Proximate Composition of Fluted Pumpkin Seed *(Telfairia Occidentalis)*, Extraction and Characterization of the Oil from the Seed

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Abstract
The proximate composition of fluted pumpkin seed (Telfairia Occidentalis), extraction and characterization of the oil from the seed was carried out using detailed analytical procedures. The samples were collected from Sabon-gari market of Kano state, Nigeria. The results showed mean moisture content of 8.54±0.07%, ash content of 3.48±0.02%, crude fiber of 15.85±0.03%, crude protein of 29.15±0.04%, crude lipid of 27.83±0.07%, and carbohydrates of 15.15±0.01%. On extraction of the oil, the physiochemical properties of the oil yield iodine value of 82.7±0.02meq/Kg, saponification value of 183.13±0.11mgKOH/g, peroxide value of 13.7±0.01meq/Kg, free fatty acid of 18.21±0.12%, specific gravity of 0.899±0.05g/cm^3, Ester value of 168.11±0.05mgKOH/g and acid value of 17.7±0.13mgKOH/g The viscosity of the oil was 87.8Cps@25^{o}C. The results revealed that the seed could be a good source of nourishment to both Man and animals. The properties of the extracted oil indicated that it could also serve as a good raw material to the soap, pharmaceutical and food industries.

Key words: *fluted pumpkin seed, proximate analysis, physiochemical properties.*

Introduction

Fluted Pumpkin (*Telfairia occidentalis*) is a cultivar of a squash plant. The thick shell contains the seeds and pulp, some exceptionally large cultivars of squash with similar appearance. Fluted pumpkin shapes ranges from round to oblong among varieties and size from less than 0.45kg to more than 4.50kg, most weight 4-8kg. Fluted pumpkin have a long shelf life of over 6 months without addition of any preservatives if stored in a cool dry place (Echiessa, *et al* 2013)

Fluted Pumpkin plant is propagated by seed; it can also be directly sown in the main field, and on hills. Planting on a raised land promotes drainage, so the root does not have to deal with constant wetness which leads to disease problem. It is planted 2 to 3 seeds per hills for better growth usually spacing varies with variety and vine size, it is advised to plant bush or short-vined of fluted pumpkin at the spacing of 0.5 meter to 1 meter in the row and 1 meter to

1.5 meter between rows. It is cultivated in Slovenia and Australia while in Nigeria is cultivated in some part of the country such as Niger delta and Abia states. (Udo *et al* 2016).
Fluted Pumpkin can be harvested whenever there is deep solid color (Greenish in colour) and the rood is hard, if vines remain healthy, harvest in late September or early October, before heavy frosts and store them in a moderate warm, dry place until Halloween, cutfluted pumpkin from the vines carefully using pruning shears and sharp knife, leave 3 to 4 inches of stem attached. Snapping the stem from the vines results in many broken or nubbinhandles, fluted pumpkinwithout stem usually do not keep well, wear gloves when harvesting fruit because many varieties have sharp prickles on the stem (Udo, *et al* 2016).

The health benefits of fluted Pumkin seed include; reducing Inflammation in the human body, nutritional aid for cancer patients, good for prostrate health, fight hair loss in human (fluted Pumpkin seed act as anhair loss remedy due to their Zinc content which helps balance hormones, thus in turn benefits hair growth.
This research works is aimed atdetermining the proximate composition of the fluted pumpkin seed, extraction and characterization of oil from seed for both domestic and industrial consumptions.

Udo and Alozie 2016 carried out the proximate and physicochemical properties of oil from (*Telfairia occidentalis)* seeds using standard methods. The proximate composition (%) includes moisture, ash, crude fat and protein and calorific values were 0.15, 0.04, 99.78, 0.18 and 898.74 % respectively. Physicochemical characteristics of the oil showed that the oil was golden yellow oil. It had a saponification value of 175.63mgKOHg^{-1}, specific gravity of 0.193gcm-^{3}, unsaponifiable matter of 4.17% and peroxide value of 8.62meq kg^{-1}. The result also revealed free fatty acid value of 6.5%, viscosity value of 13.15kg ms^{-1}, ester value of 167.35mg KOH g^{-1} and iodine value of 46.58gI per 100g. These results indicated that T. occidental is seed oil is a rich source of edible vegetable oil due to the low free fatty acid content. The high fat and calorific content will contribute significantly to the consumer's energy requirement and the low iodine value suggests that the oil is non-drying oil and therefore will have low susceptibility to deterioration hence having a long shelf life.

Christian Agatemor 2006 studied selected physiochemical properties of fluted pumkin seed and tropical almond seed oil. The oils from the seeds of fluted pumpkin (*Telfairia occidentalis* Hook F.) and Tropical almond (*Terminalia catappia* L.) were extracted with petroleum ether. The ether extract was evaluated for Wijs iodine value, saponification value, acid value and specific gravity. The result of the evaluation was compared with that of palm oil (*Eloesis guineensis*). The acid value of fluted pumpkin and Tropical almond were 3.51mgKOH/g and 7.59mgKOH/g respectively. The saponification value; fluted pumpkin (179.02mgKOH/g) and Tropical almond (183.44mgKOH/g) indicate that the oils have high molecular weight fatty acid and therefore provide good feedstock for lubricants, candles and soap production. The iodine values, fluted pumpkin (101.73) and Tropical almond (85.12) suggest a high degree of unsaturation compare to palm oil. This makes the oils good cooking oils and suitable for margarine production. Their specific gravities were also higher than that of palm oil fluted pumpkin (0.921) while Tropical almond (0.926).
Elinge *et al* 2012, carried out research on the proximate, mineral and anti-nutrient composition of pumpkin seeds. The pumpkin seeds were analysed for their nutritional and anti-nutritional composition, the results obtained were; moisture content (5.00%), ash (5.50%), crude lipid (38.00%, crude fibre (1.00%), crude protein (27.48%), Available carbohydrate (28.03%) and calorific value (564kcal/ 100g). Elemental analysis shows that

potassium is the most abundant element in the sample (273mg/100g) and manganese is least (0.06mg/100g).The anti-nutritional parameters analysed are; phytate (35.06 mg/100g), oxalate (0.02± 0.10mg/100g), hydrocyanic acid content (0.22± 0.04mg/100g) and nitrate (2.27± 002mg/ 100g). The result showed that the pumpkin seeds if properly utilized can serve as good source of minerals.

Materials and Methods

Healthy, matured and ripped fruits of the pumpkin fruits (*Telfairia Occidentalis)* were purchased from Sabon gari market of Kano State, Nigeria. The matured fruits were cut open to expose the seeds. The seeds were then manually extracted from the pods, washed with distilled water and oven dried for 48 hours at 70^{o}C (Udo 2016).

The oven dried seeds were then crushed and milled into fine powder with an electric blender. The oil was extracted from the milled seed by Soxhlet extraction with petroleum ether as solvent. Proximate composition and physiochemical properties of the oil were determined using standard methods (Ayo and Agu 2012). Analyses of the samples were performed in triplicates. Results were expressed as mean and standard deviation.

Oil content determination

Oil was extracted using the Soxhlet extractor for a period of 1 hour and 30 minutes with petroleum ether as the solvent after which the oil was dried in an oven at a low temperature for 2 hours.

Then the oil content was calculated from equation;

$$\text{Percentage of oil content} = \frac{(W_2\text{-}W_1) \times 100}{W_3}$$

Where; W_2 is weight of extraction cup,

W_1 is weight of extraction cup + oil,

W_3 is the weight of original sample (Ayo and Agu 2012).

Moisture content analysis

About 5g of the sample was weighed into an empty humidity dish. The dish was placed into an oven at a temperature of 105^{O}C for 3 hours (drying period begins when oven temperature has actually reached 105^{O}C), it was then removed from the oven and transferred to the desiccator, the sample was weighed after cooling and the drying continued until constant weight was obtained.

$$\% \text{ moisture} = \frac{(W2\text{-}W3) \times 100}{(W2\text{-}W1)}$$

W_1 = weight of the empty humidity dish

W_2 = weight of the empty humidity dish + Sample

W_3= weight of the dish + sample after drying, (Ayo and Agu 2012).

Ash content determination

A clean crucible was placed in a muffle furnace regulated at 550^{O}C for 30 minutes. The crucible was transferred to desiccator and was weighed when cool (W_1). 5g of the finely grounded sample was weighed accurately into the crucible (W_2). The sample was placed in a Bunsen flame inside a fume cupboard to dry off most of the smoke. The sample was transferred into pre-heated muffle furnace at 550^{0}C at the temperature for 7-8 hours until a white gray ash was resulted. The sample was cooled in a desiccator and was reweighed until a constant weight was obtained (W_3).

$$\% \text{ Ash} = \frac{(W3\text{-}W1) \times 100}{(W2\text{-}W1)}$$ (Ayo and Agu 2012)

Crude fiber determination

The sample was weighed 2g, and the weight of the crucible weighed 34.90, 2.5ml of sulphuric acid was measured, and 100ml of H_20 was added, the 2g of the sample was poured inside the 200ml of H_20 and was then homogenized and then was heated for 30 minute, and was then filtered, the residue was poured in another 2.5ml of sulphuric acid and was then homogenized and was heated again, and was then filtered again and the residue was weighed.

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

W_1 = Weight of the sample

W_2 = Weight of the empty crucible + sample

W_3= Weight of the crucible + ash (Ayo and Agu 2012).

Crude protein determination

About 5g of the sample was transferred into a macro digestion flask and Kjeldahl catalyst tablet added. 25ml of concentrated H_2SO_4 was added into the flask. The flask was heated on a Bunsen burner; the heat was carried gentle until frothing is complete. The heat was increased strongly with shaking at intervals. The heating was continued for an hour until mixture become clear and was cool. About 400ml of distilled water was added to digest the mixture. 50.0ml of boric acid with 1ml of methyl red indicator were added to a 500ml of volumetric flask, delivery tube was dipped in to the 2% boric acid, and 75ml of NaOH (50% NaOH containing 5% sodium thiosulphate) was added to the mixture. Air tight condition was created when distilled passed in to the boric acid. 200ml of distilled water was collected in a beaker and the distillate was titrated with 0.1N H_2SO_4.

$$\text{Total nitrogen \% by weight} = \frac{N \times T \times 6.25 \times 1.4 \times 100}{\text{Weight}}$$

N = normality of HCL

T = titration value

Protein constant= 6.25

Kjhedal constant = 1.4 (Ayo and Agu 2012).

Carbohydrate determination

The carbohydrate was determined by difference as;

% Carbohydrate = 100- (%oil +% moisture + %ash + %fiber +% protein) (Ayo and Agu 2012).

Specific gravity

A 50ml density bottle was thoroughly washed and dried in an oven. The bottle was first weighed emptied, and was the filled with water was weighed again. It was filled with the oil and was weighed and the weight was noticed (Ayo and Agu 2012).

$$\text{Specific gravity} = \frac{\text{weight of bottle x ml of oil}}{\text{Weight of the bottle x ml of } H_20}$$

Saponification value

About 2.0g of the oil was weighed in a flask resistant to action of alkaline and was boiled for an hour in a water bath with 25ml of alcohol and 0.5N of potassium hydroxide, shaking the content frequently at intervals. The content was them titrated with 0.5N HCl to determine excess alkaline using phenolphthalein as indicator. A blank determination using the same quantity of KOH was carried out at the same time as describe Ayo and Agu 2012.

If X = volume of 0.5N acid required for the blank

Y= volume of ml of 0.5N acid required for the oil
W= weight of grams of the sample used

$$\text{Saponification value} = \frac{(x-y) * 28.05}{\text{Weight of the sample used}}$$

Acid value

About 25ml of diethyl ether was mixed with 20ml of alcohol and 1ml of phenolphthalein solution was then carefully neutralized with 0.1ml of sodium hydroxide (NaOH). 5g of the oil was then dissolved in a mixture neutral solvent and titrated with aqueous solution of 0.1ml of sodium hydroxide. (Ayo and Agu 2012)

Acid value was estimated using the following formula

$$\text{Acid value} = \frac{\text{titration value (ml)} * 5.61}{\text{Weight of the sample used}}$$

Peroxide value

About 5g of the oil was placed in 30ml glacial acetic acid, chloroform and saturated solution of potassium iodide (20.0ml) was added to liberate iodine by reacting with the peroxide. The resulting solution was titrated against sodium thiosulphate (0.002M) using starch solution (1%) as indicator, until the yellow colour just disappeared. The peroxide value was calculated as follows

$$\text{Peroxide value} = \frac{2(a-b)}{\text{Sample weight}}$$

Where; a= titer value for the sample
b = titer value from the blank (Ayo and Agu 2012).

Iodine value

About 0.5g of the oil sample was placed in a 250ml conical flask and 10ml of anhydrous chloroform was added. This was followed by 30ml of wijs solution and the flask was Stoppard and was allowed to stand in the drawer for 30 minutes after the potassium iodide (10ml of 15% v/v) was added to the content of the flask so as to wash down any iodine that might be present. The resulting solution was titrated with sodium thiosulphate solution (0.1M) until the light yellow colour formed disappeared. The determination for the blank was conducted in the same manner but without the oil. The iodine value was calculated as

$$\text{Iodine value} = \frac{(b-a) * 1.269}{\text{Sample weight}}$$

Where M= molarity of sodium thiosulphate
b= blank titre value
a= sample titre value (Ayo and Agu 2012)

Unsaponifiable Matter

About 1ml of aqueous 3M potassium hydroxide solution to neutralize the liquid left after the titration for the determination of saponification value. While the extract used in the titration is still warm, it was extracted with 3x50ml diethyl ether. The ether layer was washed thrice by shaking vigorously with 20ml portion of 0.5M potassium hydroxide solution and then with 20ml portions of water until was no longer alkaline to phenolphthalein. The content in the flask was evaporated on water bath. 2-3ml of acetone was then added to complete the removal of the solvent. The content in the flask was the dried to a constant weight at 100^{o}C. The content in the flask was then dissolved in 2ml ether and 10ml of ethanol added to neutralize it. The content was the titrated with 0.1N alcoholic potassium hydroxide solution.

Calculations

Unsaponifiable matter = $\frac{M_1\text{-}0.0282V * 1000\ G/KG}{M_2}$

Where; M_1 = Mass of oil taken for saponification value

M_2 = Mass of unsaponifiable matter

V = ml of 0.1N alkali required to neutralize the residue in the determination of the unsaponifiable matter (Ayo and Agu 2012).

Percentage of the free fatty acid (FFA) determination

2g of the sample was weighed into a 250ml conical flask and 10ml of ethanol was added, the resulting mixture was titrated with sodium hydroxide (0.1M) using phenolphthalein as an indicator. The titration was done with constant shaking until pink color persisted for 30 seconds. The % FFA was then calculated from the following equation.

$\%FFA = \frac{V \times M \times 2.82\ mg}{\text{Sample weight}}$

Where V= volume of sodium hydroxide

M= molarity of sodium hydroxide solution used

2.82= conversion factor oleic acid.

The acid value=% FFA x 1.99 (Ayo and Agu 2012).

Results and Discussion

Table 1: Results of proximate analysis

Parameter	Values (mean±SD)
Moisture content (%)	8.54±0.07
Ash content (%)	3.48±0.02
Lipid content (%)	27.83±.007
Crude fiber (%)	15.85±0.03
Crude protein (%)	29.15±0.04
Carbohydrates (%)	15.15±0.01

Table 2: Results of physiochemical analysis

Parameter	Values (mean±SD)
Colour	Pale yellow
Peroxide value (meq/kg)	13.7±.001
Iodine value (gl/100g)	82.7±0.02
Specific gravity (g/cm^3)	0.87±0.05
Acid value (KOH/g)	17.7±0.13
Free fatty acid (%)	18.21±0.12
Saponification value (KOH/g)	183.13±0.11
Ester value (mg KOH/g)	168.11±0.03
Unsaponifiable matter (%)	3.9±0.01
Melting point (^{0}C)	31.89±0.12
Freezing point (^{0}C)	12.99±0.21
Calorie (Kcal)	908.11±0.18

Discussion

The proximate analysis of the seed and physiochemical properties the oil extracted from the seed fluted pumpkin was determined and the results were shown in table 1 and 2. The moisture content is the loss on drying at oven temperature of 105^{0}C. Besides water, the loss will include other volatile matter at 105^{0}C. The samples had a moisture content of 8.54%. This moisture content is safe for storage. The ash content is residue when the organic

component of the food has been burnt off in the furnace at 500^0C for about 6-8hours. The result of the ash content yields 3.48%.

The lipid content is the gravimetric estimation of the fat from the dry sample after continuous extraction with light organic solvent say petroleum ether or n-hexane. The lipid content of the sample yields 27.83%. The crude fiber content of any food sample is the residue from the process whereby the starch and protein components have been dissolved by boiling with acid and sodium hydroxide. The residue of cellulose and lignin is washed, dried, ashed and weighed. The results of the proximate analysis were similar to the results Christian 2006 and Etong *et al* 2014.

The crude fiber is the expressed in percentage of the weight of the sample. The sample yielded a crude fiber content of 15.85%. The protein content of any seed or food sample is the derived usually by estimating the nitrogen content of sample and multiplying by appropriate factor to convert to protein content. The sample had a protein content of 29.15%. The carbohydrates content of the sample was by difference from the other component as expressed in percentage and it yielded 15.15%.

Table 2 presents the physicochemical properties of the oil from fluted pumpkin (*Telfairia occidentalis*) seeds. The pale yellow colour of the oil was similar to other conventional oils from such other vegetables as melon and groundnut oils (Christian, 2006). Specific gravity is the ratio of the mass of a given volume of the substance to an equal volume of water. The specific gravity of the oil in this study was 0.899gcm $^{-3}$ which is comparable to values obtained for lard and tallow (0.893-0.904 gcm^{-3}) (Agu 2012).

The melting points of fluted pumpkin seed oil was 31.89 °C. This is higher than the melting point of Africa bush mango (*Irvingia gabonensis*) seed oil with the value of 13 °C (Etong *et al*., 2014) but comparable with the melting point range of 24.2 - 42.30 °C reported for some other seed oils such as breadnut (Nwinuka *et al.*, 2001). The melting point of the seed oil is an advantage in cold cream manufacture. The lower the melting point of seed oil, the better the oil is for making oil creams. The boiling and freezing point were 14.50 °C and 262.0 °C respectively.

Saponification value measures the average molecular weight or chain length of all the fatty acids present in the oil. The saponification value obtained in this study for *Telfairia occidentalis* seed oil was 183.13mgKOHg^{-1} which compares favorably with 176.87mgKOHg^{-1} reported for walnut oil (Isong *et al.*, 2013) and also similar to the range (177– 82mgKOHg^{-1}) reported by Ogunniyi (2006) for castor seed oil. The value obtained in this study is lower than 194 mg KOH g^{-1} for red palm oil and 213mgKOHg^{-1} for neem seed oil (Osita, 2007) but higher than 159.33mgKOHg^{-1} for *Dennettia tripatala* fruit oil (pepper fruit) (Nwinuka and Nwiloh, 2009).The result of saponification value of *Telfairia occidentalis* seed oil suggests its suitability for industrial soap making since it is within the range of oils currently used for the same purpose.

The oil was low in unsaponifiable matter (3.90%) although higher than an earlier value (0.65 %) reported for *Telfairia occidentalis* seed oil and 0.75% for *Citrillus vulgaris oil* (Yusuf *et al.*, 2006). It has been reported that oils with high ester values are more intact and therefore more suitable for consumption (Nkafamiya *et al.*, 2010). The ester value obtained

was 168.11mgKOHg^{-1}. This value is however lower than the ester values of other unconventional seed oils such as *Adansonia digitata* (224.82mgKOHg^{-1}), *Calophyllum inophyllum* (198.10mgKOHg^{-1}), *Dacryodes edulis* (193.91mgKOHg^{-1}), *Monodora myristica* (237.80mgKOHg^{-1}), *Terminalia catappa* (236.51mgKOHg^{-1}) and *Pentaclethra macrophylla* (151.42mgKOHg^{-1}) (Ajayi, 2010) but higher than 38.37mgKOHg^{-1} reported for *Musa parasidiaca* peel oil (Oladiji *et al.,* 2010) and 89.74mgKOHg^{-1} for *Tetracarpidium cocophorum* seed oil (Isong *et al*., 2013) but counteracts 230.94 for *Telfairia occidentali*reported by Ajayi (2010) which could be associated with difference in species and location of collection of sample.

Free fatty acid value is an important variable in considering the quality and suitability of oil because the lower the free fatty acid, the better the quality of the oil (Coenen, 1976). The percentage free fatty value of this seed oil was 18.21%. The value is however hiher than 9.5% reported for walnut oil (Isong *et al.,* 2013) and 0.82% for sesame oil (Elleuch *et al.,* 2007). This result indicates that *Telfairia occidentalis* seed oil can be said to be of good quality because oils intended for human consumption.

Iodine value is used to determine the degree of unsaponifiable matter of fats and oils and it has been reported that lowering the iodine value improves the stability of the oil (Nkafamiya *et al.,* 2010). In this study, the iodine value of the oil extracts was 82.70gI per 100g which is higer to the previous value (51.52gI per 100g) obtained by (Eddy *et al*., 2001) although closer to the value (98.2gI per 100g)) obtained by Alozie *et al.* (2010) for *Persea americana* seed oil. According to CODEX Alimentarius Commission (1982), a good drying oil should have an iodine value of 180 and above. The value obtained from this seed oil classifies it as non-drying oil since it is below 180 thus it cannot be used in the preparation of alkyld resins (Yahaya *et al.,* 2004). The low iodine values of the oil also reduce the risk of oxidative rancidity.

Peroxide value is used as indicators for deterioration of oil. It serves as an index of rancidity, thus high peroxide content of oil indicates a poor resistance of the oil to peroxidation during storage. The peroxide content obtained from this oil was 13.7. This value however varies from the values 0.77mEqkg-1 earlier reported for *Telfairia occidentalis* (Yusuf et al., 2006) and higher than the peroxide values for *Cocos nucifera* (0.21meq kg^{-1}) and *Colocynthis citrullus* (1.53meq kg^{-1}) (Obasi *et al.,* 2012).

On comparism with the principal vegetable oil bearing seeds, fluted pumpkin seed can be rated among the second highest oil producing seed because of its high content as clearly shown in the table 3. The saponification value of fluted pumpkin seed (101.5+0.71)mg/KOH this result agrees with work conducted by Lela's and Tsaknuss, 2002 of 189.11mg of KOH/g of oil. Acid value of fluted pumpkin seed was found to be (5+0.63) and free fatty acid of (2.5+0.31)mg/KOH/g and is comparable with the work of Lange (1994) in which oil extracted from coconut seed contain 4.753 and 2.50mg/g. acid value is an indication of deterioration of oil resulting from hydrolysis of fat and oils.

Conclusion

The results obtain from the study revealed that fluted pumpkin seed oil can serve as acceptable edible oil for use in preparation of various food products. The high saponification value suggests the potentiality of the oil in the production of liquid soaps and shampoos. Due

to its low moisture content fluted pumpkin seed oil can be stored for a longtime without deterioration or any oxidation, because of its less iodine value (less than 100%) the oil cannot be classify as dry oil due to its low acid value, which are usually indication of deterioration of oil result from hydrolysis. Fluted pumpkin seed oil it has storage stability hence it will not undergo any oxidation rancidity and therefore the oil can be used in various food product. Low content of free fatty acid indicates that fluted pumpkin seed oil is not rich in essential fatty acids. It however exhibits good physiochemical properties which make it useful for industrial application. The present study revealed that fluted pumpkin seed is a good source rich in protein, fiber, moisture, carbohydrate, and fat and oil.

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