Physiochemical and Phytochemical Characteristics of Lesser-Known Nigerian Black Melon (Ahu Agba) Seed Flour

Peter Anyigor Okorie

(Department of Food Science and Technology Ebonyi State University, Abakaliki, Nigeria.)

Abstract: The physiochemical and phytochemical properties of a lesser-known Nigerian black melon (ahu agba) seed flour was determined in this study. Foaming capacity, emulsion capacity, oil absorption, water absorption, and bulk density tests were conducted. The moisture, protein, fat, fibre, ash, carbohydrate, flavonoid, saponin, carotenoid and alkaloid contents of the flour were determined. The results show that the functional properties of the flour are: foaming capacity 0.02 %, emulsion capacity 62.00 %, oil absorption capacity 33.50 %, water absorption capacity 19.60 % and bulk density 1.49 g/ml. The proximate composition of the flour are: carbohydrate 53.18 %, protein 33.25 %, moisture 3.70 %, fat 27.00 %, crude fibre 5.75 % and ash 1.35 %. The flour has the following phytochemical composition: flavonoid 4.16 %, saponin 5.15 %, carotenoid 0.81 % and alkaloid 6.95 %. The analysis revealed that the flour could be used in soup making and infant food formulation. It could also be useful for prevention and cure of heart related diseases.

Keywords - Black melon, physiochemical properties, phytochemical properties, melon seed flour

Date of Submission: 07-06-2018 Date of acceptance: 22-06-2018

I. INTRODUCTION

The black melon simply known as ahu agba in Abakaliki is a specie of melon that is familiar to Abakaliki indigenes. It is grown during the rainy season as a source of food and oil. Melon is known to be rich vitamin B, potassium, magnesium, and zinc, with health benefits [1]. The rich in the nutrient status of melon makes it significant as a major source of food [2], especially in Africa. Melon has many species across different towns and tribes of West Africa. This quality of melon has attracted the attention of many researchers on different species of melon.

Milovanovic and Picuric-Jovanovic [3] studied the nutritional quality of melon seed and its oil seed characteristics. They reported that the moisture content, oil content and crude protein content in melon seeds were 54.5 %, 22 % and 21.8 %. The melon seed has a specific gravity of 0.914 kg/dm³ at 20 °C, refractive index of 1.473 at 20 °C, acid value of 1.00 mgKOH/g, saponification value of 188 mgKOH/g, iodine value of 119 g/100 g), peroxide value of 7.9 mmolO₂/kg, unsaponifiable matter of 1.2 %, ester number of 187 and free fatty acid of 0.52 % oleic acid. Their findings suggested that melon seeds might be a useful product with nice nutritional value.

Koocheki *et al.* [4] conducted a research to ascertain the physical properties of three common Iranian varieties of melon seeds at varying moisture content of the seeds. The three melon seeds are Ghermez, Kolaleh and Sarakhsi. It was found that increase of moisture content increases axial dimensions, surface area, emptying angle of repose, bulk and true density, sphericity, geometric and arithmetic mean diameters, and static friction coefficient on five structural surfaces, but decreases porosity and filling angle of repose. Among the varieties, Ghermez had the highest values of geometrical properties, in all moisture contents studied.

Taiwo *et al.* [5] carried out an experiment to investigate the effect of different drying methods on the quality and quantity of watermelon oil. Two drying methods, namely sun drying and oven drying were considered. Results show that the sun-dried sample and the oven-dried sample yielded 56 % and 57 % of oil/100g of the seeds. The free fatty acid and acid values were higher in oven-dried sample relative to the sun-dried sample. Other chemical properties were not affected. They suggested that the high protein content and a high concentration of the amino acids of the seed makes it appropriate for protection of foods, the oils could serve as a worthy supplement in the formulation of animal feed.

Although the physiochemical and phytochemical properties of some melon species has been studied in the past, the physiochemical and phytochemical properties of lesser-known Nigerian black melon (ahu agba) seed flour that is familiar to the Abakaliki indigenes is yet to be known. This study presents the physiochemical and phytochemical properties of the black melon seed flour that is grown in Abakaliki, a town in the southeast part of Nigeria.

MATERIALS AND METHODS II.

Sample Preparation

The black melon seeds that were sourced from the Okwo market at Ngbo town in Ohaukwu local government area of Ebonyi state, Nigeria were de-husked, sieved and screened to achieve a dirt free sample. The seeds were ground to flour, stored in an airtight polyethylene bag, and put inside containers.

Functional Properties Tests

Foam Capacity Test

2 g of the black melon flour sample was blended with 10 ml distilled water. The suspension was whipped at 1600 rpm for 5 minutes, poured into a measuring cylinder and the volume was recorded after 30 seconds. The foam capacity was calculated in line with Abbey and Ibeh [6] thus:

% whipability =
$$\frac{\text{volume after whipping - volume before whipping}}{\text{volume before whipping}} \times 100$$

After whipping, the foam was recorded at 15, 30, 60 and 120 minutes to generate foam stability according to Ahmad and Chmidt [7] (1979).

Foam stability =
$$\frac{\text{foam volume after time 't'}}{\text{initial foam volume}} \times 100$$

Emulsification Capacity Test

2 g of the flour sample was blended with 25 ml distilled water at room temperature for 39 seconds at 1600 rpm. 25 ml vegetable oil was added gradually after complete dispersion and the blending was continued for another 30 seconds. The product was transferred into a centrifuge tube at 1600 rpm for 5 minutes. The emulsion capacity was expressed according to Padmashree et al. [8].

Emulsion capacity =
$$\frac{\text{height of emulsified layer}}{\text{height of whole solution in the centrifuge tube}} \times 100$$

Water/Oil Absorption Capacity Test

The method described by Onwuka [9] was used. 5 g of the sample was put into a clean conical graduated centrifuge tube and mixed thoroughly with 10 ml distilled water/oil using a mixer for 30 seconds. The sample was then allowed to stand for 30 minutes at room temperature after which it was centrifuged at 1600 rpm for 30 minutes. The water and oil absorption capacities were expressed in grams of water/oil absorbed per gram of the sample.

$$Water absorption capacity = \frac{volume \ of \ water \ absorbed}{weight \ of \ sample} \ x \ 100$$

$$Oil \ absorption \ capacity = \frac{volume \ of \ water \ absorbed}{volume \ of \ oil \ absorbed} \ x \ 100$$

$$weight \ of \ sample$$

Bulk Density Test

The method described by Onwuka [9] was adopted. 5 g of the sample was filled in a 10 ml graduated cylinder and its bottom tapped on the laboratory bench until there was no reduction in volume of the sample. The volume was calculated thus:

Bulk capacity
$$(g/ml) = \frac{\text{weigth of sample}}{\text{volume of sample}}$$

III. **Chemical Analysis**

Moisture Content Test

It was determined according to AOAC [10]. Empty petri dish was cleansed and dried in an oven at 100 °C for 10 minutes and cooled in desiccators. The dried and cooled dish was weighed. 5 g of the prepared sample was weighed and dried in an oven with air circulation at 105 °C for 3 hours, cooled in desiccators and then weighed. The percentage amount of moisture was calculated using the formula.

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

Moisture (%) = $\frac{W2 - W3}{W2 - W1} \times 100$ Where W1 = weight (g) of petri dish, W2 = weight (g) of petri dish + sample before drying, W3 = weight (g) of petri dish + sample after drying.

Ash Content Test

It was determined in line with AOAC [10]. The crucible was washed and dried in a muffle furnace at 550 °C for 10 minutes, and cooled in desiccators for 10 minutes. 2 g of the sample was weighed with lid and charred on a hot plate until the smokes disappear. The charred sample was put in the muffle furnace at 550 °C and burned to ashes for 3 hours. The ashes was weighed after cooling for 1 hour. The amount of ashes was calculated by using the formula.

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

 $Ash\ (\%) = \frac{W3-W1}{W2-W1} \times 100$ Where W1 = weight (g) of crucible, W2 = weight (g) of crucible + sample before burning to ashes, W3 = weight (g) of crucible + sample after burning to ashes.

Crude Protein Content Test

It was determined according to AOAC [10]. 1 g of the sample was weighed in a clean testator flask. 6 ml of concentrated sulphuric acid was added and left to stand for 24 hours. After 24 hours, 3.5 ml of H₂O₂ (30 %) was added step by step. When the violent reactions stopped it was shaken and left in the rack. 3 g of accelerated reagent (a mixture of copper sulphate pent hydrate and anhydrous potassium sulphate) was added and left for 15 minutes. The mixture was digested in a digest, stored at 37 °C for 4 hours. After digestion, it was cooled in a hood on the rack and 25 ml of distilled water was added to dissolve the precipitate, 25 ml of 40 % NaOH was added to the digested sample and placed in the distiller. 25 ml of saturated solution of boric acid (H₃BO₃), 25 ml of distilled water and 3 drops of methyl red were added in the 250 ml conical flask and placed in the distiller. After distillation, about 150 - 200 ml distillate was collected and titrated with 0.1 M HCl. The amount of protein was calculated using the formula.

Protein (%) =
$$\frac{\text{titre value x } 0.0014 \text{ x } 6.25}{\text{weight of sample}} \text{ x } 100$$

Crude Fat Content Test

It was extracted according to AOAC [10]. The cleaned flask and the boiling chips were dried in the drying oven at 100 °C for 1 hour, cooled in the desiccators for 3 minutes and weighed. 2 g of sample was weighed in a thimble containing fat free cotton. The thimble was placed in the thimble holders. 50 ml of petroleum ether (boiling range of 60 - 90 °C) was poured into the flask and the thimble immersed in the petroleum ether and heated at 80 °C in the fat determination apparatus for 1 hour. The thimble was hanged and heated at the same temperature for 2 hours and then the solvent was recovered for 15 minutes. The heater was switched off and the flask dried in the drying oven at 90 °C for 30 minutes, cooled in the desiccator for 15 minutes and then weighed together with the extract. The amount of extracted fat was calculated by using the formula.

Weight of fat (Wf) =
$$\frac{Wa - Wb}{W} \times 100$$

Where W = weight (g) of sample, Wa = weight (g) of extraction flask after extraction, Wb = weight (g) of extraction flask before extraction.

Crude Fibre Content Test

It was determined using the method of AOAC [10]. 0.5 g of the sample was transferred into a 600 ml beaker and 200 ml of 1.25 % sulphuric acid was added and boiled for 30 minutes. Recording took place by placing a watch glass over the mouth of the beaker. After 30 minutes heating by gently keeping the level constant with distilled water, 20 ml of 25 % KOH was added and again boiled gently for 30 minutes, then the solution was filtered through sintered glass crucible. Subsequently, washing was done with hot distilled water, 1 % NaOH solution and finally with acetone. It was later filtered and dried in electric oven at 130 °C for 2 hours. Furthermore, it was transferred to a muffle furnace and burnt to ashes for 30 minutes at 550 °C. Finally, it was cooled again and reweighed. The crude fibre content was determined by using the formula.

Crude fibre content (%) =
$$\frac{W1 - W2}{W3} \times 100$$

Where W1 = weight (g) of crucible after drying, W2 = weight (g) of crucible after burning to ashes, W3 = weight (g) of sample.

Carbohydrate Content Test

The method of Cordenunsi and Lajolo [11] was adopted. It was determined by estimation using the arithmetic difference method. The carbohydrate was calculated and expressed as the nitrogen free extract as shown.

Carbohydrate (%) =
$$100 - a + b + c + d + e$$

Where a = % protein content, b = % fat content, c = % ash content, d = % crude fibre content, e = moisture content.

IV. **Phytochemical Analysis**

Flavonoid Content Test

It was determined according to the method proposed by Harborne [12]. 5 g of the sample was boiled in 50 ml of 2 M HCl solution for 30 minutes under reflux. It was allowed to cool and then filtered through Whatman number 1 filter paper. A measured volume of ethyl acetate was added. The flavonoid precipitate was recovered by filtration using weighed filter paper. The resulting weight difference gives the weight of flavonoid in the sample.

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Saponin Content Test

It was determined by double solvent extraction gravimetric method proposed by Harborne [12]. 5 g of the powdered sample was mixed with 50 ml of the 20 % ethanol and both extracts were pooled together. The combined extract was reduced at about 40 ml at 90 °C and transferred to a separating funnel where 40 ml of diethyl ether was added after shaking vigorously.

Carotenoids Content Test

Measured weight of the sample was homogenized in methanol using a laboratory blender. The homogenate was filtered to obtain the initial crude extract, 20 ml of ether was added to the filtrate to take up the carotenoid, and it was mixed well and then treated with 20 ml of distilled water in a separating funnel. The ether layer was recovered and evaporated to dryness at low temperature (35 °C – 50 °C) in a vacuum desiccator. The carotenoid was taken up. The dry extract was then saponified with 20 ml of ethanoic potassium hydroxide in a dark cupboard. The next day, the carotenoid was taken up in 20 ml of ether and was washed with two portions of 20 ml distilled water. The carotenoid extract was dried in a desiccator and was treated in a light petroleum and was allowed to stand overnight in a freezer at 10 °C. The next day, the precipitate steroid was removed by centrifugation and the carotenoid extract was evaporated to dryness in a weighed evaporation dish, cooled in a desiccator and then weighed. The weight of carotenoid was determined and express as a percentage of the sample weight.

Alkaloid Content Test

It was done by the alkaline gravimetric method described by Harborne [12]. A measured weight 5 g of the sample was dispersed in 10 % acetic acid solution in ethanol to form a ratio of 1:10. The mixture was allowed to stand for 4 hours at 28 °C. It was later filtered via Whitman number 1 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid precipitate was received in a weighed filter paper, washed with 1 % ammonia solution and dried in an oven at 80 °C. Alkaloid content was calculated and expressed as a percentage of the weight of the sample.

V. RESULTS AND DISCUSSION

The results of the functional properties, proximate composition and phytochemical composition of the lesser-known Nigerian black melon (ahu agba) seed flour are presented in Tables 1, 2 and 3. Table 1 shows that the foaming capacity is 0.02 %. The low foaming capacity could be because of the inadequate electrostatic repulsions, less solubility and excessive protein-protein interactions [13]. The foaming capacity value is lower from the value submitted by Arawande and Borokini [14], who proposed that melon seed flour has a foaming capacity of 23.5 %. The difference in result could be due to the uniqueness of the lesser-known Nigerian black melon seed that is peculiar to the Abakaliki people of the southeast Nigeria.

 Table 1: Functional properties of black melon seed flour

Foaming capacity	Emulsion capacity	Oil absorption	Water absorption	Bulk density (g/ml)
(%)	(%)	capacity	capacity	
		(%)	(%)	
0.02	62.00	33.50	19.60	1.49

The emulsion capacity of the black melon seed flour is 62.00 %, which is higher than the value reported by Olaofe *et al.* [15] for calabash seed flour (a type of melon) with 23.20 % as the emulsion capacity. Geographical region difference and changes in climatic condition could be the cause of the variation of the results. The oil absorption capacity is 33.50 %, which is close to the value reported by Olorode *et al.* [16] who submitted a value of 39.05 % as the oil absorption capacity of melon seed flour. It implies that the seed flour could serve as a good aroma agent, flavour retainer and be used to improve mouth feels for food. The bulk density is 1.49 g/ml. It is higher than the value reported by Fagbemi *et al.* [17] who submitted a bulk density of 0.42 g/ml for fluted pumpkin. The difference could be due to the absolute difference among the species studied. The water absorption capacity is 19.60 %. The result shows that the flour could be useful in confectionery products where hydration to improve handling is desired [18].

Table 2 shows that the moisture content of the black melon is 3.70 %. Sanful *et al.* [19] pointed out that the greater the amount of moisture in a flour, the greater the rate of decay. It implies that the lesser-known Nigerian black melon seed that has a low moisture content might have a long shelf life, which is an advantage of product stability when wrapped and kept appropriately. The protein content is 33.25 %, which is within the range reported by Fokou *et al.* [20]. The high protein value indicates that it could be used as food.

Table 2: Proximate composition of black melon seed flour

Carbohydrate (%)	Protein (%)	Moisture (%)	Fat (%)	Crude Fibre (%)	Ash (%)
53.18	33.25	3.70	27.00	5.75	1.35

The crude fibre content is 5.75 %, which is higher than the values (1.66 - 2.16 %) submitted by Abiodun and Adeleke [21] for different varieties of melon seed flour, probably due to the variety and climate condition differences. The high crude fibre content implies that the seed flour contains indigestible materials, which can reduce constipation by increasing bowl movement. The fat content is 27.00 %, which is within the range reported by Mabalaha *et al.* [2] who reported fat content value range of 24.80 - 30.00 % for melon seed flours. The result shows that the black seed flour is a good source of dietary oil. The ash content is 1.35 %, which is lower than the value (2.81 - 5.00 %) recorded by Fokou *et al.* [20]. The carbohydrate content is 53.18 %. Although it is not a perfect source of carbohydrate relative to cereals with a carbohydrate range of 72 - 90 % [22], it could serve as an alternative in a cereal scarce area.

Table 3 shows a flavonoid content of 4.16 %. It is higher than flavonoid content of 1.00 % for African eleme pulp reported by Ekoh [23]. It implies that the flour is a good source of flavonoid, which helps protect blood vessels from rupture or leakage. The saponin content is 5.15 %. The flour could be a good source of saponin for the treatment of hyper calcium in hum [24]. The alkaloid content is 6.95 %. Food with high content of alkaloid has a negative effect on humanity [25, 26]; it should be cooked very well to reduce the alkaloid content before consumption. The carotenoid content is 0.81 %. The result suggests that the lesser-known Nigerian black melon seed could lower the risk of cardiovascular disease since it has some carotenoid content [27].

Table 3: Phytochemical composition of black melon seed flour

Flavonoid	Saponin	Carotenoid	Alkaloids
(%)	(%)	(%)	(%)
4.16	5.15	0.81	

VI. CONCLUSION

The analysis of the functional properties of the lesser-known Nigerian black melon seed flour (ahu agba) shows that the flour could be used in soup making and infant food formulation. The existence of phytochemicals in the flour shows that the flour has disease defensive and healing attributes. The nutritional composition of the flour indicates that the flour is a nice source of energy, protein, fat and carbohydrate. It is suggested that research be carried out on the characteristics of other species of local Nigerian melon in the future.

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Peter Anyigor Okorie "Physiochemical and Phytochemical Characteristics of Lesser-Known Nigerian Black Melon (Ahu Agba) Seed Flour "International Journal of Engineering Science Invention (IJESI), vol. 07, no. 06, 2018, pp 34-39