*Annals of West University of Timişoara, ser. Biology, 2013, vol XV I(1), pp.1-10*

# MICROMORPHOLOGICAL STUDIES OF STARCH GRANULES IN SELECTED PROCESSED INDIGENOUS FLOUR OF SOUTH WESTERN NIGERIA

***Adeniyi A. JAYEOLA*, Oladele A. AKINSEBIKAN***
Department of Botany, University of Ibadan, Ibadan, Nigeria
**Corresponding author e-mail: aa.jayeola@ui.edu.ng*

**ABSTRACT**
*Starch granules of yam stem tubers, plantain fruits and cassava root tubers were studied using the light microscope and then compared. Yam and plantain, both monocotyledons, had morphologically similar granules between locations, being mostly oval in shape while the granules of cassava, a dicot, were predominantly globular but varied between locations. Differences in processing methods between locations might be responsible for the observed variation in cassava granules. There is a suggestion that starch granule morphology might follow lineages in a broad sense. The unique micromorphology of cassava starch granule could make it easily detectable when adulterated, substituted or contaminated with yam or plantain granules and this could provide a cost effective clue in forensic determination.*
**KEY WORDS:** *starch granules, processing method, globular, traumatic canals, forensic*

**INTRODUCTION**

For ages past, processing of cereals, tubers and fruits into flour forms has been an integral part of the food preservation culture in Africa in general and in Nigeria in particular. In south western Nigeria, different types of flour are produced from a variety of sources, notably *Dioscorea alata* L. (white yam), *Manihot esculenta* Crantz. (cassava) and *Musa paradisiaca* L. (plantain). Yam flour, cassava flour and plantain flour all belong to the generic group, "elubo"or "iyefun", in the Yoruba folk taxonomy, meaning flour. The words "isu", "lafun/ege/gbaguda" and "ogede" are the specific epiths, respectively.

Starch is the major carbohydrate of nutritional importance in the human diet and is also an important industrial material; starches from different botanical sources have different polymer compositions and structures and, hence, different physicochemical properties (Zeeman *et al*., 2010). Yam is among the oldest recorded food crops and ranks second after cassava in supply of starch in West Africa (Nweke *et al.,* 1991). Naturally, yam is rich in starch and produces energy. (*Musa paradisiaca* are used as an inexpensive source of calories (Akubor *et al.,* 2003). It is recommended to produce plantain flour from green fruits, since it has high starch content of about 35% on wet weight basis (Simmond, 1976). The traditional method of processing cassava into flour though unique for its ability to reduce the toxic cyanogenic compound to a least possible level (Numfor, 1983) imparts a strong smell to the product (Cereda and Mattos, 1996). Lafun is a fermented product from cassava, is a dry product, which can be preserved for a long time under the prevailing local environment.

Several works have been done and reported on the anatomical as well as physico-chemical properties of starch in *Dioscorea* species, *Manihot* species and *Musa* species. Rasper (1967), examining the properties of starches from some West African yams, found considerable variation between the different *Dioscorea* species. He found that the longitudinal diameter of the West African *Dioscorea rotundata* POIR starch varies from 35µm-40µm and forms gel of considerable strength.

Single sample of each major West African species, grown in Nigeria have been described in a report (Greenwood-Burton, 1961) which is not unfortunately generally available; this report includes studies with amylograph on the viscosity of the starches, and it noted very considerable variation between species.

Moorthy (1999) also reported in his work that Cassava starch granules are mostly round with a flat surface on one side containing a conical pit, which extends to a well-defined eccentric hilum. The effect of starch granule size on enzymatic susceptibility has been studied by various authors (Franco and Ciacco, 1992), and many of them have observed that the large size of cassava starch granule are responsible for less susceptibility of cassava starch to α- amylase hydrolyzation. It has been reported that enzymatic hydrolysis of granular cassava starch at temperatures below the starch gelatinization temperature results in a pitted and porous.

The larger the granules are, the lower the gelatinization temperature (Brandam *et al.,* 2003), due to less degree of association in cassava starches. But Charles *et al.* 2005 reported that gelatinization temperatures increased almost linearly with decreasing amylase molecule size and/or content in starch from different varieties of cassava. The higher the granule size, the higher the swelling power and vice-versa in cassava starches.

Gallant *et al.* (1992) proposed that both the inside and surface of cassava granules are made of tiny egg like shells but their packing differ in both crystalline and amorphous regions. The surface of granules is hard and crystalline because they are made of tightly packed bigger and harder crystalline shells.

Zakpaa *et al.* (2010) reported that plantain starch granules obtained from the cultivar “Apantu” were very large (24.3 µm) thereby increasing swelling behavior and also minor components of the starch granules affect the functional performance (pasting and gelling behaviour). Large starch granules tend to build higher viscosity, which is delicate because the physical size of the granule makes it more sensitive to shear. It was determined that there is no significant difference between plantain starch and the other starches. Plantain starch from the cultivar “Apantu” possess good physicochemical properties indicative of good quality starch with high potential for industrial use when compared to other sources of plant starches in common use.

Malomo and Jayeola (2010) reported in their work on starch grains of some selected cultivars of white yam (*Dioscorea rotundata* Pior) that both the local and hybridal cultivars have similar solubility patterns in spite of the marked granular size differences, which tends to support previous reports that there is no direct correlation between granule diameter and amylose content (Franco *et al.,* 1988). And also that Starch granular sizes of *D. rotundata* in Nigeria is in broad agreement with the figures reported for other places but observed incongruence was observed with previous findings concerning granule size and solubility, as well as and swelling powers.

The results of previous works suggested that starch granules could contain useful attributes for discrimination among economic plants in the absence of other organs. Therefore the research was conducted in order to study and compare the morphology of starch granules in the processed flours from the organs of three economically important plant species, namely, stem tuber of yam, fruit of plantain and root tuber of cassava. The study also aimed to examine the significance of starch granule evolution in plants.

**MATERIALS AND METHODS**

Yam flour or “elubo isu”, cassava flour or “elubo lafun/ege/gbaguda” and plantain flour or “elubo ogede” were prepared from three crop plants as described below using yam, plantain and cassava randomly collected from selected major farm produce outlets in south west Nigeria, namely, Central Bodija Yam Market, Ibadan, Central Sabo Yam Market, Ondo and Ayobo Market, Lagos. Each sample was carefully labeled for correctness of identification.

In order to study granule morphology, the starch specimen contained on a pinhead was suspended in a drop of distilled water and iodine-KI on microscope slide. The stained granules were observed under a Carl Zeiss light microscope fitted with camera. Photomicrographs of whole granules were taken while all permanent slides prepared for the study were deposited in the Laoratory of Structural Botany (Anatomy), University of Ibadan, Nigeria.

**RESULTS**

The photomicrographs of starch granules of the three plant species used in this study are presented in Figs. 1-18. The granule micromorphology of each sample is described below.

Shapes of starch grains vary among the sources of flour from which they were derived, namely, *Dioscorea alalata* (yam), *Manihot esculenta* (cassava) and *Musa paradisiaca* (plantain). Plantain granules were similar to yam granules as shown in Figs. 7-12 while the cassava granules were distinct from both as shown in Figs. 13-18. In some starch grains, there were concentric lamellations which varied in clarity among granules, being distinct in starch grains of yam from Lagos area (Figs.5-6) but rather faintly visible in the yam granules from Ibadan and Ondo samples. Among the plantain flour samples, those collected in Ibadan (Fig.7) showed pronounced lamellations than those collected in Ondo (Fig.9) and Lagos area (Fig.11). The starch granules of cassava from Ondo showed conspicuous straiations (Fig.16) while straiations were faint in starch grains of Ibadan and Lagos cassava samples (Figs.14 and 17). The photomicrographs of starch granules of yam flour from Ibadan and Ondo were predominantly oval or spindle-shaped but rarely circular (Figs.1-4). The granule of Ibadan yam flour appeared to comprise a range of sizes, the largest being about 20 times the size of the smallest. The granules of the Lagos samples of yam flour occurred in dense clumps, loosely bound in a transparent viscous substance (Fig.6), some conical and some oval in shape.

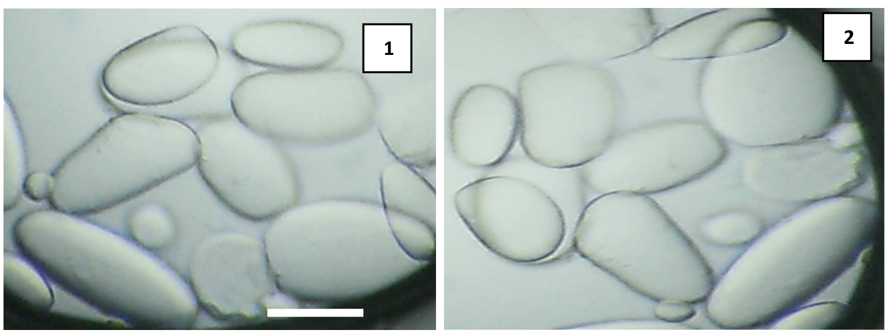



**Fig.1-2. Photomicrographs of starch grains of yam from Ibadan area**

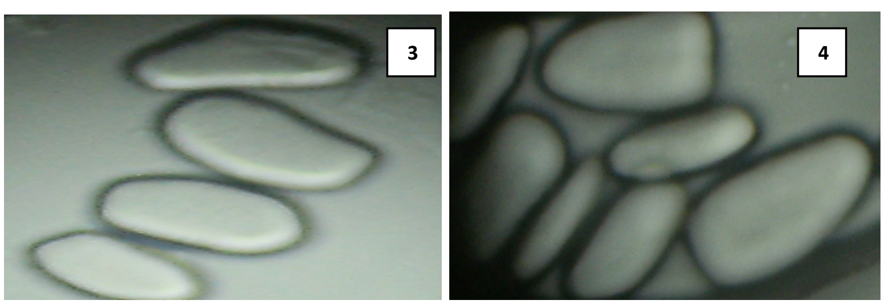



**Fig.3-4. Photomicrographs of starch grains of yam from Ondo. Bar represents 20µm**

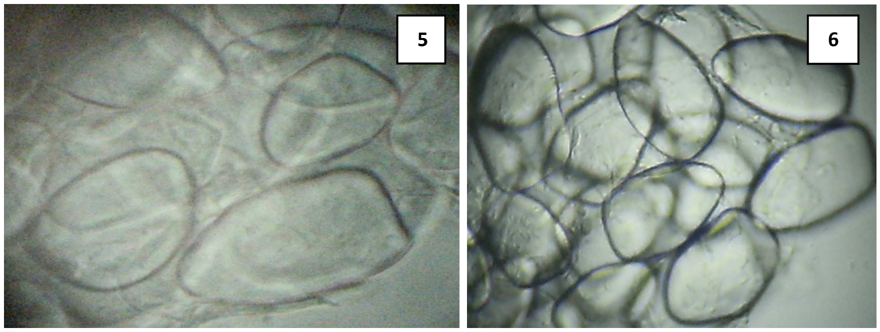
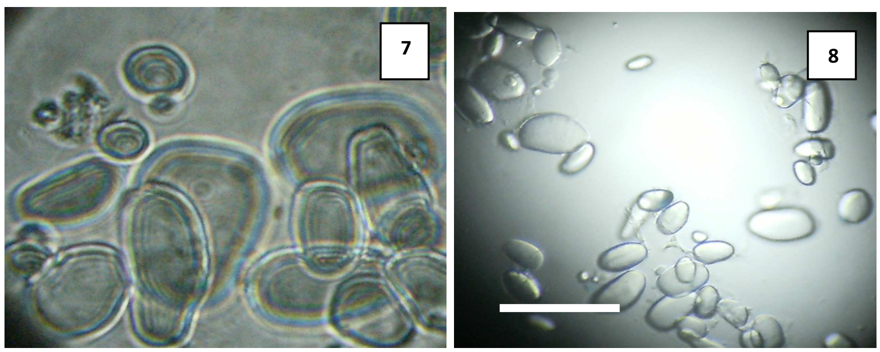



**Fig.5-6. Photomicrographs of starch grains of yam from Lagos area**




**Figs.7-8. Photomicrographs of starch grains of plantain from Ibadan area. Bars represents 20µm**

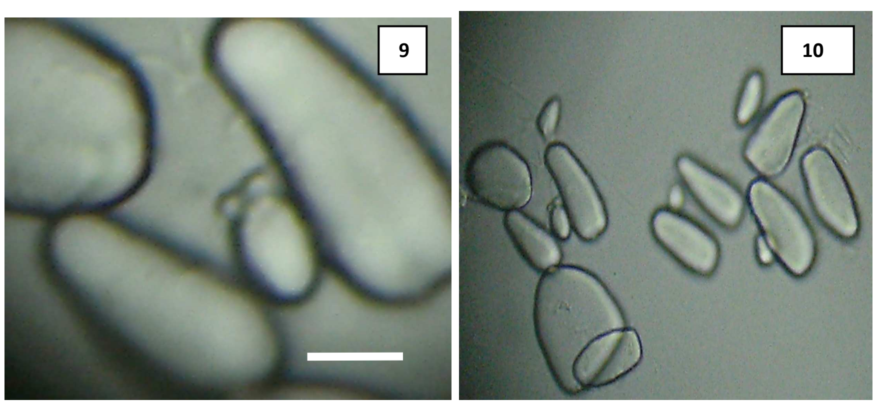



**Fig.9-10. Photomicrographs of starch grains of plantain from Ondo. Bars represents 20µm**

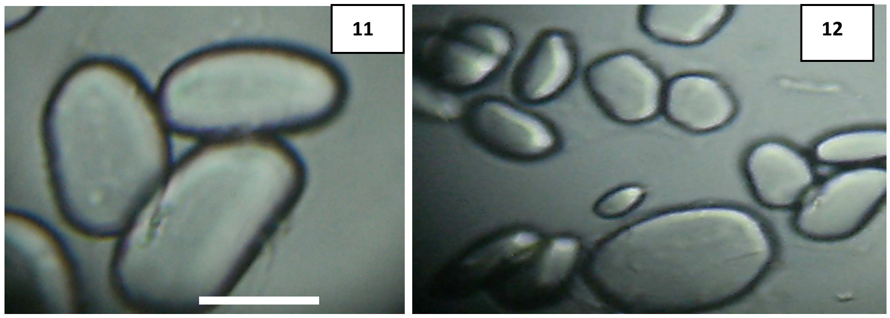



**Fig.11-12. Photomicrographs of starch grains of plantain from Lagos area. Bar represents 20µm**

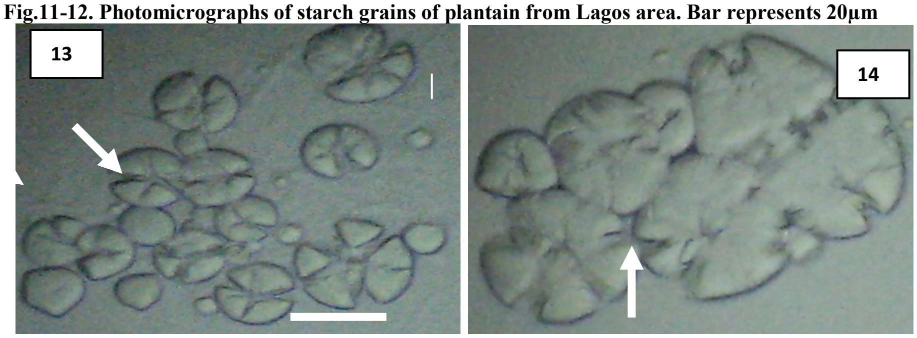



**Fig.13-14. Photomicrographs of starch granules of Cassava from Ibadan area. Arrows represent canals**

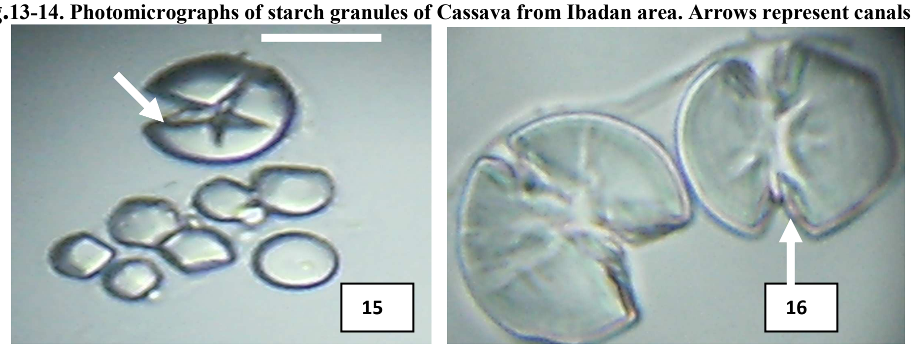



**Fig.15-16. Photomicrophs of granules of cassava from Ondo. Arrows indicate "traumatic" cannal. Bar represents 20µm**

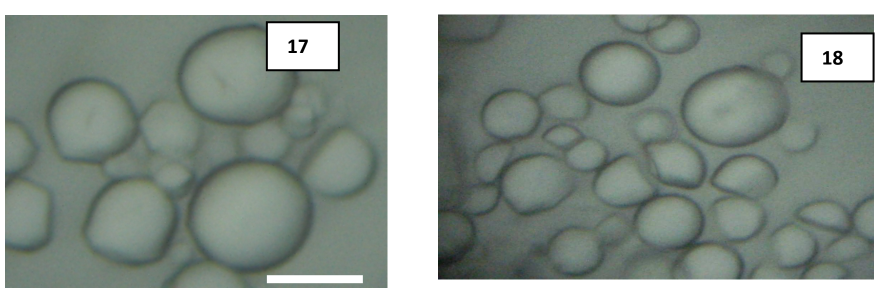



**Fig.17-18. Photomicrophs of granules from Lagos. Arrows indicate "traumatic" cannal. Bar represents 20µm**

The starch grains of plantain samples from Ondo area showed spindle-like shape, few were triangular, while some were globular in shape,but the spindle-shaped grains were prevalent than the other types of shapes. In spindle-shaped grain, lower portion is shallowly constricted while the upper portion is expanded. These grains had no concentric lines or lamellations were not visible compared to the distinct concentric lamellations of the granules Ibadan plantain flour sample.

The photomicrographs of the starch grains of plantain flour sample from Lagos area showed different shapes which range from polyhedral, spherical, and rod-like to oval (Figs.11-12). The sizes of starch grains also were sub-equal, being of different sizes within the same sample.

The photomicrographs of starch grains of cassava from Ibadan had about 95% star-shaped granules (Fig.13-14, 15-16) and 5% globular shape. This shape is similar to that of the starch grains of cassava from Ondo which were also star-shaped and partitioned by radiating canals.

The photomicrographs of starch granules of cassava from Ondo showed 95% star-shaped and 5% globular forms (Figs.13-14). This shape is similar to the granules of the Ibadan cassava samples as revealed in the photomicrographs (Figs.13 and 14). In both Ondo and Ibadan samples, granules appeared star-shaped, with radiating canal-like partitions, with somewhat subequal granule sizes.The photomicrographs of starch grains of cassava from Lagos area also showed 95% globular or round granules, with some being spherical in shape but the globular type was prevalent. These starch grains were very different compared to those of the photomicrographs of the grains of cassava from Ibadan and Ondo which were spherical and bearing radiating canal-like tracks. In both Ibadan and Ondo samples, there were small fragments of broken pieces of larger grains, arising probably, from processing trauma. The sizes of granules were also subequal.

**DISCUSSION**

This study has shown that plantain and yam granules were similar, being frequently oval to rod or spindle-shaped whereas cassava granules were consistently globular to disc-shaped and somewhat spheroidal. However, intraspecific variation was commonplace as there was variation among the shapes of the granules of the same species. This agrees with the observation of Malomo and Jayeola (2009) on the limitations of granule morphology in the delineation of cultivars of *Dioscorea alata* L. (White yam), though they studied several cultivars of a single species while this work studied three distinct species. Therefore, the broad similarity among the granules of yam flower from different localities is not unexpected. The fact that yam and plantain are both monocotyledons while cassava is a dicotyledon may suggest a more fundamental cause, possibly in their phylogeny, for the difference among the species used in the current study.

The globular shape of the starch granules of cassava from Lagos is in agreement with the finding of Itiola and Odeku (2005) in the study of cassava starch grain and yam starch grain and reported that none of the starches were truly spherical with their shape being round to ovoid. Oyewole and Sanni (1995) reported that one of the constraints in the commercialization of local fermented cassava products is that the quality of the products varies from one processor to the other and even from one processing batch to the other by the processor.

The intraspecific variation observed in the cassava granules, range from flattened shape to prominent cracks or canal-like areas could have been due to processing trauma, as a result of the levels of pressure applied on the milled cassava during fermentation. Such pressure might have deformed the granules to various degrees. One of the constraints in the commercialization of local fermented cassava products is that the quality of the products varies from one processor to the other and even from one processing batch to the other by the processor (Akingbala *et al*., 1991; Blansherd *et al*., 1994; Oyewole and Sanni, 1995). Also this variation in their shape can be as a result of different varieties of cassava, yam and plantains that are been processed to flour from different locations. Almazan (1992), Idowu and Akindele (1994), reported that the factor responsible for this variation is due to the age and the variety of the cassava root used by different processors. Kerr (1950) has reported that striations or concentric lamellations found in starch are significant clues to the organization of the granules, suggesting that these striations or lamellations represent periods of relative inactivity of deposition of starchy material. This would be more in accord with the fact that the outer layer appeared to be denser. Other layers also have different colours meaning that they refract light at different angles because of the differences in their densities. Amylopectin is the major component, typically making up 75% or more of the starch granule, then amylopectin and amylose together form semicrystalline, insoluble granules with an internal lamellar structure (Bul´eon *et al*., 1998). A large number of enzymes are involved in determining starch structure in a specific plant organ, hence the potential number of different starches that could be bioengineered by up or downregulation of the respective genes is enormous (Zeeman *et al*., 2010).

## CONCLUSION

The anatomical study of starch grains of yam (*Dioscorea alata*), cassava (*Manihot esculenta*), and plantain (*Musa paradisiaca*) and analysis of the granule micromorphology suggests that their starch grains have sufficient differences in attributes to allow for discrimination among them. While the starch granules of yam and cassava show morphological similarities, clear differences exist in the shapes of starch grains of cassava. This study has to be followed through in the line of plant lineages to fully understand when starch grains occurred in evolution and how many times that happened. This further indicates to be an efficient means by which the various starch grain identification could be achieved for application in taxonomy, industrial quality control processes (to detect adulteration and contamination) and also in forensic application. The difference in the shape of the starch granules from different cultivated plants may offer easy method of distinguishing their flour and also serve as a tool for identification of the various plants microscopically.

## REFERENCES


- Akingbala J.O., Oguntimehin G. B., Abass A.B. 1991.Effect of processing method on quality and acceptability of 'fufu' from low cyanide cassava. *Journal of the Science of Food and Agriculture*.57, 151 - 154.
- Akubor PI, Adamolekun FO, Oba CO, Obari H, Abudu IO (2003).Chemical composition and functional properties of cowpea andplantain flour blends for cookie production. Plant Foods Hum. Nutr.58(3):1-9.
- Almazan A.M. 1992. Influence of cassava variety and storage on 'gari' quality. *Tropical Agriculture, (Trinidad)*, 69, (4). 386-390.
- Blansherd A.F.J., Dahniya M.T., Poulter N.H , Taylor A.J. 1994.Fermentation of cassava into 'foofoo'. Effect of time and temperature of processing and storage quality. *Journal of the Science of Food and Agriculture,* 66, 485 - 492.
- Brandam C., Mayer X. M., Proth J., Strehaiano P., Pingard H. 2003. An original kinetic model for the enzymatic hydrolysis of starch during Mashing. *Biochemical Engineering Journal.*13: 43- 52.
- Buleon A, Colonna P, Planchot V, Ball S. 1998. Starch granules: structure and biosynthesis. *Int. J. Biol. Macromol*. 23:85–112
- Cereda M.P**.,** Mattos M.C.Y. 1996. Linamarin: The toxic compound of cassava. *J. Venom. Anim. Toxins* 2: 6 –12.
- Charles A. L., Chang Y. H., Ko W. C., Sriroth K., Huang T. C. 2005. Influence of Amylopectin Structure and Amylose Content on the Gelling Properties of Five Cultivars of Cassava Starches. *Journal of Agricultural and Food Chemistry*. 40: 5-10.
- Franco M. L. C**.,** Ciacco C.F. 1992. Factors that Affect the Enzymatic Degradation of Natural Starch Granules. Effect of the Size of the Granules. *Starch/Stärke* 44: 422–426.
- Gallant D. J., Bouchet B., Bulion A., Perez S. 1992. Physical characteristics of starch granules and susceptibility to enzymatic degradation. Eur. *J. Clin. Nutr.* 46:3-16.
- Greenwood-Barton L. H.1961. Gel volume and amylase leaching. *Cereal chemistry,* 68, 172-177.
- Idowu M.A., Akindele, S.A. 1994. Effect of storage of cassava roots on the chemical composition and sensory qualities of 'gari' and 'fufu'. *Food Chemistry*, 51, 421 -424.
- Itiola O.A**.,** Odeku, O.A. 2005. Packing and cohesive properties of some locally extracted starches *Tropical Journal of Pharmaceutical Research*, 4 (1): 363-368.
- Kerr R. W. 1950. Acid hydrolysis of starch. In: K.W. Ker (ed.). Chemistry and Industry of Starch, 2nd Ed. Academic Press, New New York.
- Malomo O**.,** Jayeola A.A. 2010. Micromorphological and Chemical Characterization of Starchesin *Dioscorea rotundata* L. (White Yam) *Not. Bot. Hort. Agrobot. Cluj 38 (1) 2010, 14-20.*
- Moorthy S.N**.** 1999. Effect of steam pressure treatment on the physicochemical properties of *Dioscorea* starches. *J. Agric. Food Chem*. 47: 1695-1699.
- Numfor A . 1984. An improved technique for the processing of cassava fufu. In: Terry ER, Doku EV, Arene OB,Mahungu, NM (eds.). Tropical root crops: production and uses in Africa.. Ottawa, Canada, pp. 11 –113.
- Nweke F.L.,Ugwu B.O., Asadu, C.L. 1991. Production cost in the yam based cropping stem in south Eastern Nigeria (RCMP) *Research monograph N0. 6 RCMP*, IITA Ibadan, Nigeria Pp. 6.
- Oyewole O.B**.,** Sanni, L.O. 1995. Constraints in traditional cassava processing - the case of 'fufu' production. In: *Transformation Alimentaire du Manioc.* Editors: T. Agbor-Egbe, A. Brauman., D. Griffon and S. Treche, ORSTROM, Paris. Pp. 523 - 529.
- Rasper V., Coursey, D. G. 1967. Properties of starches of some West African yams*. Journal of Science Food and Agric*18:240-244.
- Simmond N.W**.** 1976. *Food uses of Banana*. Macmillan Publishers, Ibadan Sons Ltd, New York.Pp 86-87.

- Zakpal H. D.,Mak-Mensah E.E., Adubofour J. 2010. Production and characterization of flour produced from ripe "apem" plantain (*Musa sapientum* L. var. paradisiacal; French horn) grown in Ghana. *J. Agric. Biotechnol. Sustainable Dev*., 2(6): 92-99.
- Zeeman S.C, Kossmann J., Smith A.M. 1910. Starch: Its Metabolism, Evolution, and Biotechnological Modification in Plants. *Annu. Rev. Plant Biol*., 61:209-234.