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Evaluation of Selected Physico-Chemical Properties and Anti-Trypsin Activity of Fluted Pumpkin Telfairia Occidentalis Hooker (*Cucurbitaceae: Cucurbitales*) Seed and Seed Oil

Johnson JT[1]*, Ekpo GI[2], Gbashi S[3] and Peter H[4]

[1]*Department of Biochemistry, Federal University Otuoke, Nigeria*

[2]*Department of Biochemistry, University of Calabar, Nigeria*

[3]*Department of Biotechnology and Food Technology, University of Johannesburg, South Africa*

[4]*Department of Food Science and Technology, University of Calabar, Nigeria*

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***Corresponding author:** Johnson Joel Theophilus, Department of Biochemistry, Faculty of Sciences, Federal University Otuoke, PMB 126, Yenagoa-Bayelsa State, Nigeria, Email: theophjoe@yahoo.com

Abstract

Oil extract from seeds of *Telfairia Occidentalis* fluted pumkin, Hooker (*cucurbitaceae: cucurbitales*) using n-hexane gave 50-53% content. The oil with golden yellow appearance was liquid at room temperature with specific gravity of 0.921 at 20 °C and refractive index of 1.481. The result of the evaluation showed that the oil has high saponification value of 186.8, iodine value of 148.2 and hydroxyl value of 65.3. Other characteristics assayed which had low value were the peroxide 0.97, unsaponifiable matter 2.2 and acid value 0.84. Compared to data obtained from well known researched such as soya bean oil, the fluted pumpkin seed oil could be useful as edible oil as well as in soap and epoxy resin manufacture. Moreover, the anti-trypsin substance activity of graded aliquots of extract of fruited pumpkin seed (*Telfaria occidetalis*) and treated samples were determined.

The different treatments were:

1) Roasting at 105 °C for one hour,

2) Roasting at 50 °C for 24 hours,

3) Roasting at 50 °C for 36 hours,

4) Boiling with roasting at 50 °C for 24 hours and

5) Boiling alone for 24 hours at 50 °C.

The raw seed was shown to contain a low level of anti-trypsin factor (s) (1.1%/ mg of protein). Treatment 3 and 4 resulted in loss of all anti-trypsin activity even at higher concentration. There was however, a residual anti-trypsin activity in seeds which were roasted at 105 °C for 1 hour (treatment 1) and 50 °C for 24 hours (approximately 2%). Boiling the seeds did not also remove the anti-tryptic activity completely. This study also shown that fluted pumpkin seeds contain some anti-tryptic substance (s). It has also been revealed that roasting alone at varying temperatures for a short period as specified in this research will not completely remove trypsin inhibitor substance(s) activity. Fluted pumpkin seeds may compliment soya-bean and other high proteinous plant food item(s) in meeting the nutritional requirements of Nigerians and Africans, particular in the rural areas with high prevalence of nutritional disorder among the populace. Finally, the histo-mophological evaluation of the liver and kidneys of rats that were fed with various treated fluted pumpkin seed mixed showed no physible or detectable abnormalities or distortion.

Keywords: Anti-trypsin activity fluted pumpkin; Physico-chemical properties; seed oil

Introduction

Fluted pumpkin is a tropical tendril climbing, herbaceous annual plant which belongs to the family cucurbitaceae Hooker (*cucurbitaceae: cucurbitales*). The creeping vegetative shrub can spread low across the ground with large lobed leaves and long twisting tendrils [1]. The leaf is a popular traditional pot herb in the south-South, south-east and south-western part of Nigeria. The female plant yields seeds protected by pulp and contained

in a pod which resembles a large okro (Lady Finger). The seeds could be boiled, seed coat removed and the cotyledon eaten or dried and eaten as nut. The cotyledon could also be blended and used as a soup thickener or fermented into a food condiment. The mineral composition of fluted pumpkin leaves have been investigated and reported and are known to be a good source of four minerals required in human nutrition [2]. Fermentation of the seed have also been shown to increase thiamine and riboflavin content [3].

The oil and protein contents of the seed have been reported as 47% and 31.1% respectively with the oil having a fatty acid constitution of over 63% oleic and linoleic acid and 31.85% for stearic and palmitic acid [4]. Extraction of lipids from the seed was reported to contain a total lipid of 31.14% dry weight which on fractionation yielded 58% phospholipid, 26% glycolipid and 16% neutral lipid (Odemena and Onyeneke, 1988). A low oil content of 13% of the seed was reported by Okoli and Nyanayo [5]. The oil of fluted pumpkin seed has been reported to be used for cooking [1], marmalade manufacturing [6], cookie formulations [7]. The oil has also been recommended as good feedstock, for lubricants, candles and soap production, in addition to cooking and margarine production [8].

Due to the fact that the major sources of edible oils in Nigeria: oil palm, peanut, soya bean are unable to meet the increasing demand in some areas occasioned by the cost, there is need to source for commonly available local oil-bearing seeds with even high nutritional value to complement the existing ones. The characteristics of oil from fluted pumpkin seeds sold in Lagos, Nigeria are yet to be documented. This study was therefore carried to assess the physical and chemical properties of the oil from T. occidentalis with a view of providing information towards its effective utilization both for domestic and industrial purposes.

Materials and Methods

Collection and preparation of samples

Fluted pumpkin pods used for the studies were obtained locally from Otto market in Lagos State Nigeria. The pods were cut open and the seeds separated from the pulp. The seeds were boiled for about 30-35 minutes in 200ml distilled water and when soft, the cotyledons were removed from the coat, weighed and dried in G150 air circulating oven overnight at 50 °C. The weight after drying was also noted. The seeds were milled into flour using a domestic electric blender.

Extraction of Oil

The oil was obtained by exhaustive extraction of the flour with n-hexane using a soxhlet extraction apparatus. The hexane was distilled off using a rotary evaporator. The oil was then dried at 50 °C for 15 minutes and left at 20 °C until used [9].

Physico-chemical properties assessment

The characteristics of the oil such as specific gravity, saponification and iodine number, peroxide acid and hydroxyl values, refractive index and unsaponifiable matter were determined by procedures in AOAC [10].

Treatment of seed for enzyme activity

Roasting: Cotyledons (without the thin membrane found in between) were roasted at 105 °C for 1hr, at 50 °C for 24hrs and 36hrs using a circulating hot air oven [11].

Boiling: Seeds with seed coat intact were boiled for 30 minutes alone or followed by roasting at 50 °C for 24hrs [11].

Trypsin inhibitor activity

Phosphate Buffer (PH 7.6, 0.1M); 0.2M NaH_2SO_4 was used to adjust 0.2M Na_2HPO_4 to PH 7.6. The solution was then made up to 200ml with distilled water.

1.0% Casein solution: 1.0g of casein was suspended in 80ml phosphate buffer and dissolved by heating on a steam bath for 10 minutes. The cooled solution was made up to 100ml with phosphate buffer.

Trypsin solution: 0.125g trypsin was dissolved in 25ml of 0.1m phosphate buffer [11].

Preparation of fluted pumpkin seed extracts

Crude extracts of the raw and various treated fluted pumpkin seed were prepared by shaking 0.5g seed flour with 25ml of 0.1N NaOH for 1hr, the PH being maintained at 9.0. The suspension was diluted 1:10 using distilled water. We chose the stated dilution since we assumed that fluted pumpkin seed is not a legume and therefore, may not contain a high level of anti-trypsin factor. This was done using the method of Kakade [12] & Hemen [9].

Determination of enzyme activity

Estimation of trypsin inhibitory activity of the extracts was based on the method developed by Kakade [12]. Stock trypsin solution (0.5ml) was pipette in triplicate into clean test tubes followed by 0.4ml HCl (0.001N). Various volumes of the fluted pumpkin seed extract were then added. The final volume of each tube was adjusted to 2.0ml with phosphate buffer (PH 7.6; 0.1m). The tubes were incubated at 37 °C water bath for 10 minute. To one set of tubes, 6.0ml of 5% (W/V) trichloroacetic acid (TCA) was added. This served as a blank. To each tube was then added 2.0ml of 1% casein solution previously brought to 37 °C. Incubation was continued for a further 20 minutes. At the end of the incubation period, 6.0ml of TCA solution was added to each tube in the second and third sets to stop the reaction. The suspension was centrifuged at 1000g for 10minutes. The Absorbance of the supernatant was measured at 280nm in a spectrophotometer (uv/vis sp 17500) against the blank.

Expression of activity

Enzyme activity is expressed in trypsin units (TU). In this method, one trypsin unit is defined as an increase of 0.01 absorbance unit at 280nm in 20 minutes for 10.0ml of reaction mixture under the conditions described. The trypsin inhibitory

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activity is therefore the number of TU inhibited (TUI; 5). The results are average of three (triplicate) different determinations [13].

Statistical Evaluation

Data obtained were expressed as Mean ± Standard Error of Mean (SEM) and analyzed using the Analysis of Variance 'ANOVA; f-ratio' [14], student't' test and statistical package for social sciences (SPSS version 20) where applicable. Values at $P<0.05$ were regarded as significant in comparison with appropriate controls.

Results and Discussion

Table 1: Some properties of fluted pumpkin seed oil.

Species	Oil in seed (% Dry Weight)	Colour	Room Temperature	At -40C
Telfairia occidentalis	50-53	Golden yellow	Liquid	Liquid

The lipid extracted from fluted pumpkin was golden yellow in colour and liquid both at room temperature and 40 °C with a distinct aroma which disappeared with time. The lipid content of the fluted pumpkin seeds on dry weight basis was 50-53% (Table 1). This oil content is higher than the 47% earlier reported for fluted pumpkin seed by Asiegbu [4] and other related studies. The high content of oil in these seeds investigated could contribute to a favourable consideration of fluted pumpkin seed oil as one of the edible oils in Nigeria. It is known that the major sources of edible oils in Nigeria have been from peanut (*Arachis hypogoea*) and oil palm (*Eloesis guineensis*), mainly used as cooking oils and for production of soap, margarine and cosmetics [15]. The increasing demand for these common sources of oil has led to the search for alternative sources of other local oil-bearing seeds such as fluted pumpkin and soya bean.

Table 2: Physico-chemical properties of fluted pumpkin seed oil

Property	Value(s)
Iodine value	148.2
Saponification number	186.8
Acid value	0.84
Hydroxyl value	65.3
Unsaponifiable matter	2.2
Refractive index	1.481
Specific gravity (at 20 °C)	0.921

The physicochemical properties of the oils are shown in Table2. The saponification value 186.8 of oil from Telfaria occidentalis in this study was outside the range of 188-196 for most oils of plant origin [16] and less than 199.10 for palm oil but higher than 179.20 for tropical almond [8]. This saponification value observed indicates the presence of low molecular weight fatty acids which could be of use in soap making. The iodine value of 148.2 in this study was higher than the iodine values of 101.73 reported for fluted pumpkin, 85.12 for tropical almond and 56.10 for palm oil [8]. This high iodine value is indicative of the high degree of unsaturation of the oil. Thus, the lipid could be classified as semidrying oil which could be of advantage in the manufacture of epoxy resin esters mainly used as protective and decorative coatings. The low acid value of 0.84 demonstrates good quality suitable for cooking. The refractive index- a physical attribute of triglyceride was 1.481, while the specific gravity at 20 °C was 0.921 which was in line with the value reported for fluted pumpkin by Agatemor [8].

The hydroxyl value was high (65.3) and indicates the presence of hydroxyl groups. The peroxide value which is an indicator of deterioration of fat was low in this study (0.97), but poor storage condition should be avoided as this can lead to oxidative rancidity as the oil has a high proportion of unsaturation. After saponification of oil with potassium hydroxide and extraction with suitable solvents, the unsaponifiable matter includes insoluble hydrocarbons, sterols and fatty alcohols. The low unsaponifiable matter in this oil suggests that the lipid may not be a suitable illuminant.

Table 3: Trypsin inhibitory activity of fluted pumpkin seed.

Trypsin Unit (TU) Volume of extract (ml)	T1	T2	T3	T4	T5	T6
0.5	35.20±4.02	37.60±0.57	39.10±3.01	37.40±1.11	39.30±1.09	42.20±5.67
1	38.10±3.97	38.90±6.10	42.70±4.78	38.20±3.74	35.30±6.04	40.00±5.23
1.5	37.30±5.05	39.20±2.33	36.40±1.12	39.30±2.61	36.5±2.22	39.10±4.78
2	35.40±1.03	36.60±0.45	38.10±0.99	36.90±3.72	38.6±3.10	38.80±5.66

T1: Roasting at 105 °C for one hour, T2: Roasting at 50 °C for 24 hours, T3: Roasting at 50 °C for 36 hours, T4: Boiling with roasting at 50 °C for 24 hours, T5: Boiling alone for 24 hours at 50 °C, T6: Roasted at 105 °C for 1 hour and boiling at 50 °C for 24 hours. Data where express as Mean±SEM.

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Table 3 shows trypsin units (as defined in the text) of raw fluted pumpkin seed and following the various treatments, while the percentage inhibition of trypsin activity in untreated and heat treated samples are presented. As can be clearly seen, anti-trypsin substance (s) is present in fluted pumpkin seeds although at a low concentration. Heat treatment also affects this substance; the original activity of the enzyme without addition of fluted pumpkin extract was treated as control value. Trypsin unit inhibited (TUI) and percentage of inhibition were calculated after subtracting the absorbance from that of the control value.

Boiled seeds followed by roasting at 50 °C for 24hrs, and roasting alone for 36hrs, lost all the anti-tryptic activity and at all concentrations used. Roasted seeds at 105 °C for 1hr and 50 °C for 24hrs retained some activity only at higher concentrations, while boiling alone for 30 minutes resulted in low activity at only low concentrations of anti-tryptic activity.

Like soya bean and most other leguminous seeds, fluted pumpkin seed contain trypsin inhibitor substance(s). However, the activity of this substance as found in the raw sample was much lower than has been reported for, say, soya bean (66-223/mg protein) depending on the variety [12,17,18], or other leguminous plants e.g. winged bean [19]. The loss of some/all anti-tryptic activity following heat treatment, indicate the labile nature of the inhibition activity. This is contrary to what is known about other trypsin inhibitor substances which are usually denatured at that temperature for a period. It is possible; therefore, that the substance(s) may be of different nature from that present in soya or other leguminous seeds. The low levels of anti-tryptic activity found in fluted pumpkin seed migh still reduce the dietary effectiveness of protein if improperly cooked. A long period of heat exposure might be necessary particularly when trypsin inhibitor concentration is high. The toxic components of fluted pumpkin seeds have never been reported or documented. Beside trypsin, others e.g. haemoglutinnin activity, cyanide or saponin might be present and require further investigation which is currently ongoing in our laboratory.

Conclusion

The above properties of the oil from Telfairia occidentalis seeds therefore allow the oil to be of value in soap making, cooking and its epoxy components in protective and decorative coatings; though some other possible uses may be discovered. The use of this oil as source of energy in the formulation of diet suitable for protein energy mal-nourished children will be the subject of further investigation. More so, fluted pumpkin seeds with its high content of protein and oil but low TUI have the potential to be cultivated on a large scale. In developing countries, where daily intake of protein and energy is rather low especially in rural communities, fluted pumpkin might well be considered in future as a complement or supplement for soya bean and it products. A considerable level of nutrition education of the rural populace where this seed is not commonly eaten will be necessary to make possible appreciable harvest and utilisation of the pod.

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