present the extraction, analysis and characterization of pumpkin seed oil and other physiochemical properties. Thus, this study's objective was to determine fatty acid composition and physicochemical properties of Ethiopian pumpkin (*Cucurbita maxima*) seed oil, and compare these results with other previously reported data from different countries.

EXPERIMENTAL

Instruments and apparatus

Electronic balance (ARA520, China), grinder (high speed multifunctional grinder, Shanghai, China), Soxhlet apparatus, rotary evaporator (Heidolph Instrument, and Co. KG, Germany), burette, (super Tec 25 mL, Switzerland), Abbe refractometer (Kirkland, WA, USA), density meter (Anton Paar, Austria), and Agilent gas chromatograph equipped with a mass spectrometer detector and Agilent automatic injector spectrometer (Agilent Technologies, 7890A GC–MS, USA) were used.

Chemicals

Petroleum ether (99% Labscan, Thailand), n-hexane (99% Labachemic Pvt. Ltd, India), dichloromethane (99% Aldrich, Germany), methanol (99.9%, Carlo Erba, Italy), potassium hydroxide (Research-Lab Fine Chem Industries, Mumbai, India), anhydrous sodium sulfate (Sigma-Aldrich, UK), phenolphthalein, potassium iodide (98.5%, England), and starch (99.9%, England) and fatty acid standards (99%, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were used as received.

Sample collection and preparation

In Ethiopia, pumpkin (*Cucurbita maxima*) is called "*Dubba*". Five pumpkin samples were collected from the local market in Ethiopia's capital, Addis Ababa. Following collection, the seeds were manually separated from the fruits using a sharp steel knife. The seeds were cleaned and dried for two weeks under shade. All the pumpkin seeds (200 g) were mixed and ground into a consistent size using an electric grinder. The ground pumpkin seed was used for the extraction of oil.

Extraction of oil from pumpkin seed

According to Uba and Muhammad [19], the Soxhlet extraction was utilized to extract the crude oil from pumpkin seeds with a few changes. The pulverized pumpkin seeds (50 g) were appropriately packed into the thimble of the Soxhlet extractor, and petroleum ether (300 mL) was put into the Soxhlet extractor's 500 mL round bottom flask. The oil from pumpkin seeds was extracted using the Soxhlet extraction system with petroleum ether (boiling point 60-80 °C). The extraction process took 6 hours. The extract was filtered using filter paper before being dried in a rotary evaporator at 40 °C. The recovered oils were stored in a vial at 4 °C until further examination was required. The pumpkin seed oil yield was calculated as:

$$\text{Oil content (\%)} = \frac{W_3 - W_2}{W_1} \times 100$$

where, W_3 = weight of extraction cup + oil, W_2 = weight of extraction cup, W_1 = is weight of original sample.

Determination of fatty acid using gas chromatography-mass spectrometry (GC-MS)

Preparation of fatty acid methyl esters (FAME). Conversion of fatty acids into its derivatives such as fatty acid methyl ester is required so that it can be measured by gas chromatography-mass spectrometer (GC-MS) analysis. According to Uba and Muhammad [19], 1 g extracted oil and 2% methanolic KOH (6 mL) were added to a 50 mL round bottom flask. The condenser was connected to the round bottom flask, and the mixture was heated in a water bath for 1 hour at 60-70 °C with constant stirring. Allowing the reaction mixture to cool to room temperature was the next step. After adding 40 mL of n-hexane to the solution, it was transferred to a separatory funnel. The organic layer (upper layer) was separated and filtered through Whatman filter paper (110 mm) after being dried over anhydrous sodium sulfate. Rotary evaporation was used to remove the solvent to reduce the volume and concentrate the esterified sample. It should be noted that it is possible to selectively remove the solvent (n-hexane) from the esterified samples because it is more volatile than the ester (esterified sample). The methyl ester was kept at 4 °C until the GC-MS analysis. A similar procedure was used for methylation of the standards of free fatty acids. For GC-MS analysis, a transesterified sample was produced at a concentration of 10 mg/L.

Gas chromatography-mass spectrometry. The fatty acid compositions were determined using an Agilent Technology 5977E MSD with an auto-sampler and an Agilent 7820A GC system (USA). The chromatographic separation was performed on a DB-1701 micro-column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) at an 8 psi pressure and a 1 mL/min flow rate. At constant

flow mode, ultra-high purity helium was used as the carrier gas. In a total run time of 16.67 min, an Agilent G4567A auto sampler was used to inject 1.0 µL of the sample into the inlet heated to 275 °C using a split less injection mode. The oven temperature was set to 100 °C for 2 min, with the beginning column temperature set to 100 °C. The temperature of the column was raised at a rate of 15 °C/min to 220 °C, then increased at a rate of 3 °C/min until it reached 240 °C. The temperatures of the transfer line and the ion source were 280 °C and 230 °C, respectively. The electron energy was set to 70 eV. Ions with mass to charge ratio of 40 to 650 were collected. The fatty acids were identified by comparing the retention times of a standard mixture to the retention times of the fatty acids, and by comparing with NIST spectral library. Area normalization was used to calculate the relative content of individual fatty acids.

Determination of physicochemical proprieties of pumpkin seed oil

Determination of saponification value. In a conical flask, 5 g of oil and 25 mL of 0.5 M ethanolic potassium hydroxide solution were heated under reflux for 60 min. The saponified mixture was titrated with 0.5 M HCl after adding 2.0 mL of 0.05% phenolphthalein. When the pink color becomes colorless, the resultant end point is reached [35]. The titration was also performed on the blank solution. The saponification value was calculated by the following formula:

$$\text{Saponification value (mg KOH/g)} = \frac{\text{V HCl (mL)} \times \text{M KOH} \times 56.1}{\text{Mass of the sample (g)}}$$

where, V is the volume of HCl used for titration, M is concentration in molarity of HCL and calculated by subtracting the total volume needed for titration of the blank solution and the sample (V HCl for blank - sample).

Determination of iodine value. Iodine value determination using a simple, quick, and environmentally friendly visual approach based on Shimamoto *et al.* method [36] with slight modifications (by reducing the sample weight and volume of distilled water). An aliquot of the oil sample (0.5 g) was weighed and transferred to a 250 mL Erlenmeyer flask, where it was dissolved in 15 mL 99% ethanol for 5 min with vigorous magnetic swirling. After stopping the stirring, add 20 mL of the 0.1 M ethanolic iodine solution. After 5 min of stirring, the solution was slowed down for the addition of 100 mL of cold distilled water. The flask was kept covered for 5 min and gently swirled with a magnetic bar, taking care not to lose any I_2. The solution was titrated with a standard 0.1 M sodium thiosulfate solution until it was pale yellow in color. After that, 3 mL of a 1% starch solution was added. The titration was carried out until the blue color vanished, leaving a milky solution. A blank was created and examined in the same way as the sample, but without the sample. Iodine content was measured in milligrams per 100 grams of oil.

$$\text{Iodine value} = (\text{V } Na_2S_2O_3 \text{ (mL)} \times \text{M } Na_2S_2O_3 \times 12.691)/(\text{Mass of the sample (g)}$$

where, V $Na_2S_2O_3$ is a volume needed for titration, M is concentration of $Na_2S_2O_3$ in molarity and calculated by subtracting the total volume needed for titration the blank solution and the sample (V $Na_2S_2O_3$ for blank - sample).

Determination of peroxide value. Peroxide value was performed by weighing 5 g of the oil sample into a 250 mL conical flask. This was dissolved in 30 mL of chloroform and a ratio of glacial acetic acid (2:3). The mixture was forcefully shaken for exactly one minute. After that, 30 mL of distilled water was added. The solution was then titrated with sodium thiosulfate 0.1 M until it turned into a pale yellow solution. To this solution, 1 mL 1% starch solution was added and titration continued until the blue color disappeared [37]. The peroxide number was calculated by the following equation:

$$\text{Peroxide number (meq/kg)} = (\text{V } Na_2S_2O_3 \text{ (mL)} \times \text{M } Na_2S_2O_3 \times 1000)/(\text{Mass of the sample, g})$$

Determination of acid value. Weighed 5 g of oil samples were into a 250 mL conical flask, then 100 mL of neutralized ethanol (warmed to 60–65 °C) was added, along with 2 mL of 1% phenolphthalein, and titrated with an ethanolic KOH 0.1 M up to light pink color [36]. Acid value was used to calculate (mg KOH/g oil) of AV [36]. The following equation was used to get the sample's acid value:

$$\text{Acid value (mg/g)} = \frac{\text{V KOH (mL)} \times \text{M KOH} \times 56.1}{\text{Mass of the sample (g)}}$$

where, V = volume of KOH, and M (molarity) = concentration of KOH solution.

Determination of refractive index. The refractive index (RI) was determined using an Abbe 60 Refractometer, as specified by Amin *et al.* [28]. The instrument was then let to stand for a few minutes before reading to allow the sample temperature to equilibrate at (40 °C). Between readings, the prisms were cleaned by wiping away the oil with a soft cloth, then wiping with cotton saturated with petroleum ether and leaving to dry to avoid repeating the low times.

Determination of density. Density is a physical parameter that plays a vital and important role in all material states, whether solid, liquid, or gaseous. It is measured throughout the industry to gain insight into materials, for example their purity, concentration of components, and composition. The density (and concentration) of liquid products greatly impact their quality, behavior, and use. In this study the density of pumpkin seed oil was measured using the density meter.

Viscosity. According to Bwade *et al.* [38], the viscosity of the oil sample was determined using an Ostwald-U-tube viscometer. The viscometer was suspended at room-temperature. The instrument was filled with oil to the mark at the top of the lower reservoir using a pipette instrument into the tide arm, leaving the tube above the mark dry. The oil was then moved into the other arm by means of the pressure on the respective arm of the tube, so that the meniscus was 1 cm above the mark at the top of the upper reservoir. The liquid was then allowed to flow freely through the tube, and the time it took for the meniscus to pass from the mark above the upper reservoir to the mark at the bottom of the upper reservoir to the mark at the bottom of the upper reservoir was recorded. Calculation: Viscosity of oil = $(T - T_0)/T_0$, where: T = flow time of the oil, T_0: flow time of distilled water.

P/S Indices. According to the WHO/FAO report, dietary trans-fatty acids (TFAs) and saturated fatty acids (SFAs) have negative impacts on blood lipoprotein profiles and coronary heart disease (CHD). The ratio of polyunsaturated to saturated fatty acids (P/S index values) can be used to calculate the nutritional value of oils and any associated health hazards. Higher than one P/S index value for oils and fats are seen as having a positive nutritional impact. Less lipid is deposited in the body when the P/S index is higher. According to Gedefaw *et al.* [39] the P/S index was measured by taking the ratio of total polyunsaturated fatty acid to the total saturated fatty acid.

Statistical analysis of data

Results were provided as mean ± standard deviation (SD) (n = 3) following the use of triplicate measurements for all tests. The ratio of the overall peak area was used to calculate the relative peak areas of the fatty acids. Origin 6.0 was used to carry out the statistical analysis (Microcal Software, Inc., Northampton, USA).

RESULTS AND DISCUSSION

Oil content of pumpkin seed (%)

In this study, the oil content of pumpkin (*Cucurbita maxima*) was found to be 43.6±0.8% (Table 1). The oil content in this study was higher than the values that had previously reported from Uganda [1], Algeria [4], Bangladesh [28] and Nigeria [35] (Table 1). The oil content discovered in this study,

however, was less than the oil content reported previously from Ethiopia [33, 34] and Nigeria [19, 38] (Table 1). This variance could result from a number of variables, including the extraction procedures, extraction solvent and the environment in which the pumpkin was grown.

According to studies, compared to the oil content of other seeds, pumpkin seed oil was higher than cottonseed oil (22–24%), sunflower oil (30–35%), soybean oil (18–22%), rapeseed oil (40–48%), and olive oil (12–50%) [40]. According to Kukeera *et al.* [1], the oil content of numerous Curcubitacae crops, including the egusi (30%), sesowane (24.8%), wrewre (27.5%), tsama (24.8%), and melon (28%) varies. According to the Food and Agricultural Organization (FAO), any seed containing more than 17% oil is considered an oil seed and can be used as a feedstock for biodiesel synthesis [41]. As a result, a pumpkin seed can be used in industrial vegetable oil processing.

Table 1. Oil content of pumpkin seed oil in comparison with other previously reported from different countries.

Country origin	Oil content (%)	References
Ethiopia	43.6±0.8%	This study
Uganda	35.6	[1]
Algeria	33.5	[4]
Nigeria	45.2	[19]
Bangladesh	23.0	[28]
Ethiopia	50.5	[33]
Ethiopia	35.8-56.2	[34]
Nigeria	42.1	[35]
Nigeria	55.8	[38]
Nigeria	52.8	[38]

Fatty acid compositions of pumpkin seed oil

The FAMEs chromatogram on a gas chromatograph revealed that pumpkin seed oil is made up of four distinct esters of fatty acids, with retention times of 11.42, 13.29, 13.35, and 13.44 min, respectively. The methyl esters were identified as methyl esters of palmitic acid, oleic acid, linoleic acid, and stearic acid by comparing the retention times of the sample with the retention times of a standard mixture (Figure 1A and B). Linoleic acid, the major fatty acid in the oil, was found to be the most prevalent fatty acid in the studied pumpkin seed oil, accounting for 50.7% of the total (99.8%) fatty acids. The percentages of oleic, palmitic, and stearic acids were found to be 18.8%, 17.9%, and 12.4%, respectively (Table 2).

The pumpkin seed oil has a high quantity of polyunsaturated fatty acids, particularly C18:2, and a low content of saturated fatty acids. Pumpkin seed oil is a rich source of a valuable essential fatty acid (linoleic acid C18:2-omega-6 and oleic acid C18:1-omega-9), according to the current fatty acid composition. Table 2 demonstrates higher than 50% of the oil contains the essential fatty acid omega-6 (linoleic acid). Omega-6 fatty acids are polyunsaturated fatty acids that perform essential functions in the human body. Omega-6 fatty acids play a variety of significant roles in the body. They aid in the development and maintenance of cell membranes and participate in the control of gene activity within the cell. In Ethiopia, most people discard the seeds of pumpkins; nevertheless, this study discovered that the pumpkin seed oil has a high quantity of polyunsaturated fatty acids, particularly C18:2, and a low content of saturated fatty acids. It is now well known that eating a diet rich in polyunsaturated fatty acids and low in saturated fatty acids is helpful to one's health.

Table 3 contrast the fatty acid (FA) content of pumpkin seed oil with other pumpkin seed oils that have been previously discussed in the literature from different country origins. According to Benalia *et al.* [4], the pumpkin seeds oil produced in Algeria showed that the seeds were high in oil with linoleic acid (48%) and oleic acid (39%) ranking first and second in terms of major unsaturated fatty acids, and palmitic (20.0%) ranking first in terms of major saturated fatty acids, respectively. The study did not, however, mention stearic fatty acid. Linoleic content is high (58.2%), followed by oleic content (40.5%), palmitic content (18.2%), and stearic content (3.56%) in the fatty acid composition of

pumpkin seed oil produced in Kenya [18]. Uba and muhammad [19] stated that the pumpkin seed oil from Nigeria contained (8.13%) stearic acid, (17.5%) palmitic acid, and (53.4%) linoleic acid, but no oleic fatty acids were identified.

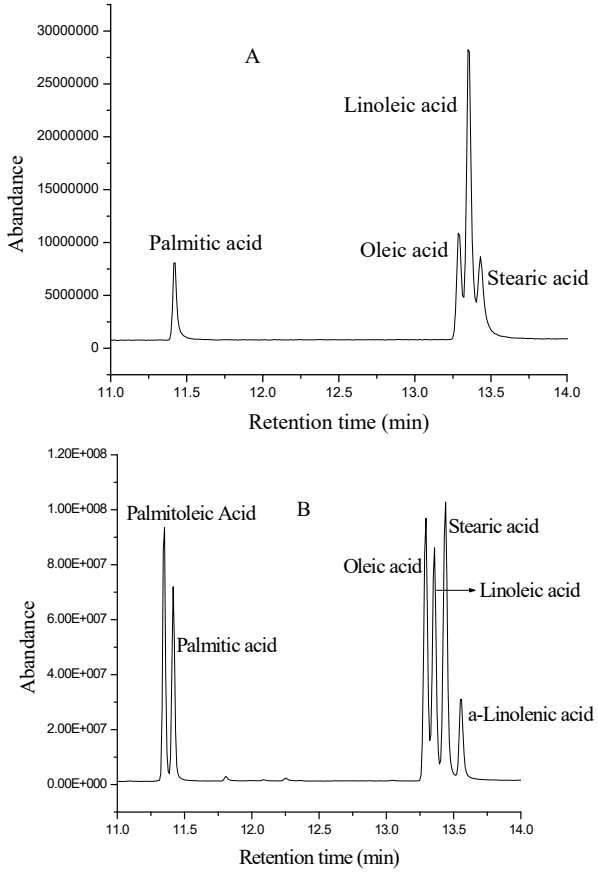

Figure 1. The gas chromatograph chromatogram of fatty acid (A) pumpkin seed oil and (B) standard mixture the different methyl esters (40 mg/L of each).

Rezig *et al.* [26] found that *Cucurbita maxima* seed oil produced from Tunisia contained (44.1%) oleic, (34.7%) linoleic, and (15.9%) palmitic acids. The study did not, however, mention stearic fatty acid. Ethiopia and Dessalegn, [33] reported the presence of four fatty acid (44.1%) oleic, (34.8%) linoleic, (15.9%) palmitic and (4.68%) stearic acids in pumpkin seed oil cultivated in Ethiopia. In addition, Redrouthu *et al.* [34] reported (40.6%) linoleic, and (37.6%) oleic acids were identified in pumpkin seed oil from Ethiopia. The study did not, however, mention palmitic and stearic fatty acid.

Velikovi1 *et al.* [42] from Serbia observed that linoleic acid (46.1%) was the most abundant component in *Cucurbita maxima* seed oils, followed by oleic (29.4%), palmitic (14.8%), and stearic (6.0%). Schinas *et al.* [43] from Greek discovered four main fatty acids: linoleic (43.7%), oleic (37.0%), palmitic (12.5%), and stearic acid (5.43%) in the *Cucurbita pepo* seed oil. The pumpkin seed oil from Italy was found to be linoleic (37.2%), oleic (41.7%), palmitic (14.2%), and stearic acid (5.80%) [44].

It should be noted that the percentage of oleic acid is 2 to 3 times lower in the present study than that reported in other literature. There are several factors that change the content of fatty acids, even

when similar types or species of samples are examined. Several factors have an impact on the fatty acid profile and oil content of pumpkin seeds (extraction condition, growing region, climate, ripeness, etc.). The proportion of saturated, monounsaturated, and polyunsaturated fatty acids, as well as the quantity of essential fatty acids like, linoleic and oleic acids (omega-3, omega-6, and omega-9), differs significantly amongst oils [6, 10, 28, 29].

According to Sew *et al.* [45], the main component of winter melon seed oil is linoleic acid (67.3%), followed by palmitic acid (17.11%), oleic acid (10.21%), and stearic acid (4.83%). Seed oils from *C. melo* and *C. pepo* could be investigated as a source of omega-6 nutritional supplements. The high concentration of essential linoleic acid in pumpkin seed oil implies that it is a nutrient-dense food.

The present study discovered four fatty acids; thus, linoleic acid is the most prevalent. According to the above information the fatty acid composition of pumpkin seed oil produced from Algeria [4], Kenya [18], Nigeria [19], Ethiopia [34], Serbia [46] and Greek [43] all agreed with the finding of this study. On the opposite sides, higher oleic acid levels were seen in samples from Tunisia [26], Ethiopia [33] and Italy [44] (Table 3). The present study and the majority of other studies from various nations have identified the four major fatty acids as palmitic, oleic, linoleic, and stearic. Redrouthu *et al.* [34] from Ethiopia, on the other hand, did not mention the palmitic and stearic fatty acids. Oleic acid was not mentioned in a study from Nigeria [19], while studies from Algeria [4] and Tunisia [26] did not mention stearic fatty acids either (Table 3). In general, all pumpkin seed oils obtained from various countries indicates higher polyunsaturated fatty acids, as shown in (Table 3). On the other hand, the quantities of polyunsaturated and other fatty acids varied between the species examined. These variations could be caused by environmental conditions, harvesting timing, seed drying conditions, seasonal variation, fruit seed maturity and others.

Table 2. Compositions fatty acids in pumpkin seed oil.

Peak number	Chemical name	Common name	RT	Area peak (%)	Matching quality (%)
1	Hexadecanoic acid	Palmitic acid (16:0)	11.42	17.9 ± 0.7	98
2	cis-9-Octadecenoic acid	Oleic acid (18:1)	13.29	18.8 ± 1.1	99
3	cis-9,cis-12-Octadecadienoic acid	Linoleic acid (18:2)	13.35	50.7 ± 0.5	99
4	Octadecanoic acid	Stearic acid (18:0)	13.44	12.4 ± 1.2	99

Table 3. Comparison of four fatty acid content (A%) of pumpkin seed oil found in this study with other published literature report from various country origins.

Fatty acids	Linoleic	Oleic	Palmitic	Stearic	Country origin	References
Content (A%)	50.7 ± 0.5	18.8 ± 1.1	17.9 ± 0.7	12.4 ± 1.2	Ethiopia	This study
	48	39	20.0	NR	Algeria	[4]
	58.2	40.5	18.6	3.56	Kenya	[18]
	53.4	NR	17.5	8.13	Nigeria	[19]
	34.7	44.1	15.9	NR	Tunisia	[26]
	34.8	44.1	15.9	4.68	Ethiopia	[33]
	40.6	37.6	NR	NR	Ethiopia	[34]
	46.1	29.4	14.8	6.0	Serbia	[42]
	43.7	37.1	12.5	5.43	Greek	[43]
	37.2	41.7	14.2	5.80	Italy	[44]

NR = not reported.

Physicochemical properties of pumpkin seed oil

The physicochemical characteristics of the analyzed pumpkin (*Cucurbita maxima*) seed oil were determined and presented in Table 4. The acid value or acid number, and the free fatty acid content are employed for the determination of oils and fats and as quality control parameter. The higher the acid number and free fatty acid content, the lower the quality of the oil. The acid number increases further

with the age of an oil, because triglycerides are changed into glycerol and fatty acids as an effect of time. The acid value is a measure of how much potassium hydroxide (mg) is needed to neutralize the free acids in one gram of fat [36]. The result obtained for acid value was 1.32 mg KOH/g (Table 4). This number is comparable to the results of Ethiopia and Dessalegn [33] and Samuel *et al.* [47] who have reported 1.23 and 1.41 mg KOH/g, respectively (Table 5). The acid value of pumpkin seed oil discovered in this study is lower than that of pumpkin (*Curcubita pepo*) 62.6 mg KOH/g and pumpkin (*Curcubita maxima*) 12.6 mg KOH/g published by Bwade *et al.* [38], and 6.9, 8.4 and 3.7 mg KOH/g reported by Uba and Muhammad [19], Amin *et al.* [28], and Yusuf *et al.* [35], respectively (Table 5). An oil sample with a low acid value includes fewer free acids, minimizing its risk of rancidification [36]. In comparison to other seed crops, the acid value of pumpkin seed oil in this study is lower than that of soybean (9.86 mg KOH/g), coconut oil (6.36 mg KOH/g), and cotton seed (5.75 mg KOH/g) [1].

The saponification value is employed as a quality control parameter and for the characterization of oils and fats. The saponification value carries the information on the average molecular weight of all fatty acids which are present in the sample. The higher the saponification number, the lower the molecular weight of all fatty acids, and vice versa [35]. The saponification is measured in milligrams of potassium hydroxide (KOH) necessary to saponify one gram of fat. The saponification value was found to be 191 mg KOH/g (Table 4). This study's saponification value was lower than those reported for pumpkin seed oil by Uba and Muhammad, [19], Boujema *et al.* [20], and Yusuf *et al.* [35], which were 261, 236, and 210 mg KOH/g, respectively (Table 5). The saponification values of pumpkin seed oil are comparable to existing literature results of 175, 184-187, 189, and 199 mg KOH/g reported by Jiao *et al.* [25], Kouba *et al.* [27], Ethiopia and Dessalegn, [33], and Bwade *et al.* [38], respectively (Table 5). The saponification value in this study was lower than cocosnucifera oil (246 mg KOH/g), but higher than the saponification values published by others [48] for moringa seed oil (171.9 mg KOH/g) and cashew seed oil (161 mg KOH/g). Furthermore, the saponification value of pumpkin seed oil was found to be consistent with that of melon seed oil (190.4 mg KOH/g) and sesame seed oil (189 mg KOH/g) [48] (Table 5). The findings also indicate that pumpkin seed essential oil is more suited for making liquid soap, cosmetics, shampoos, and creams.

For the characterization of oils and fats, and as a quality control parameter, the iodine number is utilized. As a sum parameter, it permits quantification of the amount of unsaturated fatty acids present in oils and fats. The more unsaturated fatty acids, the more iodine reacts with the double bonds, resulting in a higher iodine value. Iodine value is a measure of the degree of unsaturation of oils and fats [35]. It is defined as the amount of iodine that can be added to 100 g of oil [1]. The iodine content of the pumpkin seed oil was found to be 114 g I_2/100 g (Table 4). The iodine value of the pumpkin seed oil in the present study was greater than the previously reported values of 100 and 15-16 g I_2/100 g for pumpkin seed oil [19, 38], respectively (Table 5). However, the iodine value in this study was lower than that of pumpkin seed oil [26, 35], which was 183 and 153 g I_2/100 g, respectively (Table 5). The more iodine in the oil, the more unsaturated it is. When the iodine level is too high, however, the oil's stability suffers since it is more likely to oxidize [19, 33].

The peroxide number supplies information about the amount of peroxide compounds present in the oil and so can be utilized as an indicator of the quality and age of the edible oil. The lower the peroxide numbers the better and/or fresher the oil. The peroxide value is used to determine the extent of rancidity of oils during storage, therefore, it can be used as an indicator in determining stability of fats and oils. It is primarily used to determine the oxidation of lipids [37]. The milliequivalent (meq) of oxygen per 1000 g of fat is the peroxide value, which is used to represent the degree to which a fat has been oxidized [47, 49]. Pumpkin seed oil had a peroxide value of 3.6 meq O_2/kg (Table 4). This study's peroxide value is higher than the pumpkin seed oil peroxide values of 2.3, 2.5, and 2.5 meq O_2/kg published by Kukeera *et al.* [1], Rezig *et al.* [26], and Bwade *et al.* [38], respectively (Table 5). However the peroxide value of this study is within the range of 3.09 - 5.17 meq O_2/kg recorded by others [50] (Table 5).

The molecular weight, fatty acid chain length, and degree of unsaturation all influence the refractive index of oils [19]. The refractive index of *Cucurbita maxima* seed oil was determined to be 1.47 in this investigation (Table 4). Uba and Muhammad, [19], reported a lower refractive index of 1.12 (Table 5). The current value, on the other hand, was consistent with the refractive index (1.46-1.47) of pumpkin

seed oil previously reported by Kukeera *et al.* [1], Benalia *et al.* [4], Rezig *et al.* [26], Amin *et al.* [28], Redrouthu *et al.* [34], Shimamoto *et al.* [36], and Gedefaw *et al.* [39] (Table 5).

The viscosity was found to be 24.3 cps. In this work, the viscosity of pumpkin seed oil was shown to be lower than previously reported by Stevenson *et al.* [32], and Magu *et al.* [37]. Amin *et al.* [28] reported a viscosity of pumpkin seed oil that was lower than the current value (Table 5). The World Health Organization has recommended a minimum P/S index for edible oils to be >1 [46]. Edible oils with higher P/S index have more poly unsaturated compounds compared to unsaturated ones [39]. The higher value of the P/S index has direct relation to the extent of oil solubility in the blood stream. SFAs have the capacity to stick tightly to cell membranes unlike poly unsaturated fatty acids. Those SFAs with carbon number between 12 and 16 have the same impact on serum lipoproteins; particularly, they increase low-density lipoprotein (LDL) cholesterol [39]. In this work the P/S index for the oil was calculated to be 1.67 which is in the acceptable range that was recommended by the WHO.

Table 4. Mean value ± standard deviation of physicochemical properties pumpkin seed oil.

Parameters	Results
Saponification value (mg KOH/g)	191 ± 0.6
Iodine value (g I_2/100 g)	114 ± 0.8
Peroxide value (meq/kg)	3.60 ± 0.3
Acid value (mg KOH/g)	1.32 ± 0.2
Density	0.91 ± 0.1
Refractive index	1.47 ± 0.1
Viscosity (centipoise)	24.7 ± 0.8
P/S Index	1.67

Table 5. The physicochemical properties of pumpkin seed oil compared with other previously reported literatures from different countries.

Parameters	AV mg KOH/g	SV mg KOH/g	IV G I_2/100 g	PV meq/kg	Density	RI	V cps	Country of origin	References
Oil content	1.32	191	114	3.6	0.94	1.47	24.7	Ethiopia	This study
	3.70	NR	NR	2.5	0.92	1.47	NR	Uganda	[1]
	28.0	199	NR	NR	0.89	1.47	NR	Algeria	[4]
	6.90	261	100	NR	0.89	1.12	NR	Nigeria	[19]
	7.54	175	153	2.3	0.91	1.46	NR	Algeria	[26]
	13.0	236	113	NR	0.90	1.50	4.7	Bangladesh	[28]
	1.23	189	97.4	NR	0.91	1.46	35	Ethiopia	[33]
	1.18	174	98.4	3.2	NR	1.50	NR	Ethiopia	[34]
	8.40	210	183	2.5	0.88	1.46	NR	Nigeria	[35]
	13.0	184	16.0	NR	0.93	1.46	45	Nigeria	[38]
	63.0	187	15.0	NR	0.93	1.46	44	Nigeria	[38]

AV = acid value, SV = saponification value, IV = iodine value, PV = peroxide value, RI = refractive index, V = viscosity, cps = centipoise. NR = Not reported.

CONCLUSION

The findings of this study revealed that pumpkin seeds are rich in oil and their fatty acid composition places them in the same linoleic-oleic group as cottonseed, corn, sesame, sunflower, and soybean oils. The oil content of pumpkin (*Cucurbita maxima*) was found to be 43.6%. Linoleic acid (50.7%), oleic acid (18.8%), palmitic acid (17.9%), and stearic acid (12.4%) were identified in the pumpkin seed oil. Pumpkin seed oil is high in omega-6 (linoleic acid) and omega-9 (oleic acid) important fatty acids. The seeds contained a larger proportion of unsaturated fatty acids than saturated fatty acids, which is significant in human diet since it helps to avoid different disease. As a result, a pumpkin seed can be used in industrial vegetable oil processing. The physicochemical analysis also revealed that the

pumpkin seed oil samples could be good for industrial ingredients in soap production, cosmetics, pharmaceuticals, and food additives, among other things.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Chemistry, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia for proving laboratory facilities. Mulu Hagos is thankful to Ethiopian Police University, Ethiopia, for sponsoring her study.

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