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Physicochemical Properties of Oil Extracted from Pumpkin (*Cucurbita pepo*) Seeds

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Abstract

Pumpkin (*Cucurbita pepo*) is a member of the family Cucurbitaceae and its fruit parts are edible. This study evaluates the physicochemical properties of extracted oil from the seed of pumpkin. The results revealed a percentage yield of 41.08 ± 2.33, refractive index of 1.47 ± 0.18, relative density of 0.09 ± 0.02 g/cm^3; saponification value of 184.60 ± 1.67 mg/KOH/g, Acid value of 2.64 ± 1.31 mgKOH/g, iodine value of 80.27 ± 2.09 g of I_2/100g, and peroxide value of 11.0 ± 1.0 mEq/Kg. The result shows that pumpkin seed oil may find application either in food industry as food addictive as it may pose a good quality shelf life or energy generation as biofuels.

Keywords: Pumpkin, physicochemical, seed oil, food industry

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Introduction

There is limited utilization of most vegetable oils in their original form, because of their chemical properties and physical characteristics of having a high content of polyunsaturated fatty acids despite possessing an excellent nutritional profile. This makes the use of this oil unsuitable for usage in high temperatures of frying, due to the oxidative formation of compounds that are injurious to health [1].

Since the mid-1970s, the global consumption of vegetable oil which is an important food commodity has consistently increased by over 5% per annum [2]. The reason for the increase includes, growth in population, improvement in living standards, changing diets and production of biodiesel, has led to a growing demand for conventional vegetable oils, especially sunflower, soybean, rapeseed and palm oils [2, 3]. This trend combined with adverse effect of climate change has led to a gradual reduction of vegetable oils supplies in the world. Therefore, looking at these challenges, there is a need to find alternatives that can replace some of the conventional oils from unconventional oils sources and thus lower their price hike that accompany the insufficiency in oil supplies.

In Nigeria, vegetable oil demand has continually been on an increase as industrialist depend heavily on the conventional vegetable oils such as soya bean oil, palm kernel oil, coconut seed oil, and cotton seed oil for production of different products [4, 5]. Pumpkin (*Cucurbita pepo*) is a member of the family Cucurbitaceae. The pumpkin seeds characterised to have valuable nutritional and medicinal qualities besides being the source of good-quality edible oils. Pumpkin seed extract and other parts of the plant has been reported to have antidiabetic, anti-hypercholesterolemia, anti-cancer, anti-tumor, anti-mutagenic, anti-oxidant anti-bacterial, anti-hypertensive, anti-inflammation, immunomodulation, intestinal anti-parasitic, and antalgic activities [6, 7].

The global hunger index (GHI) shows a worrisome gap between the current rate of progress in combating hunger and undernutrition and the effort required to eradicate hunger. The data states that the people suffering from acute hunger is approximately 124 million, with is a conspicuous increase from the 80 million two years back, while the reality of undernutrition and hunger continues to have a huge effect on the next generation [8].

Also, the study of the physicochemical characteristics of pumpkin seeds and oil is important as it would help nutritionist and medical practitioners to know the physicochemical values of pumpkin in the diets and human health. The properties of oils from various sources were dictated mainly by their chemical compositions. Oil from any single source cannot be suitable for use in every application hence, the study of their constituents and characterization is germane.

This study aims to determine some physicochemical properties of extracted oil from the seed of pumpkin of Jama'are Emirate, Bauchi State, Nigeria.

Materials and Methods

Plant material

The pumpkin seeds were procured from Azare market which is located at 11.67 latitude and 10.19 longitude and 413 meters above sea level. The temperature of Azare is at 96°F and 16% humidity.

Equipment
Soxhlet extractor, reflux condenser, density bottle, thermometer, water bath, separating funnel, burette, oven, gooche crucible, Refrigerator, Refractometer, metal dish.

Chemicals and reagents
n-hexane, petroleum ether, potassium hydroxide, distilled water, ethanol, hydrochloric acid, phenolphthalein, acetone, diethyl ether, Wij's solution, iodine trichloride, glacial acetic acid, carbon tetrachloride, potassium iodine solution, sodium thiosulphate solution, starch indicator, chlorofoam, chloroglucinol.

Methods
Oil extraction procedure
The pumpkins seeds were washed to remove contaminants and dried at 25°C. The dried seeds were pulverised with a grinder (Toos Shekan, Iran). Hot extraction process was adopted using Soxhlet apparatus at 25°C to extract oil with n-hexane as the solvent (1:4 w/v). The solvent was evaporated in the oven at 100°C to dryness. The extracted oil was sealed and stored in a dark bottle until analysis.

Chemical analysis of the extracted oil
Refractive index
Refractive index was determined by the method described by AOAC [9] using a refractometer. Briefly, few drops the oil sample placed between the refractometer double prisms and left some minutes for the sample temperature to equilibrate with the instrument before taking reading. The refractive indices of all samples were determined at 35 – 40°C.

Relative density of the oil at 20°C
This is the ratio of the mass in air of a given volume of oil at 20°C to the mass of the equal volume of water at 20°C. A cleaned, dried and weighed relative density bottle was filled with distilled water and equilibrated to 20°C on water bath. The density bottle was weighed after wiping it dry. The same procedure was the undertaking to obtain the weight of the oil at 20°C.

Saponification value
This is the number of milligram (mg) of potassium hydroxide (KOH) that would neutralize the acid resulting from the complete hydrolysis of a gram of the oil sample. Two grams (2 g) of the oil was place into a conical flask and 25 ml of the alcoholic potassium hydroxide solution was pipetted. On boiling water bath, a reflux condenser was heated for an hour with occasional shaking. A millilitre (1 ml) of phenolphthalein solution was used as the indicator and titrated while hot with the standard hydrochloric acid (a ml). A blank determination was carried out (b ml).

Unsaponifiable matter
A milliliter of 3M potassium hydroxide solution was added to the neutralized liquid left from the titration of the determination of saponification value. It was transferred to a separator, using a quality of water 50 ml less the volume (i.e. 50 ml of 0.5 HCL used in neutralizing) of 0.5M HCL used in the titration for saponification value. Warm extraction with diethyl ether (3× 50 ml) was done. The ether layer was wash thrice by shaking vigorously with 20 ml portion of aqueous 0.5 M KOH and then with 20 ml portions of water until the water is no longer alkaline to phenolphthalein. The ether extract is collected into a conical flask and the ether is evaporate off. Then 2 – 3 ml acetone added while heating on a water bath. Complete the removal of the solvent with a current of air. Dry to constant weight at 100°C. The content of the flask is dissolved in 2 ml of ether. Then add 10 ml of neutralized ethanol and titrate with 0.1N alcohol sodium or potassium hydroxide solution. The weight of unsaponifiable matter is corrected for free fatty acid (1 ml 0.1N alkali = 0.0282 g oleic acid.).

Iodine value (IV)
Iodine value (IV) is the amount in milligrams of iodine absorbed by one-gram fat. The proportion of unsaturated constituents present in fat is measured with IV. The reaction involves the addition of halogen to attack the double bonds of the unsaturated fatty acids, the amount of halogen added is expressed in terms of iodine (iodine number) [10]. IV indicates the total amount of unsaturationin any particular oil or fat [11]. About 0.2-0.5 g of the oil (the approximate weight of the oil to be taking is obtain by dividing 20 by the highest expected iodine value), was weighed into a glass Stoppard bottle of about 250 ml capacities. Ten (10 ml) milliliter of carbon tetrachloride (CCl_4) was then added and dissolved followed by addition of 20 ml of Wij; solution and insertion of the Stoppard which has been moisten with potassium. The mixture is mixed and allowed to stand in the dark for 30 min. Fifteen milliliter (15 ml) potassium iodide solution and 100 ml water were mixed and titrate with the standard thiosulphate solution using starch as indicator just before the end point (titration =a ml). A blank test was carried out simultaneously, omitting the oil (titration =b ml).

Acid value
The acid value (AV) is the amount potassium hydroxide in milligrams that would neutralize the free fatty acids in one gram of oil or fat sample. The extent of decomposing glycerides in oils by lipase action is measured through AV [11]. A volume of 25 ml diethyl ether was mixed with 2 ml ethanol and, 1 ml of 1% phenolphthalein solution was added. The solution was neutralized by titration with 0.1M sodium hydroxide solution. Five grams (5 g) of oil was contained in the mixture and titrate with 0.1M sodium hydroxide solution.

Peroxide value
Peroxide value is an oxidation index lipid oxidation at early stages [12]. The PV of oils and fats is an important indicator for detecting storage oil quality. The PV of oil should not be more than 10 milliequivalent of peroxide.

The test was carried out in subdued day light. A clean dry boiling tube was used to collect one gram (1 g) of oil. Then 1 g powdered potassium iodide and 20 ml solvent mixture was added. The mixture was boiled in water bath for a min. Afterwards, water content was poured and the washing was added to 20 ml potassium iodide solution in a conical flask. The tube was washed twice with 25 ml portion of water and the washings was added to the titration flask. It was then titrated with 0.002 m thiosulphate, using starch as indicator (v ml).

Matter volatile

The matter volatile at 105°C (moisture) on 5 g of the sample was determined in a metal dish dry to constant weight in an oven.

Insoluble impurities

Fifty grams (50 g) of oil was filtered and was then washed down with light petroleum ether until free from oil. It was dried in an oven at 100°C for 1 h and cooled in a desiccator and weigh.

Kreist test for rancidity

Ten milliliter (10 ml) of oil was placed in a stopper test tube, followed by addition of 10 ml of 0.1% chloroglucinol solution in ether and 10 ml concentrated hydrochloric acid. The mixture was **s**haken vigorously for 20 seconds. A pink colour indicates incipient rancidity. If the oil is diluted 1 in 20 with kerosene and the test is still positive, the rancidity will be noticeable to the test and smell.

Results and Discussion

Tables 1 show the physico-chemical properties of Pumpkin seed oil. The percentage yield of oil from the seed of pumpkin was 41.08 ± 2.33%. The oil showed iodine value of 80.27±2.09, which may indicate content of unsaturated fatty acid.

Table 1: The physicochemical properties of extracted oil from pumpkin seed

Parameters	Results
Percentage yield (%)	41.08 ± 2.33
Refractive index 40ºC	1.47 ± 0.18
Relative density	0.09 ± 0.02
Saponification value (mg KOH/g)	184.60 ±1.67
Acid value (mg KOH/g)	2.64 ±1.31
Iodine value (g of I_2/100g)	80.27±2.09
Unsaponifiable matter (% of oil)	15.04 ± 0.05
Matter volatile % m/m	0.14 ± 0.07
Insoluble impurities% m/m	0.07±0.02
Peroxide value (mg Eq/Kg)	11.0 ±1.0

Values are presented as Mean ± SD, n = 3

The result of this study as shown in Table 1 reveals that the oil content of pumpkin seed (41.08%) was in close, agreement to the pumpkin yield of 39 and 42% obtained by Siano *et al*. [13] and Odunukan *et al*. [14], respectively. Although, the slight difference may be due to species related factors or seasonal differences. Furthermore, our result is within the range of value (40-50%) of pumpkin seed oil reported by Bavec *et al.* [15]. In comparison to other oil sources, a higher yield of 53.0, 56.24, 58.4% was reported in fluted pumpkin works of Longe *et al*. [16], Akwaowo *et al.* [17] and Eddy *et al*. [18], respectively. The lipid content reported for cashew nut was between 42.15 and 48.7% (Eddy *et al*., 2011; Fetuga *et al*., 1973; Longe *et al*., 1983). Generally, the values obtained for the lipid content of the pumpkin seeds is comparable to the range of values (42.10 – 70.0%) reported for some oil-bearing seeds [18]. The calculated physiological fuel value of oil that can be obtained from the consumption of 100 g of pumpkin seed will liberate 41.08x 9 = 369.72 kcal of energy (1 g of lipid = 9 Kcal of energy). The recommended daily intake of lipid is within 80 – 100 g also, lipid from plant should be made of not be less than a quarter (20-25 g) per day of unsaturated fatty acids of the daily lipid consumption [20]. Therefore, the pumpkin seed may be excellent source of lipids.

Our result on refractive index is within the physical and chemical indices of the Codex Standard range of value. The refractive index values of oils are closely related to their molecular masses or relative density. It is comparatively in close agreement to the refractive index value was reported previous for pumpkin seed oil of 1.4662, and 1.465 reported by Ardabili *et al*. [21], and Tsaknis *et al*. [22], respectively. It is also in agreement with oils from other from sources such as cashew nut oil (1.498), fluted pumpkin (1.462) [18] and *Trichosanthescucumerina* seed oil (1.45) [23] .

The saponification value of our pumpkin seed oil (184.6 mg/KOH/g) result compare favourably with that from *Trichosanthescucumerina* seed oil (184.9 mg/KOH/g) [23] *C. lanatus* melon seed oil (181.12-189.26 mg KOH/g) and *A. horridus* melon seed oil (180.48 and 186.19 mg KOH/g) [24] which is suggestive of possible utilization in cosmetic products [23]. This result is within the pumpkin seed oil range of Nichols and Sanderson [25], who reported 174-197 mg KOH/g. But, when compared to other pumpkin studies, it is lower to the values 206 mg KOH/g [20] and 201 mg KOH/g [22] and higher than 132.3 mg KOH/g [26]. Although pumpkin seed oil saponification value is higher than that of cashew nut oil (92.57 mg KOH/g), there are some vegetable oils with higher values such as coconut oil (253.0 mg KOH g-1), palm kernel oil (247.0 mg KOH g-1) and butter fat (225.0 mg KOH g-1) (Aremu *et al*., 2006). The higher the molecular weight of the fat and oil, the smaller is its saponification value [27]. Essentially, this means there is an inverse relationship between the saponification value and the molecular weight of lipid content.

The lower the saponification values of the oil, the better the quality of the oil in production of soaps and detergents [18]. The saponification value of pumpkin oil we obtained is high when compared with that of the oil commonly used in soap making, suggesting it may not be a good oil source for soap and detergent making.

The degree of unsaturation of oil is inferred from the iodine value [28].

The iodine value of pumpkin seed oil (80.27 g I_2/100g) was higher when we compared it with the iodine value obtained on the samples of pumpkins studied by Ali *et al*. [29], cashew nut oil (47.20 g I_2/100g) and fluted pumpkin oil (51.52 g I_2/100 g) [18]. While the iodine values of *C. lanatus* melon (110.28 to 118.61 g of I_2/100 g) and *A. horridus* melon (111.03 g of I_2/100 g) [24] seed oils were higher. The oils with iodine values of less than 130 are regarded as non-drying oils and are suitable for paint making [18], and within the range of 6 – 150 g I_2/100 g specified by CODEX for edible oils Therefore, the pumpkin seed oil sample may be used for soap making or purified for edible purpose. The low iodine value of the oil is indicative of few unsaturated bonds and also reduces the susceptibility of deterioration and oxidative rancidity [4]. Non-drying oils are not suitable for ink and paint production.

The peroxide value of pumpkin seed oil (11.0 mg Eq/kg) was lower than the peroxide value of cashew nut oil (15.23 mg Eq/kg) and higher to that of fluted pumpkin (1.53 mg Eq/kg) [14]. The low peroxide value of the oil sample is an indication that the oil is stable and may not be susceptible to oxidative rancidity. The CODEX ALIMENTARIUS [30] stipulated a permitted maximum peroxide level of not more than 10 mequivalent of oxygen/kg of the oils; therefore, some of the oils reviewed may not be suitable for consumption.

The lower iodine and peroxide values by implication suggests pumpkin oil has a good shelf life. Furthermore, the acid value of pumpkin seed oil (2.64 mg KOH/g) was lower than the acid value of cashew nut oil (3.74 mg KOH/g). Cheikyou [24] reported the acid values of *C. lanatus* melon seed oil ranging from 0.95 to 1.63 mg KOH/g while that of *A. horridus* melon seed oil was between 0.643 and 0.708 mg KOH/g. The acid and peroxide values of pumpkinseed oils are within the acceptable levels of the standards for edible oils of cold pressed origin [30].

Conclusion

The results obtained from this study shows that pumpkin seed oil may find application either in food industry as food addictive. The result is also suggestive of the pumpkin oil to be a posing a good quality shelf life. This oil may be explored for energy generation as biofuels.

Competing interest: The authors declared no competing interest.

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