Evaluation of Physicochemical, Functional and Morphological Characteristics of Starch Extracted from Defatted Conophor Seed(*Tetracapidiniumconophorum*).

Abstract

This research work was conducted to evaluate thephysicochemical, functional and morphological characteristics onstarch extract from defatted Conophor seeds. Conophor seedswerewashed,shelled manually, oven dried and then milled. The defatted Conophor flour was obtained by extraction using n-hexane and then soaked in distilled water. A physicochemical, functional and morphological property of the starch was carried-out.The physicochemical analysis showed that the starch has Carbohydrate (77.26%), Moisture (12.10%), Protein (4.46%), Fat (1.99%), Fibre (1.20%) and Ash content (2.99%), Calcium (194.17mg/g), Copper (0.05mg/g), Manganese (0.42mg/g), Magnesium (4.26mg/g), Iron (0.34mg/g), Zinc (1.59mg/g), Phosphorus (26.1mg/g), Sodium (23.20mg/g), Potassium (37.13mg/g), Na/K (0.62) and Ca/P (7.44) andthe functional analysis showed the starch bulk density to be(0.42g/ml), water absorption capacity (513.33%), oil absorption capacity (347.57%), foaming capacity (12.69%), Foaming stability (3.26%), emulsion capacity (45.48%), emulsion stability (50.00%), swelling index (246.67%), gelation capacity (12.00m/v), Amylose (20.5%), Amylopectin (79.5%), and In-vitro digestibility (63.68%). This finding revealed that thedefatted Conophorstarch is very rich in nutrient and cantherefore be used as food supplement.

Keywords: Emulsion; Kernel; Defatted; Dirt; Absorption.

Introduction

Traditional food is asignificant part of African culture and history.Conophor (*Tetracarpidiumconophorum*), a member of family Euphorbiaceae is a climber found in the wet parts of Africa especially in Nigeria. The seeds are planted and usually cooked before consumption(Enujiugha and Ayodele, 2003). Conophor is a legume seed withhighamino acid and caloric values (Ekumankama and Okeke, 2009). It is rich in protein and a goodsource of oil that can be incorporated into some poor nutritional food which can be of greatbenefit to people with very low nutritional status. It has also been stated that Conophor seedhas the highest antioxidant activity among plants or legume species (Sirilakshmi, 2011). Conophor seed possessesphytochemicals such asphenolic compound,flavonoids and luteolin and also known to be a rich source of minerals. Increase in its consumption has been considered good for human health (Pradhan, *et al*., 2021).

Naturally, Starch is an insoluble, non-structural carbohydrate found in plants like Conophor seed in small granules or cells that are produced and stored in tissues of green plants. It is a common carbohydrate in human diet and used as a renewable raw material in industries(Adebowale and Lawal, 2003). Starch granules have two polysaccharide polymers which include amylose and amylopectin.Starches extracted from different plants vary in terms of functional properties such as final viscosity of paste or paste stickiness and the end uses. The structure of starch also affects its digestibility in human gut. Starch with reduced digestibility like amylose starches are highly valued because of its health-promoting factors which can be used to prevent against condition such as colorectal cancer and diabetes(Raigond*et al*., 2014).

Understanding the physico-chemical, functional and morphological structures of starches extracted from different starchy crops are of great importance as it representsa prerequisite for the targeted improvement of starch crops. Hence, in this study, thephysico-chemical, functional, and morphological characteristics of starch extracted from the defatted Conophor seed (*Tetracapidiniumconophorum*) were discussed.

Materials and Method

Acquisition of Materials

Conophor seeds were obtained from the Ojefarm in Ibadan, Oyo State, Nigeria. The seeds were authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure.

Sample Preparation

Production ofConophorFlour

The Conophor seeds were washed thoroughly and shelled manually to remove the kernel from the seeds. The removed seeds were washed and sliced (2-3mm) for easy drying. The shredded Conophorseeds were oven dried at 70ºC for 24hours and milled intoflour according to the method of Barber and Obinna-Echem (2016).

Production of Defatted ConophorFlour

Defatted Conophorflour was obtained by solvent extraction method to extract oil from the Conophor flour using Soxhlet Extraction method and starch was extracted by method of Muazu (2007).

Proximate Composition ofDefatted ConophorStarch

Proximate composition was evaluated in terms of moisture content, crude protein, fat, ash content, crude fiber and carbohydrate content according to the standard methods A.O.A.C. (2012).

Determination of Mineral Composition of Defatted Conophor Starch

The mineral composition of the defatted Conophor starch was carried-out by ashing (dry ashing). The atomic absorption spectrophotometer (Buck Scientific, 2004) was used in the determination of the concentration of the minerals (A.O.A.C., 2012).

Determination of the Functional Properties ofDefatted ConophorStarch

Bulk Density

The Bulk density was conducted according to the method describe by Asoegwu*et al.*, (2006).

Water absorption capacity (WAC)

The water absorption capacity was carried-out with the procedure of Sathe *et al.,* (1982) as modified by Adebowale *et al.,* (2005).

Oil absorption capacity (OAC)

Oil absorption capacity is an index of the amount of oil retained within a protein matrix under certain conditions. The oil absorption capacityof Conophor starch was determined using the method of Sathe and Salunkhe (1981) as modified by Adebowale *et al.,*(2005).

Swelling index determination

The swelling index of the starch was carried-out by the method of Ukpabi and Ndimele (1990).

Foaming Capacity and Stability

The method of Coffman and Garcia, 1977 was used with slight modification in the determination of foaming capacity and stability of the starch.

Emulsion Stability

The emulsion capacity and stability of Conophor starch was carried-out according to the procedure described by Beuchat (1977).

Gelation Capacity (GC)

The gelation properties of the starch was carried-out by the method used by Adebowale *et al.* (2005b)

Swelling power and Solubility

The swelling power and solubility of the starch was conducted at temperature 30, 50, 70 and 90 ºC respectively using the method of Walizewskiet al. (2003)**.**

Freeze-Thaw Stability

The freeze-thaw stability of the starch was conducted according to the procedure described by Simi and Abraham (2008)

Pasting Property

The pasting property of the defatted Conophorstarch was assessed using the RVA (Rapid Visco Analyzer).

The Amylose and Amylopectin of Defatted Conophor Starch

The amylose and amylopectin of the defatted Conophorstarch was assessed using themethoddescribedbyZhu*et al.,* (2011).

In-Vitro Starch Digestibility

The in-vitrostarch digestibility of Conophor starch was conducted by themethod used by Singh *et al*. (1982).

Colour Attributes of Defatted ConophorStarch.

Colour was determined using a colour meter PCE-CSM 2 (Deutschland GmbH) connected to a CQCS3 software (PCE Instruments UK Ltd, United Kingdom). Whiteness Index (WI) was calculated as follows**:**

$WI = [(100 - L^*)^2 + a^{*2} + b^{*2})^{1/2}]$

Where,

WI = Whiteness index,
L = Lightness
a = Green to red
b = Blue to yellow

Scanning Electron Microscopy (SEM) of Defatted Conophor Starch

The granular morphology of Conophor starch was characterizedby using SEM(JEOL JSM-6390, Tokyo, Japan). The starch was first coated with platinum before imaging at a voltage of 3.0kV and X1200 magnification.

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics software version 21. Analysis of variance (ANOVA) was used to compare the mean values of the flour blend at $p<0.05$.

RESULTS & DISCUSSION

The proximate compositions of defatted Conophor starch arepresented in table 1.Moisture content obtained from defatted Conophorstarch is 12.10%. The result obtained is higher than that of Wayah and Safiya (2015) who examined the nutritional component of tigernut starch.Moisture plays an important role in product storage stability. According to Potter and Hotchkis 2006), the result obtained in milled food products with moisture less than 13% are considered to be stable from moisture deterioration.

The value of protein content ofdefatted Conophor starch was found to be 4.46%. Okoli et al. (2012) reported that protein is made up of amino acids. The value obtained is higher than the protein obtained from cassava starch as reported by Ogunbode et al. (2019).

The amount of crude fat of the defatted Conophor starch as shown in table 1 is 1.99%. The amount of fat content in food determines its shelf-life. An increase in the amount of fat composition in food could speed upfood spoilageby promoting rancidity which could result to the production of off-flavours and odours. Also, diet high in fat predisposes consumers to different illnesses like obesity, heart diseases (Omoba and Omogbemile, 2013).

The value of Ash content obtained from the defatted Conophor Starch in the study as shown in table 1 is 2.99%.The percentage of ash content from this study is lower than that of cassava starch reported by Ogunbode et al. (2019). The amount of ash present in a food material could be used as a measure of the percentage mineral constituent of the food since ash is the inorganic residue remaining after water and organic matter have been removed by heating in the presence of an oxidizing agent (Sanni *et al.,* 2008).

Crude fibre value (1.20%) in defatted Conophorstarch was higher than (0.5.00%) reported by Zubair et al., (2020).Crude fibre content is an intrinsic component of diet, it increases stool bulk and decreases the time waste materials spend in the gut.

The carbohydrate content of the defatted Conophorstarch obtained was 77.26%. The carbohydrate content obtained was higher compared to the sample reported by Zubair et al., (2020).

Table 1: Proximate Composition of DefattedConophorStarch

Variables	% Amount
Moisture (%)	12.10±0.19
Protein (%)	4.46±0.07
Fat(%)	1.99±0.01
Fibre (%)	1.20±0.18
Ash (%)	2.99±0.05
Carbohydrate (%)	77.26±0.50

Values are expressed as mean ± standard deviation of triplicate readings.
Means with different letters in the same column are significantly different in the same column at the p≤0.05 level.

The mineral compositions of defatted Conophor starch arepresented in table 2. It was observed that defatted Conophorstarch has Calcium (194.17mg/g), Magnesium (4.26mg/g), Copper (0.05mg/g), Manganese (0.42 mg/g), Iron (0.34 mg/g), Zinc (1.54 mg/g), Phosphorus (26.10 mg/g), Sodium (23.2 mg/g) and Potassium (37.13 mg/g). Studies have reported that Zinc, Magnesium and Calcium play a vital role in glucose metabolism. They serve as co-factors for enzymes involved in glucose metabolism which can increase the insulin action by activation of insulin receptor (Masood *et al.*, 2009; Ramaswany*et al.*, 2016). Mineral elements are very important in metabolic processes in maintenance of osmotic pressure, regulating muscle contractions, transmitting of impulses, acid-base balance, absorption of glucose,bone formation, etc. (Peters *et al*., 2016). Potassium plays vital role in amino acid and protein synthesis (Oshodi *et al*., 1999). The molar ratio of Na/K in the defatted Conophorstarch is within the recommended value of (< 1.0), hence, it may be recommended for people with high blood pressure. The Ca/P molar ratio of defatted Conophorstarch is 7.44; this implies that the Ca/P molar ratio of defatted Conophorstarch is within FAO recommendation (>1.0). Therefore, Conophor starch can serve as calcium booster to prevent osteoporosis in an adult and ricket in children. Food is considered 'good' if Ca/P ratio is above one and poor if the ratio is less than 0.5, while Ca/P ratio above two helps to increase the absorption of calcium in the small intestine (Nieman *et al.*, 1992).

Table 2: Mineral composition of DefattedConophor Nut Starch

Mineral	Amount (mg/g)
Calcium	194.17±0.29
Copper	0.05±0.01
Manganese	0.42±0.02
Magnesium	4.26±0.01
Iron	0.34±0.02
Zinc	1.59±0.01
Phosphorus	26.10±0.01
Sodium	23.20±0.26
Potassium	37.13±0.32
Na/K	0.62±0.00
Ca/P	7.44±0.00

Values are expressed as mean ± standard deviation of triplicate readings.
Means with different letters in the same column are significantly different in the same column at the $p \leq 0.05$ level.

Functional properties of defatted Conophor starch arepresented in table 3. The bulk density of defattedConophorstarch is 0.42 g/ml. The result obtained from this study is low compared to the report by Zubair et al., (2020). It has been reported by Akpata and Akubor (1999) that food with low bulk density can be used as food complement.It enhances easy digestibility in children with immature digestive system (Gopaldas and John, 1992). The water absorption capacity of the defatted Conophorstarch obtained was 513.33%. Water absorption capacity in food indicates the amount of water content it can absorb and retain. It also enhances food digestibility (Ijarotimi and Keshinor, 2012). Excessive amount of water cancause foodto deteriorate which could result in spoilage(Ocheme*et al*., 2010). The value of oil absorption capacity obtained for defatted Conophorstarch as shown in table 3 is 347.00% which is higher than that of banana and plantain starch as reported by Olatunde et al. (2017). It has been reported by Balogun and Olatidoye (2010) that high oil absorption capacity promotesflavour and mouth feel when used in food preparation.The value of foamingcapacityfromthisstudyis 12.69%.The foaming capacity of Conophorstarch was well compared with 14.363% obtained from open sun-dried cassava starch and higher than 11.417% obtained from solar drying of cassava starch respectively reported by

Robert (2019). The foam capacity of all starch can be rated as low since they do not contain considerably high amount of protein as foaming capacity is influenced by the surface activity of proteins (Robert, 2019). Foaming stability ofdefatted Conophorstarch 3.26% is lower than 60.193% obtained for oven dried cassava starch reported by Robert (2019). Emulsion capacity (EC) is related to the amount of oil, non-polar amino acid residue present on the surface of protein, water and other components in the food. The defatted Conophorstarch has emulsion capacity content of 45.48% which is lower compared to other foods; this may be because starches are polysaccharides that mostly act as stabilizer in emulsions by enhancing the viscosity of the aqueous phase (Lael-Calderon *et al.,* 2007).The value of emulsion stability obtainedfrom defattedConophorstarch is 50.00%. . Emulsion stability is the measure of the steadiness of emulsion formed by protein. The nature of proteins withthe composition of charged, non-chargedpolar and non-polaraminoacids make them potential emulsifiers and surfactants that possesses bothhydrophilic and hydrophobic properties and are able to interact withboth water and oil components in food systems (Ulloa, Rosas-Ulloa and Ulloa-Rangel,2011). The percentage swelling index of the defatted Conophorstarch is 246.67%. The swelling index shows the degree of exposure of the internal structure of starch granules to action of water. It is a measure of the hydration capacity (Arinola *et al.*,2016).The gelation capacity obtained for Conophor starch was 12.00%. Gelation capacity is the formation of gel from a food system that has biopolymers (Oliveira, 2008; Ahmad *et al.,* 2016) such as starch, protein etc. Gelation properties are dependent on the nature of the protein and other non-protein component in a food sample. (Omowaye-Taiwo *et al.,* 2015).

Table 3 : FunctionalProperties of DefattedConophornutStarch

Component	Amount (%)
Bulky density	0.42±0.02
Water absorption capacity	513.33±11.54
Oil Absorption capacity	347±10.75
Foaming capacity	12.69±0.01
Foaming Stability	3.26±0.08
emulsion capacity	45.48±0.03
emulsion stability	50.00±0.00
swelling index	246.67±11.55
Gelation capacity	12.00±0.00

Values are expressed as mean ± standard deviation of triplicate readings.
Means with different letters in the same column are significantly different in the same column at the $p \leq 0.05$ level.

Table 4 shows the pasting properties ofthe defatted Conophorstarch.Pasting property of a food material is the changes that take place in food when heat is appliedin the presence of water. The texture, digestibility and end use of the food product may be affected (Ochemeet *al.*, 2018). Peak viscosity of Conophorstarch flour was 14.0 RVU, the value is lower than 270.5 RVU reported by Awoluet al. (2017) for nativetigernut starch. Peak viscosity is the maximum viscosity attainable soon after or during cooking. It is an indication of water binding capacity of the starch (Adebowale *et al.*, 2011). Peak viscosity indicates the strength of the paste formed from gelatinization during food processing. According to Swinkles (1985) high peak viscosity corresponds to high thickening power of starch. The result for peak viscosity of Conophorstarch in this study suggests low thickening capacity. The setback value for Conophorstarch from table 4 is 4.00 RVU. This value is significantly lower than 85.3 RVU reported by Awoluet al. (2017) for tigernut native starch. According to Maziya-Dixon et al. (2007), the higher the setback value, the lower the rate of synresis and weeping. The low setback value obtained for Conophorstarch in this study indicates a high retro-gradation value.Pasting temperature is said to be the temperature at which viscosity begins to rise (Swinkels, 1985). The pasting temperature for Conophor starch obtained for this study was zero (0) which implies that the starch ofConophorwas not viscous. Low pasting temperature ensures swelling, gelatinization and gel formation during processing (Eke-Ejiofor, 2015).Breakdown viscosity of defatted Conophorstarch obtained from this study was 2.00 RVU. Breakdown viscosity is a reflection of the stability of peak viscosity during processing (Moothy, 1985). Starch with lower breakdown viscosity has higher tendency to withstand heating and shearing during cooking. Peak time is referred to as the measure of the cooking time (Adebowale *et al.*, 2005). It is the time required for starch to attain the highest viscosity. The peak time obtained for Conophor starch was 5.67 min.The final viscosity of Conophorstarch obtained was 16 RVU. Final viscosity is an indication of the ability of starch to form a viscous paste. Awolu and Olofinlae (2016) informed that modification using acetylation and oxidation increased final viscosity of native water yam starch. A high final viscosity of starch indicates that the paste is more resistant to mechanical shear and may easily form a more rigid gel (Zhang *et al.*, 2011) but, the reverse is the case for the value obtained for Conophorstarch in this study. High final viscosity is required in food products such as; soups, sauces and dressings.The trough viscosity which indicates the ability of pastes to withstand breakdown during cooling was low (12.00 RVU) in this study. This observation implies that Conophorstarch flour will easily break down during cooling.

Table 4: Pasting Properties of Defatted ConophorStarch

Pasting Properties	(RVU)
Peak Viscosity	14.00
Trough Viscosity	12.00
BreakdownViscosity	2.00
Final Viscosity	16.00
Set-back Viscosity	4.00
Peak Time (Min)	5.67
Pasting Temperature (ºC)	err

Amylose and Amylopectin content ofdefatted Conophorstarch flour is presented in figure 1. Amylose is an important part of starch composition which is about 30% in content while amylopectin consists of the remaining 70% (Behail and Howe, 1995; Afolabi *et al.*, 2016). The amylose and amylopectin content of defattedConophorstarch flour were (20.50%) and (79.50%) respectively. Amylose content determines the degree of starch digestibility in food. The amylose content plays a critical role in the digestion of starches as starches having low amylose and amylopectin content are more digestible than those with high amylose and amylopectin content. From this study, it was observed that the amylose content of defatted Conophor starch was consistent with the amylose content of native starches (15-30%) noted by Bertoft (2017), 10.1-20.2% for sweetpotatoes (Tortoe*et al.*, 2017) and 17.9- 23.6% reported for cassava by Deflooret al. (1998). The amylose content observed in this study was lower than that of cocoyam and yam (26.7%) (Amani *et al.*,2007). Foods with high amylose content are digested more slowly than those with low amylose content, which are less likely to increase blood glucose. The in-vitro starch digestibility of the defatted Conophor starch flour is presented in figure 1. A moderately high starch digestibility (63.68%) was observed in this study and this could be as a result of its low fiber, and high amylose and amylopectin contents. This result is comparable to that obtained for miracle berry seed starch flour reported by Olatunde et al. (2021). Report has shown that the amount and nature of crude fiber in foods could influence starch digestibility. High amount of crude fiber in food may reduce the level at which starch is digested by trapping starch granules within viscous protein-fiber-starch network (Chinma*et al.*, 2012). The starch present in defatted Conophor in this study may therefore not be suitable for the production of functional food for weight reduction and diabetes treatment.

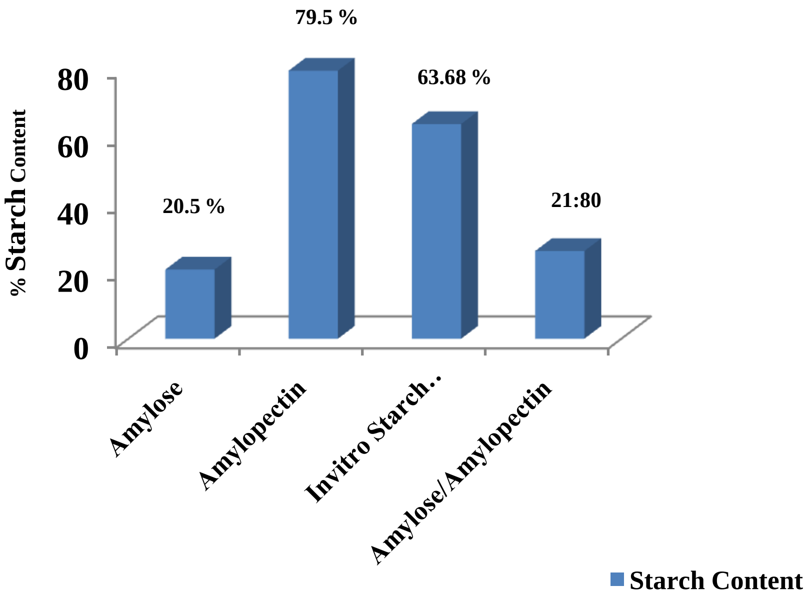

Figure 1: Defatted Conophor Starch Content

The phenomenon of freeze-thaw stability refers to the ability of starch to withstand some physical changes that occur during freezing and thawing (Karim *et al.*, 2000). It particularly affects products that require low temperature storage. The freeze-thaw stability of defatted Conophornut starch gel at 4^0C for 24h and 48h in this study is presented in figure 2. From the result, it was observed that syneresis increased with an increase in freezing days which range from 32.63% – 38.06%. The increase in the amount of water exuded from defatted Conophor starch gel during the process of freezing and thawing from 0 – 48h may be related to its low setback value (4.00 RVU). According to Dixon et al. (2007) the higher the setback value, the lower the rate of syneresis and weeping. From the result obtained, defatted Conophorstarch gel has a tendency to retrograde faster since syneresis relates to the tendency of starch to retrograde. Akonor (2019) reported higher syneresis value for yellow (65%) and black (68%) maize. The result obtained in this study was closer but higher than the 22.3% reported by Rodriguez-Torres et al. (2017) for starch syneresis of fedearroz rice variety. The report of Arocaset al. (2013) on samples produced with native corn starch suggests their susceptibility to syneresis while those produced from modified starches may improve the visco-elastic properties, freeze-thaw and heat stability of products.

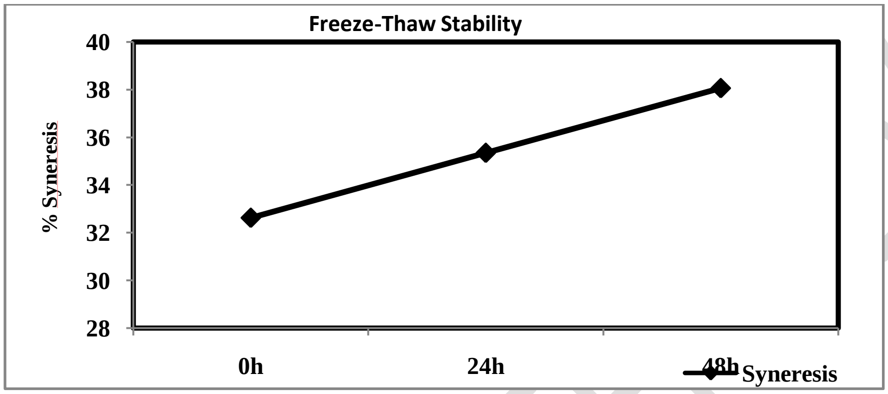

Figure 2: Freeze-Thaw Stability of Defatted ConophorStarch.

The swelling power and solubility of defatted Conophorstarch is shown in figure 3. The swelling power of defatted Conophorstarch ranged between 3.15 – 4.72%. Swelling power increased with an increase in temperature from 30ºC to 90ºC. From this study, it was observed that defatted Conophorstarch had a low swelling power compared to that of cassava flour 9.233 – 12.513% reported by Agbemafle (2019). The low swelling power of defatted Conophorstarch could be attributed to the strength and character of the micellar network within the starch granules which is the major contributing factor to the swelling behavior of starch. According to Agbemafle (2019), a highly associated starch with extensively strong-bonded micellar network structure is readily resistant to swelling. This implies that the increase in temperature decreased the strength and character of the micellar network of the starch. The higher the amylose content, the lower the swelling power. The swelling power of starch depends on the ability of amylose to solubilize in water thereby allowing water to be absorbed by starch granules (Nuwamanya *et al.,* 2011). The value obtained for swelling power suggests that an increase in temperature weakened the starch granules by allowing interaction between the amylose (water soluble fraction) molecules sited in bulk amorphous regions and the branched segment of amylopectin (water insoluble fraction) in the crystalline regions. The swelling power of flour samples relate mostly to their protein and starch contents. Higher protein content in flour may cause the starch granules to be embedded within a stiff protein matrix which minimizes the access of starch to water and restrain the swelling (Aprianita*et al.,* 2009). The solubility of defatted Conophor starch ranged from 1.98 – 3.17%. A slight increase in solubility was observed as temperature rose from 30ºC to 70ºC (1.98-

1.99%) and at 90ºC, solubility increased to 3.17% a margin of 1.18%. These values are very much lower than the 7.57% and 10.46% reported by Baafi and Safo-Kantanka (2008) for cassava starch. The low solubility obtained in this study could be attributed to the amylose content, since the solubilized amylose molecules leach from the swelled starch granules of flour (Dengate, 1984).

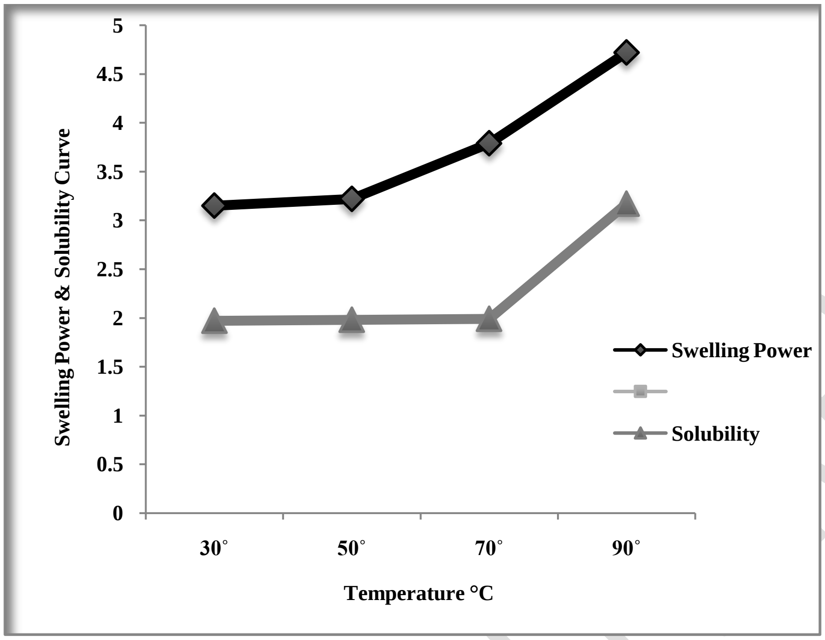

Figure 3: Swelling Power & Solubility Curve of Defatted Conophor Starch

The colourproperties of defatted conophor starch are presented in figure 4. Values are 79.73 for Lightness (L*), 8.11 for Red-green (a*), 20.45 for Yellow-blue (b*), 22.00 for Chroma (c*) and 68.37 for Hue (h*).

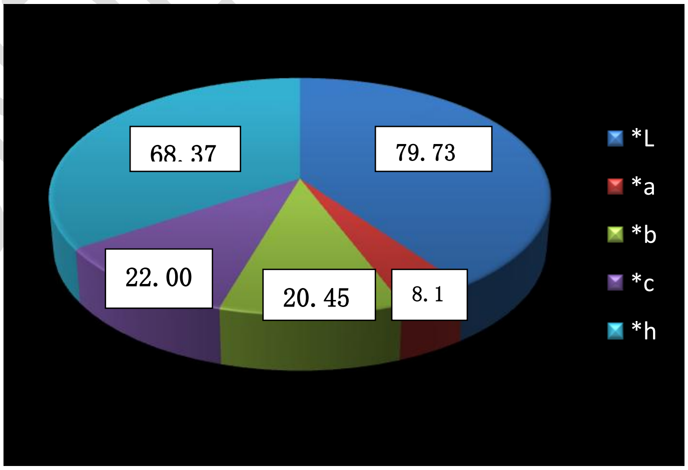

Figure 4; Colour Properties of Defatted ConophorStarch

The scanning electron micrograph of defatted Conophorstarch is shown in figure 4. Starch granules occurred in a single rough irregular cluster having sharp tips. Residual protein and drying condition have been reported as possible causes of clusters of starch granules (Ashogbon and Akintayo, 2012). An oval smooth surface granule with varying sizes was reported by Awolu*et al.* (2017) for native tigernut starch. Lawal and Adebowale (2005) reported oval and round shaped granules for native jack bean starch.

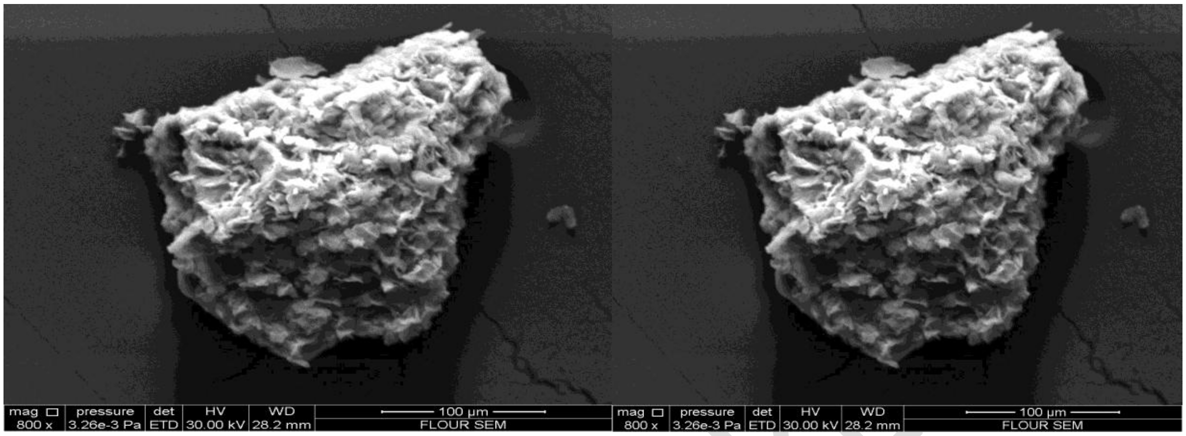

Figure 5: Scanning Electron Micrograph of Conophor Starch

Conclusion

From this study, it has been established that defatted Conophorstarch is a good source carbohydrate. It also contains some amount of minerals which increase the metabolic activities in the body, development of bones, regulation of acid base balance and the transmissions of nerve impulses.This revealed that defattedConophorstarch is an interesting source of nutrient with its potential use in food industries.The research workrevealed thatdefatted Conophorstarchhas a lowprotein content that needs to be supplemented with other food material to meet the recommended allowance for both children and adults.

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