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EFFECT OF FERMENTATION ON CHEMICAL COMPOSITIONS OF AFRICAN CUSTARD APPLE SEEDS (*Annona senegalensis*) OBTAINED FROM NIGER STATE, NIGERIA

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ABSTRACT

The chemical compositions of fermented (24 and 48 h) and unfermented seeds of *Annona senegalensis* obtained from Niger State, Nigeria were determined using standard analytical methods. The food functional properties like the foaming capacities ranged from 2.00 ± 0.17 (unfermented) to 7.92 ± 0.31 % (fermented for 48 h), oil absorption ranged from 0.66 ± 0.58 (fermented for 24 h) to 0.78 ± 0.08 mg/g (unfermented) and water absorption ranged from 0.98 ± 0.11 (fermented for 48 h) to 1.30 ± 0.10 mg/g (unfermented), bulk densities ranged from 0.46 ± 0.71 (fermented for 48 h) to 0.78 ± 0.22 g/cm³ (unfermented), while swelling capacities ranged from 3.96 ± 0.24 (fermented for 48 h) to 5.60 ± 0.15 % (unfermented) and emulsification capacities ranged from 40.17 ± 0.42 (unfermented) to 52.36 ± 0.50 % (fermented for 48 h). The metabolites present were quantitatively determined with alkaloids contents ranging from 0.86 ± 0.30 (fermented for 48 h) to 1.60 ± 0.38 mg/100g (unfermented), flavonoids concentrations ranges from 0.55 ± 0.40 (fermented for 24 h) to 0.69 ± 0.36 mg/100g (unfermented) while, fermentation at 48 h was not detected. The plant seeds were rich in glycosides with 13.54 ± 0.34 (fermented for 48 h) to 17.30 ± 0.41 mg/100g (unfermented) ranges. Also, it was observed that fatty acid compositions decreases as the fermentation period increases. Thus, large-scale production of fermented *Annona Senegalensis* seeds will be a valuable means of improving the nutritional quality of *Annona Senegalensis* seeds for man and his animals.

KEYWORDS: *Annona senegalensis*, foaming capacity, fermentation, seeds

INTRODUCTION

The role of fermented food products in human diets cannot be overemphasized. They are vital in their contribution to the livelihoods of residents of rural and urban areas with respect to revenue generation and enhancement of food security (Adesulu and Awojobi, 2014). Several fermented food products are known to exist, which are very useful in meeting the large dietary needs of the rapidly growing global population (Beveridge *et al.*, 2013).



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Current studies have well documented the availability of several shrubs and trees in Nigeria. Most of these plants produce fruits; some which have pods and seeds that can be investigated in other to promote nutrient availability and formulations of drugs (Franzo *et al.*, 2013). There is need for effective promotion of most underutilized crops including seeds, pods and other portions that are mostly disposed due to their under assessment in terms of nutritional compositions (Bello, 2014).

African custard apple plant is a tiny tree which sometimes occurs as a wild shrub that grows up to about 7 meters or more. It has been cultivated as a medicinal herb due to the active compounds present in the fruits, barks, stems. The leaves are simple, oblong and alternating in nature. It has yellow hairs with underdeveloped branches that are usually lost during growth. The diameter of the flower is 35mm while the stalk extends up to 30mm in length. The fruit usually has a diameter of about 45mm and may be found fused with ovate carpels (Mathew *et al.*, 2018a).

At the developmental stage, it appears as a dark green which gradually changes into yellow and finally orange at maturity. The internal whorl is curvy in shape which covers the stamens and ovaries (Coates, 2002). It is prevalent in northern Nigeria and commonly found along rivers, streams and open bushes. It is found as wild plant in Northern Nigeria, mainly in the states of Kaduna, Kano, Plateau and Federal Capital Territory (FCT), Abuja (Alqasim, 2013). More recently, many crops and fruits have been well recognized, however there is dearth in information with respects to their nutritional compositions and values as well as the active compounds responsible for their dietary relevance (Federal Ministry of Health, 2016). The present study therefore aims at determining the effect of fermentation on the chemical contents of African custard apple seeds (*Annona senegalensis*) collected from Niger state, Nigeria.



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MATERIALS AND METHODS

Sample Preparation

The seeds of *A. senegalensis* were collected from farmland in Muwo village, Mokwa Local Government Area of Niger State, Nigeria between the months of May and August, 2018. Identification and authentication of the plant was done by a plant taxonomist from the Department of Medicinal Plant Research and Development (MPR&TM) of National Institute for Pharmaceutical Research and Development (NIPRD), Idu Abuja, Nigeria. The removal of the cotyledons from the dried seeds was done manually.

The cotyledons from the seeds were dried further to obtain a constant weight. 250 g of the sample was weighed and ground into fine form using mortar and pestle. This was sieved using a mesh of 0.5mm, then stored for further analysis. Fermentation of the locust beans was done using the traditional method with little modifications. 100 g of *A. senegalensis* seeds powder was weighed into 250 cm³ conical flask, 30 cm³ of distilled water was added and 1 g of yeast (*Saccharomyces cerevisiae*) was also added to the mixture. It was covered after thorough mixing and then subjected to fermentation for 24 hrs. The fermentation process was also repeated for 48 hrs. The process of fermentation was then ended using a freeze drier and the samples kept for subsequent analysis (Mathew *et al.*, 2018a).

GC/MS Analysis of Fermented and Unfermented of *A. senegalensis* Seeds

The oil extracted from the fermented and unfermented samples of *A. senegalensis* was subjected to GC-MS analysis. Extraction of the oil prior to the analysis was done using petroleum ether as solvent (Orishadipe *et al.*, 2010). The operating conditions of the GC column are as follows: temperature (70 °C), linear velocity (49.2 cm/s), purge flow (3.0 cm³/min) and pressure (116.9 kPa). 8.0 µl of the sample was injected into the column with a split ratio of 20:0. Calculation of the peak area was done through a comparison of its average peak area with the total area (Mathew *et al.*, 2018b).



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Determination of Food Functional Properties of Fermented and Unfermented of *A. senegalensis* Seeds

The analysis of absorption capacity of oil and water was determined according to method described by Mathew *et al.* (2013a). AOAC (2006) method was employed in the determination of the swelling capacity while the calculation of foam solubility from swelling capacity was carried out using the method of Ndamitso *et al.* (2020). AOAC (2006) procedure was also used in the determination of gelation properties and bulk density.

Physico-chemical Properties of the Oil Samples of Fermented and Unfermented *A. senegalensis* Seeds

Analysis of viscosity, peroxide value, saponification value and specific gravity were carried out according to methods by Mathew *et al.* (2014a). Acid value, iodine value, free fatty acid content, temperature and pH were determined as described by AOAC (2006).

Quantitative Phytochemical Analysis of Fermented and Unfermented *A. senegalensis* Seeds

The contents of steroids, flavonoids and alkaloids were determined according to methods required by Mathew *et al.* (2013b); while terpenoids and glycosides contents were determined using modified method (Abubakar *et al.*, 2015).

Statistical analysis

Triplicate determinations were carried out in all analysis. Statistical analysis of the results obtained was done using Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

The result of fatty acid composition of the lipids of unfermented and fermented seeds of *A. senegalensis* are shown in Table 1. There are 9 saturated and unsaturated fatty acids recorded for unfermented, fermented for 24 h and fermented for 48 h respectively. The total saturated fatty acids (TSFA) were 44.00, 38.73 and 34.70 % for unfermented, fermented for 24 and 48h. The total unsaturated fatty acids (TUFA) were 56.00, 61.27 and 65.30 % for unfermented, fermented for 24 h and fermented for 48 h respectively. However, the ratio of



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TUFA/TSFA were 1.27, 1.50 and 1.88 % for unfermented, fermented for 24 and 48 h respectively. Similar result was obtained for the unfermented, fermented for 24 and 48 h seeds of *Cissus populnae* (Mathew *et al.*, 2018b).



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Table 1: Fatty Acid Compositions of the Oil Samples

Fatty acid	Molecular formular	Molecular weight	unfermented	fermented for 24 h	fermented for 48 h
			% Composition		
Linolelaidic acid	$C_{18}H_{32}O_2$	280	18.99	17.05	16.23
Oleic acid	$C_{18}H_{34}O_2$	282	19.03	18.38	16.18
Linoleic acid	$C_{18}H_{34}O_2$	282	7.89	5.04	4.92
Stearic acid	$C_{18}H_{36}O_2$	284	11.57	9.88	7.60
Arachidic acid	$C_{20}H_{40}O_2$	312	9.09	8.69	8.01
Petroselinic acid	$C_{18}H_{34}O_2$	282	12.70	12.11	12.00
Palmitic acid	$C_{16}H_{32}O_2$	256	14.73	15.02	14.11
Pelargonic acid	$C_9H_{18}O_2$	158	6.00	6.70	4.99
Vaccenic acid	$C_{18}H_{34}O_2$	282	5.00	4.43	4.56
TUFA			56.00	61.27	65.30
TSFA			44.00	38.73	34.70
TUFA/TSFA			1.27	1.50	1.88

TUFA = Total unsaturated fatty acid, TSFA = Total saturated fatty acid



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Table 2 presents the results of the physicochemical parameters of oils extracted from the fermented and unfermented seeds of *A. senegalensis*. The color of the oils were dark brown, brown and light brown for the unfermented, 24 hr fermented and 48 hr fermented respectively. Ademola *et al.* (2013) in a similar study, reported related colors for oil obtained from seeds of fermented *P. biglobosa*. For the unfermented, the texture was fine while the fermented was slightly smooth and smooth for 24 hrs and 48 hrs respectively. The unfermented seeds gave pleasant smell, while the 24 hr and 48 hrs fermented had irritating and offensive odour.

The low values of iodine in the sample imply low contents of unsaturated fatty acids. This is an indication that the oil will not be easily prone to deterioration due to oxidation when stored, hence can be readily stored for a long period of time (Falade *et al.*, 2010). The iodine contents ranged from 63.15 ± 0.20 (fermented for 48 h) to 70.29 ± 0.15 gI₂/100 g (unfermented). A decrease in the iodine content was observed with a rise in the duration of fermentation. This could be attributed to the drop in the fat contents through the activity of microorganisms during the process of fermentation. These values were lower than 81.94 ± 1.03 gI₂/100g obtained for groundnut oil (Nkafamiya *et al.*, 2010), but higher than 38.71 gI₂/100g reported for groundnut oil (Atasie *et al.*, 2009).

Saponification value is vital in assessing adulteration of oils. It is a measure of the reactive alkali groups in fats and oils and expressed as the mass of potassium hydroxide in milligrammes which reacts with a gramme of the sample (Oluremi *et al.*, 2013). The saponification values for the samples investigated varied from 101.06 ± 0.40 (unfermented) to 125.00 ± 0.41 mg KOH/g (fermented for 48 h). The values were found to be lower than 9.04 ± 1.60 mgKOH/g obtained for seed oil of *T. occidentalis* (Bello *et al.*, 2011).

Acid value is a measure of the content of free fatty acids in terms of percentage present in a given amount of oil. It is an indicator of the extent to which the triglycerides that are present in the oil are broken down into fatty acids through the action of lipase. This value depends on the degree of rancidity which is used as an index of freshness (Ochigbo and Paiko, 2011). The acid values range from 4.52 ± 0.16 (unfermented) to 7.20 ± 0.44 mgKOH/g (fermented for 48 h). The



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values obtained were observed to be higher than the 1.48 ± 1.60 mgKOH/g reported for seed oil of *T. occidentalis* (Bello *et al.*, 2011). They were however lower when compared to 8.89 ± 0.32 mgKOH/g reported for pea nut oil of bari (Shad *et al.*, 2012). The low acid value is an indication of less susceptibility to spoilage on storage.

Table 2: Physico-chemical Properties of the Oil Samples

Parameters	Unfermented	Fermented for 24 h	Fermented for 48 h
Texture	Fine	slightly smooth	smooth
Smell	Pleasant	irritating	offensive
Colour	Butter	dirty brown	light brown
pH	5.70 ± 0.35^a	7.80 ± 0.12^b	7.80 ± 0.21^b
Temperature (°C)	19.00 ± 0.32^a	27.00 ± 0.21^c	26.00 ± 0.30^b
Viscosity	73.45 ± 0.18^c	70.00 ± 0.32^b	68.95 ± 0.25^a
Iodine value (gI ₂ /100g)	70.29 ± 0.15^c	65.77 ± 0.26^b	63.15 ± 0.20^a
PV (Meq KOH/g)	4.13 ± 0.27^c	3.49 ± 0.24^b	3.00 ± 0.16^a
SV (mg KOH/g)	101.06 ± 0.40^e	116.30 ± 0.11^b	125.00 ± 0.51^c
Specific gravity	0.95 ± 0.22^b	0.93 ± 0.10^b	0.89 ± 0.32^a
Acid value (mg KOH/g)	4.52 ± 0.16^a	5.92 ± 0.33^b	7.20 ± 0.44^c
Refractive index	1.4566 ± 0.12^a	1.4569 ± 0.18^a	1.4571 ± 0.20^a
FFA (mg KOH/g)	2.26 ± 0.25^a	2.96 ± 0.34^b	3.60 ± 0.31^c

Values in the same row with same superscripts alphabets are not significantly different at $p \geq 0.05$. SV= saponification value, PV= Peroxide value, FFA= Free fatty acid

Peroxide value of oil is an indication of the extent of its rancidity. Thus a sample with high peroxide value shows a low resistance of the oil to peroxidation when stored (Mohammed and Hamza, 2008). High peroxide values of between 20 to 40 brings about rancid taste hence the low values from this study further affirms the stability of this oils (Akubugwo and Ugbogu, 2007). The range of the peroxide values was from 3.00 ± 0.16 to 4.13 ± 0.27 Meq KOH/g. The 48 hrs fermented sample had the least value, while the unfermented recorded the highest. The results for peroxide value from this study were lower than 5.26 ± 0.06 Meq KOH/g reported for *T.*



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occidentalis (Mathew *et al.*, 2014b). They are higher when compared to 1.50 Meq KOH/g reported by Atasie *et al.* (2009) for groundnut oil. The values for peroxide contents showed that the oils will not deteriorate quickly (Mathew *et al.*, 2020). Refractive index of oil is an indication of its level of optical clarity in relation to water. The values of oil samples in this study varied from 1.4566 ± 0.12 to 1.4571 ± 0.20 corresponding to the unfermented and 48 hrs fermented samples respectively. Bello *et al.* (2009) in a related study reported an index of 1.460 ± 0.08 for *T. occidentalis*. Samples from this study however had higher values when compared 1.449 for ground nut oil (Atasie *et al.*, 2009). This indicate that the oils from the seeds selected are of high quality and could be useful in the pharmaceutical industries due to their unique properties.

Free fatty acids are prone to lipid oxidation resulting to rancidity and production of foul odour when compared to the intact acids present in the triglycerides (Oluremi *et al.*, 2013). The values of free fatty acids varied from 2.26 ± 0.25 for the unfermented to 3.60 ± 0.31 mgKOH/g for the sample fermented for 48 hrs. Similar value (3.01 mg/KOH/g) for *C. populnae* seeds (Mathew *et al.*, 2020). These values were however higher than the 1.74 ± 0.49 mgKOH/g for seed oil *T. occidentalis* (Bello *et al.*, 2009). The low values obtained from this study indicate that the oils extracted from the selected seeds will not be prone to rancidity.

Table 3: Food Functional properties of the Samples

Parameters	Unfermented	Fermented for 24 h	Fermented for 48 h
Bulk density (g/cm ³)	0.78 ± 0.22^c	0.52 ± 0.12^b	0.46 ± 0.71^a
EC (%)	40.17 ± 0.42^a	48.03 ± 0.44^b	52.36 ± 0.50^c
GP (%)	30.50 ± 0.31^a	35.03 ± 0.20^b	37.61 ± 0.43^c
FC (%)	2.00 ± 0.17^a	5.24 ± 0.25^b	7.92 ± 0.31^c
WAC (mg/g)	1.30 ± 0.10^c	1.02 ± 0.34^b	0.98 ± 0.11^a
OAC (mg/g)	0.78 ± 0.08^c	0.66 ± 0.58^a	0.76 ± 0.16^b
Foaming stability (%)	95.11 ± 0.20^a	98.21 ± 0.35^b	99.65 ± 0.46^c
Swelling capacity (%)	5.60 ± 0.15^c	4.80 ± 0.41^b	3.96 ± 0.24^a

Values in the same row with same superscripts alphabets are not significantly different at $p \geq 0.05$, EC = Emulsification capacity, OAC = Oil absorption capacity, WAC = Water absorption capacity, FC = Foaming capacity, GP = Gelation properties.



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Table 3 presents the results for the functional properties of the fermented and unfermented *A. senegalensis* samples studied. Swelling index of food sample is an indication of the amount of water that it can absorb and its extent of swelling around a define time range (Ogunyinka *et al.*, 2017). This parameter is affected by factors such as protein and carbohydrate contents, temperature and water availability (Yellavila *et al.*, 2015). The values for swelling capacities ranged from 3.96 ± 0.24 (fermented for 48 h) to 5.60 ± 0.15 % (unfermented). The fall in the swelling potential of the samples with duration of fermentation could be because the unfermented seeds had more of their starch to be held by intermolecular bonds thus allowing the absorption of water and swelling when compared to the fermented ones (Ibeabuchi, 2014). The values were observed higher when compared to 1.41 ± 0.24 (unfermented) to 2.05 ± 0.01 % (fermented for 48 h) for pigeon pea (Mbaeyi-Nwaoha and Obetta, 2016). The values were found to be lower than 15.00 ± 0.50 (unfermented) to 4.17 ± 0.29 % (fermented for 48 h) (Ibeabuchi, 2014) for *Lagenaria siceraria*. The values were also lower than 6.50 % reported for unfermented pigeon pea seeds (Adebowale and Maliki, 2011).

Foaming capacity is vital in the maintenance of texture, appearance and compositions of food which require aeration (Ogunyinka *et al.*, 2017). The values obtained for this property ranged from 2.00 ± 0.17 to 7.92 ± 0.31 % for the unfermented and the 48 hrs fermented respectively. The observed increase with fermentation could be due to increased configurations of the protein molecules (Mohammed *et al.*, 2014). Close values were obtained with 3.24 ± 0.10 (unfermented) to 7.03 ± 0.14 % (fermented for 48 h) reported for *Haematostaphis barteri* seed (Mathew *et al.*, 2018c). The values were also found to be lower than 10.00 ± 0.20 % reported for *P. biglobosa* seeds ((Ogunyinka *et al.*, 2017). They were however higher than 0.62 ± 0.02 % (unfermented) to 0.92 ± 0.30 % (fermented for 48 h) reported for African oil beans seed in a related study (Osagie-Eweka and Alaiya, 2013).

Proteins function as emulsifiers due to the presence of hydrophilic and hydrophobic groups that enhance interactions with water and oil present in food (Mao and Hua, 2012). The emulsification properties were observed to rise with fermentation. The unfermented recorded the least



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(40.17±0.42) while the 48 hrs fermented sample had the highest (52.36±0.50 %). The observed increase in the emulsification property with fermentation process could be attributed to the tenability due to the proteins present in the sample thereby bringing about lowering of the tensions at the surfaces of the water and oil (Igbabul *et al.*, 2014). The remarkable emulsification properties reveal the potential application of these as useful additives in food industries (Oyeleke *et al.*, 2012). The values from this study were however lower than 85.00±4.10 % for *Cucumeropsis mannii* seed flours (Ogunbusola *et al.*, 2012) but higher than 35.40±0.18 to 43.16±0.70 % reported for *H. bartei* seeds (Mathew *et al.*, 2018c).

Bulk density gives the material handling as well as packaging requirements of a food sample (Otutu *et al.*, 2015). For this study, the bulk densities obtained ranged from 0.46±0.71 to 0.78±0.22 g/cm³. The 48 hr fermented sample had the least value while the unfermented recorded the highest. A decrease in the values was observed as fermentation continued which may be due to various factors such as particle sizes, number of contact points, poor intensity of attraction and other forces holding the particles together (Igbabul *et al.*, 2014). Osagie-Eweka and Alaiya (2013) in a related study reported similar value (0.39±0.00 to 0.47±0.00 g/cm³) for African oil seeds. The values were however lower when compared to 0.80±0.05 g/cm³ reported for cow pea (Appiah *et al.*, 2011). They were also lower than 0.65 and 0.80 g/cm³ for fermented and unfermented pigeon pea seed flour (Adebowale and Maliki, 2011).

Table 4: Quantitative Phytochemical Analysis (mg/100g) of the Samples

Parameters	Unfermented	Fermented for 24 h	Fermented for 48 h
Alkaloids	1.60±0.38 ^c	1.27±0.42 ^b	0.86±0.30 ^a
Flavonoids	0.69±0.36 ^b	0.55±0.40 ^a	ND
Glycosides	17.30±0.41 ^c	14.10±0.22 ^b	13.54±0.34 ^a
Steroids	6.20±0.40 ^c	5.00±0.40 ^b	4.52±0.32 ^a
Terpenoids	16.23±0.36 ^c	11.70±0.29 ^b	7.31±0.33 ^a

Values in the same row with same superscripts alphabets are not significantly different at p≥0.05

Key: ND: not detected



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Table 4 presents the results obtained for the quantitative phytochemical compositions of both the unfermented *A. senegalensis* seeds and those fermented for 24 and 48 h. The glycoside content ranged from 13.54 ± 0.34 to 17.30 ± 0.41 mg/100 g. The highest amount was obtained in the unfermented sample while the sample fermented for 48 hours had the least. There was a decrease in the content of glycoside on fermentation which was adduced to the leaching of substances that occurs during fermentation. Similar finding was documented in their study on fermented african oil beans (Okorie and Olasupo, 2014). The values from this study are higher than 2.40 ± 0.10 mg/100 g (Olasupo *et al.*, 2016) and 1.04 ± 0.01 mg/100 g reported for *M. oleifera* (Ijarotimi *et al.*, 2013).

The contents of terpenoids varied from 7.31 ± 0.33 to 16.23 ± 0.36 mg/100 for the fermented (48hr) and unfermented samples respectively. The values were observed to decrease with fermentation which could be as a results of the washing away of the associated secondary metabolites during the fermentation process. The contents of terpenoids reported from this work were lower than 25.00 ± 0.13 mg/100 g obtained for *M. oleifera* seed flour (Ijarotimi *et al.*, 2013). The flavonoids contents were 0.69 ± 0.36 and 0.55 ± 0.40 reported for unfermented and fermentation at 24 h respectively. The result is low compared to the 1.28 ± 0.03 mg/100g reported for *Vigna unguiculata sub spp. sesquipedalis* seed (Musah *et al.*, 2020).

The concentrations of steroids were observed to vary from 4.52 ± 0.32 to 6.20 ± 0.40 mg/100 g, for the fermented and unfermented samples respectively. A decrease in the contents of the steroids was noticed in the fermented sample which could be attributed to the possibility of leaching that occurs during the process of fermentation. Olasupo *et al.* (2016) reported 0.78 mg/100g as the value obtained for steroid contents in African oil bean seeds this value is lower than values obtained in the present study. The values from this study are in agreement with 5.43 mg/100 g reported for African bean seed (Okorie and Olasupo, 2014).



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CONCLUSION

Results obtained from this study have shown that there is reduction of fatty acids contents of the studied samples due to fermentation, most especially after 48 hours. Assessment of the phytochemical compositions of the samples further showed the suitability of the seeds for the formulation of drugs especially in the unfermented form. The samples studied have been found to have good functional properties. From the findings of this study, it can be deduced that the flours investigated have unique functional properties hence can be incorporated into food as dietary protein supplements for humans. This will also be vital for baked and soup products. There is need to further promote researches and food processing techniques targeted towards the utilization of flours from various plants.

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