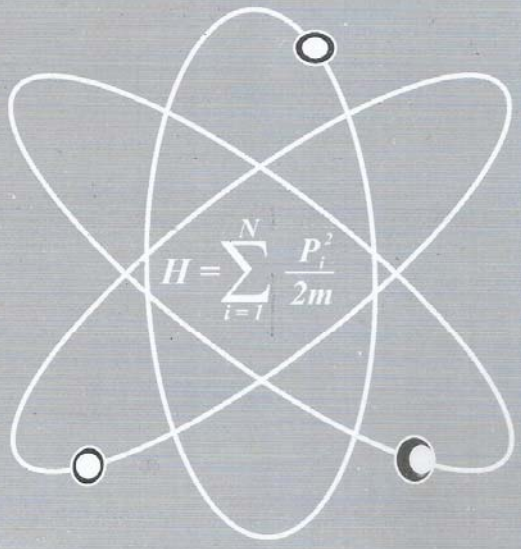
BAYERO JOURNAL OF PHYSICS AND MATHEMATICAL SCIENCES



VOLUME 10, No. 1
JUNE, 2019.

Published by: THE DEPARTMENTS OF PHYSICS AND MATHEMATICAL SCIENCES, BAYERO UNIVERSITY, KANO.

MEMBERS OF THE EDITORIAL BOARD

TABLE OF CONTENTS

BAJOPMAS JOURNAL 10(1): 1 – 11
Printed in Bayero University, Kano - Nigeria.

STRUCTURAL, MICROSTRUCTURAL AND UV-VIS SPECTROSCOPY STUDIES OF GAMMA-IRRADIATED STARCH FROM *Dioscorea Rotundata*

Umaru, A.; Ndakpayi, M. H.; Isah, K.U. Agida, M. and Bature, M.[1]
Department of Physics, Federal University of Technology, Minna, Nigeria
[1]Department of Physics, Federal University, Gusau, Nigeria
Corresponding author: u.ahmadu@yahoo.comTel:08066034360

Abstract
Starch from Dioscorea rotundata (white yam) of Kwasi species was irradiated with gamma rays at different doses (0.05, 0.1, 0.2, 0.6 and 1.0 kGy) from Cesium-137 gamma source. The native and irradiated specimens were characterised by X-ray diffraction, SEM and UV-Vis spectroscopy. Proximate analyses shows it contains moisture of ~9.77%, crude protein of 1.18%, crude fat of 0.39%, ash content of 1.13%, crude fibre 1.09%, crude carbohydrate 86.44% and Amylose content of 22.48%. The effect of increased irradiation shifted the 2θ values below the native sample peak which implies increase in the size of the unit cell. The strongest diffraction peaksare all at ~17° 2θ and confirms that Kwasi starch has typical B-type diffraction pattern. Their microstructure has average grain size maximum of 31.70 μm for 1kGy and minimum of 15.28 μm 0.6 kGy samples, all of which are either characterised by ellipsoidal, ovoid or oval-shaped grains. The crystallize sizes determined from Willamson–Hall plots are 27.79±16.01 nmmaximum, for native and 13.72±4.48 nm minimum, for 0.1 kGy. UV-Vis spectroscopy studies show maximum absorbance of 1.373 at 290 nm for 0.2 kGy sample. The TGA plot shows that kwasi starch is stable from 699°Cwith a weight loss of ~14.19 %. All the doses absorbed are in the ultra-violet region (190-400 nm) and thus no indication of absorbed light which makes Kwasi starch colourless. It is concluded that kwasi starch structure is not altered in any significant way by gamma irradiation at the applied dose and may thus be suitable for applications in the food and non-food industries.

Keywords: *Starch, gamma-irradiation, Microstructure , XRD, UV-Vis.*

1. Introduction

Starch is very useful for industrial applications spanning more than fourty areas. However, less than 1% of all studies on starches have been carried out on *D. rotundata*[1].There is thus the need not only to carry out studies on this family but to also correlate their physico-chemical and material properties within the context of different starch sources and varying irradiation regimes in order to evaluate their properties for suitable applications.

One of the most significant irradiation techniques that have been used predominantly is gamma irradiation. It is used to extend the shelf life of food products, modify their physical properties [2] and is an effective means of inhibiting sprouting in *D.rotundata* yams. Gamma irradiation has been used to control insects from starch obtained from sweet potato [3] and is attractive as it reduces the application of chemicals to food and has non residual features as an ionizing radiation.

Starch is widely available as naturally occurring carbohydrates reserve in plant tubers and seed endosperm where it is found as granules. World production of starchhas been reported to reach 27.5 million tons [4]. As a natural polymer, there has been growing interest in its use for various applications such as food, paper, textile, pharmaceutical and medical industries, among others [5]. It is used in the polymer industry because of

its environmental friendliness due to many advantages such as being safe, renewable and biodegradable, among others. However, many of the physical properties of its native form need to be studied and correlated for the proper exploitation of its functional properties. This is due to several disadvantages of native starch [5,6] which make the modifications of its properties necessary by chemical, enzymatic and genetic modifications [5-8]. These methods however, are often expensive, complex, time consuming, unsafe and often associated with environmental concerns. Starch from different sources has been exposed to different sources of irradiation such as electrons, X-rays and gamma irradiation. The effect of gamma irradiation depends on dose rate or dose applied and physiological factors, among others. It is therefore difficult to predict the behaviour without carrying out a study from low (threshold) to high irradiation levels (destructive).

Therefore, gamma irradiation has been found to be cost effective, environmentally friendly, requires simple sample preparations and is fast. Different starch sources show different thresholds for their structure to be modified by gamma irradiation [6-9] as the results depended on the various doses used for functional, structural and microstructural properties, among others. Gamma irradiation decreases the average molecular size and viscosity but increases the solubility of waxy and normal starch [2] when used in the range 5 to 20 kGy with increasing irradiation. Gamma irradiation of sweet potato starch in the range 0 to 0.4 kGy decreases swelling power while the solubility is increased with increase in irradiation. Increase in crystallinity of wheat starch irradiated between 1 to 3 kGy has been reported without change in microstructure, although structural change was expected [10].There has also been report of a complete disorganization of the crystalline and carboxyl contents on starch irradiated at 0.5 kGy from potato cultivars in the irradiation range 0 to 0.5 kGy [6].

The structural and microstructural properties of starch are fundamental to their potential applications. Thus, in this work, the effect of different gamma irradiation doses (0.0, 0.05, 0.1, 0.2, 0.6 and 1.0 kGy) on the structural, morphological and UV-Vis spectrum of *kwasi* starch from *dioscorea rotundata* species, locally grown in Nigeria, has been evaluated to assess its suitability for relevant applications in the food and radiation environments, at the same time contextualised within and outside the species.

2. Experimental

Tubers of *dioscorea rotundata* (D.R.), locally known as*Kwasi,* were purchased fresh at Tatiko village Minna, Nigeria and were taken to laboratory for extraction. Each tuber of yam was washed and weighed before peeling with top loading balance. The peeled tuber, was cut into pieces and blended with 1000 ml of distilled water for purity of starch using electrically powered blender. The blended tuber was sieved through an 80-mesh sieve with 5 litres of distil water, the sieve was allowed to settle for 10 mins and decanted. Decantation was repeated 3 times to obtain pure starch sediments. The starch collected was spread on a clean plastic tray and allowed to dry at room temperature for 24 h. The dried starch was weighed and the percentage starch content was determined using the equation below:

$$Percentagestarch = \frac{weightofthedrystarch}{weightofthepeeledyam} \times 100\ \% \qquad (1)$$

The dried lumps of starch were further ground into powder form using ceramic mortar and pestle and sieved with 80 μm-mesh sieve. Fine samples were put in universal sample bottle.

Elemental Analysis was carried out on the native sample only in order to determine its constituent elements using a portable AMPTEK(R) Energy Dispersive X-ray ray Fluorescence (EDXRF) technique. Similarly, Proton Induced X-ray Emission (PIXE, 1.7 MV Pelletron Tandem accelerators) was used to determine trace amounts of phosphorus (P) content in the native starch. For the EDXRF, the pelletized native sample was inserted into the sample holder of the XRF system and bombarded with X-ray fluorescence spectrometer with silver (Ag) anode at a voltage of 25 kV and a current of 50 μA. Spectrum acquisition was carried out using ADMCAR software. The samples were irradiated with gamma rays at different doses of 0.05 kGy, 0.1 kGy, 0.2 kGy 0.6 kGy and 1.0 kGy from Cesium-137 gamma source having activity of 1850 MBq and a dose rate of 100.9918 Sv/hr. The activity of the source was converted to dose rate using Radpro calculator software.Thermogravimetric Analysis measurement was carried out using a thermogravimetric analyzer (TGA 4000 PerkinElmer, U.S.A.). 38.18 mg of the native starch sample was weighed directly into an aluminum pan. The pan was heated from 30°C to 700°C at a heating rate of 10°C/min, under N_2 gas flow at 20 ml/min. The weight loss and weight derivatives were determined from the TGA curves by means of Universal Analysis 2000 software. X-ray diffractograms of starch powders were recorded using D8 Advance X-ray diffractometer (BRUKER AXS Germany). Cu-$K\alpha_1$ radiation, with wavelength of 1.5406 Å, was used in the experiment and the radiation was generated at a voltage of 40 kV and current 40 mA.

The sample was placed in the XRD sample holder and then scanned from 10 to 79°2θ with scan step size of 0.034 ° and scan step time of 0.5 s in a continuous scan mode at a temperature of 25°C. The procedure was repeated for both the native and irradiated starch samples.Whole pattern and peak width fitting, together with indexing was carried out using GSAS II suite of programs while estimate of the crystallinity was determined using *WinPlotr* within the Full prof program.Scanning Electron Microscope was used to study the surface morphology using Phenom Pro X at an accelerator potential of 15 kV. 1 g each of native and the irradiated samples were stuck separately on a specimen holder using a silver plate and then coated with palladium in a vacuum evaporator to reduce the deposited charge on the sample surface. Particle size distributions were determined by laser light diffractometry using a dry feeder (Malvern 2600C, Malvern Instruments, Worcestershire, U.K.). The feeder was set at a pressure of 400 kPa and the injector to a pressure of 6 kPa .The focal distance was 300 mm and the measuring time was 25-35 s. The mean particle size was determined in quadruplicate.

The apparent particle densities of specimens of all equilibrated starches were determined by helium pcnometry (Acupye 1330, micrometrics, Norcross, GA, USA) in triplicates.Bulk and tap densities were determined in a 250 ml cylinder suing a volumeter (stampfvolumeter Stav 2003, J.Engelsmann AG, Ludwigshafen, Germany). Determinations were also made in triplicate.

Proximate composition analysis for ash and lipids contents was carried out according to *Association of Official Analysis Chemists* AOAC [11] methods. Protein content was estimated from the nitrogen content determined by elemental analyses based on a conversion factor of 1.25 [12]. The phosphorus content was determined from the starch ash by mixing the starch with 1%(w/w) sodium carbonate and ignited in a furnace at 550°C for 6h.The phosphorus content in the starch was determined colorimetically based on the method described by [13].The amylose content was determined colorimetically using the method described by [14]. All determinations were done in triplicate and the results were presented as mean and standard deviations. An

ultraviolet-visible spectroscopy study was carried out in the wavelength range of 200-600 nm for both native and irradiated specimens. 0.05 g of each sample was dissolved in 30 ml distilled water. The absorbance of the starch samples were measured by means of a UV-Vis spectrophotometer (Jenway 6405).

3. Results and Discussion

The TGA/DTA curves of native starch are shown in fig. 1. The TGA curve shows mass loss in two steps. The first mass loss is observed at temperature range 30-130°C with~89.63% loss, almost corresponding to the DTG valley at 88.18°C, at -1.36 %/min. This range is in accordance with literature [15,16] for cassava starch. The initial weight loss is attributed to the loss of water and water of hydration. The second mass loss is observed at 371°C with weight loss of ~ 21.09% and a second peak at temperature 391.19°C. This temperature range is similar to that reported by other workers [8,17-18]. The TGA plot shows that *kwasi* starch is stable at ~ 699°C.

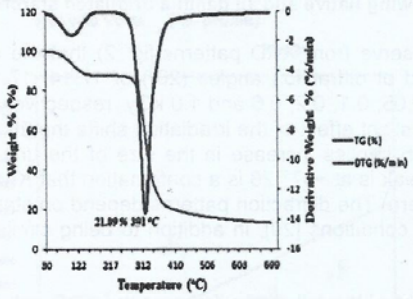


Figure 1. TGA/DTA plots for native starch showing relevant mass losses and transformation temperatures

In order to understand the constitution of the native starch, proximate analysis was carried out. The analyses shows it contains moisture of 9.77%, crude protein of 1.18%, crude fat of 0.39%, ash content of 1.13%, crude fibre 1.09%, crude carbohydrate 86.44% and Amylose content of 22.48%. The report [19] on *D.R.* species showed higher amount of moisture (11.96±0.06%) and amylose (28.830±0.65%) contents, compared to the average value obtained for the same parameters in this work. The low moisture content determined for the species is far below the threshold of 13 %(w/w)[19]which makes them safe for storage. But the amounts of crude proteins, lipids and ash reported [19] in their work (0.28±0.08%, 0.02±0.01% and 0.015±0.01%, respectively), are much smaller than our results. The phosphorus determined from proximate analysis in their work (0.022±0.001%), is lower than the value obtained in this work (0.028±0.002%).

The crystalline and amorphous natures of the samples at different doses were obtained by X-ray diffraction. Figure 2 shows the composite XRD patterns of both native and irradiated *kwasi* starch. The diffraction patterns clearly indicate that the samples are semi-crystalline in nature. The similarity of their X-ray diffraction patterns indicate that the organization of crystalline structure of starch was not affected by gamma irradiation in any significant way. There is no peak at higher than 50° 2θ, which is considered an area for amorphous structure. Gamma irradiation of *Kwasi* starch did not show any reasonable molecular movement of starch, so there may be very little interaction between starch molecules that would lead to change in crystallinity as presented in table 1.

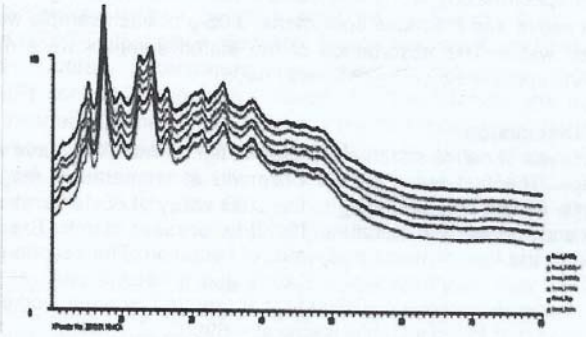


Figure 2. X-ray diffraction pattern of *kwasi* starch samples at diffraction angular range of $10 \leq 2\theta \leq 79$ ° showing native and all gamma irradiated starch at different doses.

Similarly, one can observe from XRD patterns(fig. 2) that the strongest peaks for all samples are observed at diffraction angles ($2\theta^{o}$) of 17.14, 17.12, 17.12,17.12, 17.13 and17.13 for native, 0.05, 0.1, 0.2, 0.6 and 1.0 kGy, respectively. These are very close indeed, indicating the slight effect of the irradiation shifts the 2θ values generally below the native peak, which implies increase in the size of the unit cell. The fact that the strongest diffraction peak is at ~17° 2θ is a confirmation that *Kwasi* starch has a typical B-type diffraction pattern. The diffraction patterns depend on starch origin as well as on environmental growth conditions [20], in addition to being similar to those reported by other workers [21].

Figure 3 shows the plot of the full profile fitting of the XRD pattern of the native starch as a representative. Similar plots were obtained for the irradiated samples.

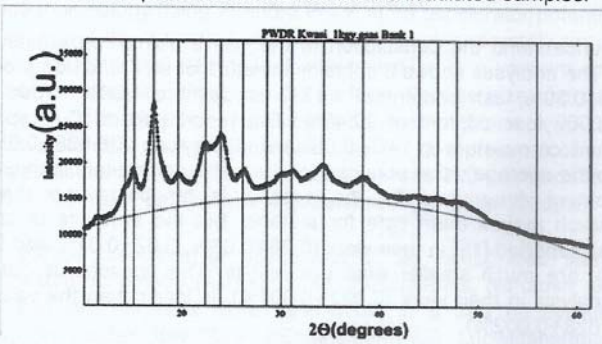


Figure 3. Full profile fitting of the XRD pattern of native starch. The red curve is the background fit; the crosses are the data points while the continuous blue line is the fitting.

Whole profile fitting for the native starch and its subsequent refinement were carried out with GSASII program for peak position, intensity and background, while the peak widths were fitted using Gaussian and Lorentzian fits and their convolution, as shown in fig. 3. The blue lines are the generated peak positions by GSASII. Those that do not coincide with the experimental data in the 2Θ range 10-60°(beyond this range is a flat background) and those that were counted twice were deleted. Beyond the high angle range the plot is essentially flat background. In GSASII the background is automatically

drawn once the generated peak positions are correctly chosen and refined. The pseudo-Voigt profile function shapes were used. The peaks were refined systematically and sequentially for background and intensity, peak position and peak width. The least squares refinements of the peak widths were constrained to follow an instrumental broadening equation. The main plot is intensity vs 2Θ in degrees. The red curve seen in all the plots represents the background, the crosses are the data points and the continuous blue line is the fitting. The low value of R_{wp}(1.83%) is due to the high background and amorphous content of the samples. Similar results were obtained for the irradiated samples.

The crystallite sizes were determined from the Williamson–Hall plots using XPowder program based on Pseudo-Voigt analysisand is presented in table 1, while fig.4 is a representative plot of *native kwasi.* The samples have varying crystallite sizes as the irradiation is increased, with the native having a maximum of 27.79 ± 16.01 nm, while those irradiated have lower values. Also the native has the largest uncertainty in its size and there seem to be little or no microstructural modification at all doses of irradiation.

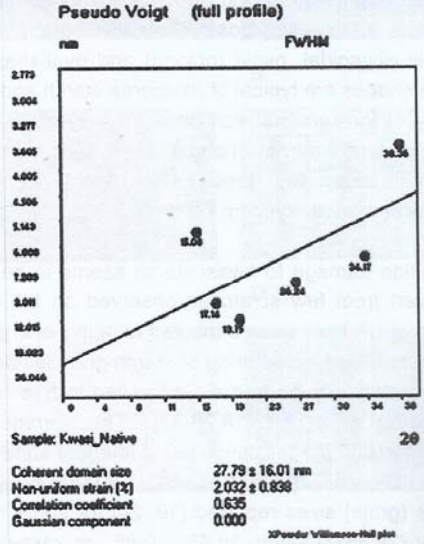


Figure4. Williamson–Hall pot of native sample based on Pseudo-Voigt (full profile) analysis using XPowder program. The black dotted points and associated numberings refer to the 2Θ positions while the details below the points are the generated data.

Table 1 lists the values of % crystallinity for each sample determined using the *Winplotr* program within the *Fullprof* program. Five to six background points were selected from the profile in order to estimate the relative crystallinity rate and this was done in quadruplicate. The average and standard deviations(approximately zero) were determined for each profile. The %crystallinities are not only approximately the same for native (34%) and irradiated starch (about 33%) and similar to those reported in literatures [8] for cassava and waxy maize starch but also within the standards reported for starches of various origins, 15-45% [22], which is an indication that the starches are of relatively high crystallinities. There is no clear trend in the values of the results obtained for native and irradiated parameters nor are there correlation between crystallinity, crystallite size and grain size, but the crystallinities are so close to suggest irradiation had little effect on the starch.

Table 1.Summary of crystallinity, crystallite size, grain size and crystal type of *kwasi* starch

Samples (kGy)	Crystallinity (%)	Crystallite size (nm)	Average Grain Size (μm)	Crystal type
Native	34	27.79±16.01	35.33(773)*	B
0.05	32	21.00±10.11	17.31(132)	B
0.1	33	13.72±4.48	37.41(21)	B
0.2	33	15.69±6.31	18.20(27)	B
0.6	33	20.29±7.95	15.28(119)	B
1.0	32	17.48±6.42	31.70(25)	B

*Numbers in parenthesis represent the number of grain counts used to determine the average grain size from Image J software.

The native and gamma irradiated (0.05-1.0 kGy) starch samples were examined using SEM scanned at x1000 magnification. Fig.5(A-F) are representative for native and irradiated starches in order of increasing dose. The native starch granules and gamma-irradiated samples show ellipsoidal, ovoid (oblong) and oval-shaped granules with a smooth surfaces. These shapes are typical of *dioscorea* starch and have been reported by other workers [6,23-24] for potato starch because they are root and tuber crops. Similarly, the granule structure was not changed even after treatment with 1 kGy of gamma irradiation, in agreement with previous findings [7,9,25]which reported the absence of evident physical damage on corn starch at this dose.

Therefore, gamma radiation damage to *kwasi* starch seems to be absent even at the microstructural level, apart from few scratches observed on the granule surfaces of some irradiated samples which have been attributed to highly energetic and penetrating radiations, and to the source. Some clustering of starch granules on irradiated samples at 0.1 kGy and 0.2 kGy was observed and are attributed to free radicals produced by gamma irradiation on starch molecules [18,26-27]. The average grain size (μm) of *kwasi* starch measured at x1000 magnification using image J software show maximum value of 37.41 μm at 0.1 kGy, while the least is 15.28 for 0.6 kGy, as presented in table 1. The values of particle (grain) sizes reported [19, 24] for some native starch of *D.R* species are much higher (29.85±0.17μm, 18.68 ± 0.85 μm, respectively) compared to the result obtained in this work. Other studies[28] on six species of *D.R.* reported average grain sizes in the range 18.4±5.0 to 40.9±3.0 μm, with some workers [23] reporting polygonal and rod-like shapes for the grains which have mean diameters of 27.33μm.This shows that the values obtained in this work are generally within the typical range for starches. The apparent density obtained from this work for *kwasi* starch is 1.46g^{-3}, almost the same as that reported in literature (1.53g^{-3})[19].

Elemental analysis indicates the constituent elements in the native sample and their concentrations (and uncertainties by weight %) are K(0.01031±0.007), Ca(0.0096±0.0005), Mn(0.0218±0.0019),Zn(0.0229±0.0012), Fe(0.0752±0.0031) and Cu(0.0350±0.0016).The spectrum is shown in fig. 6. Qualitative work reported on mineral compositions of *D.R.* species found in south west Nigeria [29]show the elements Mn, Fe, Zn, Ca and Mg, all of which were detected in the native sample, except the last. On the other hand, quantitative work reported [30] on *D.R.* species

show the presence of elements(w/w%) K (0.475±0.003), Na (0.070±0.004), Ca (0.100±0.005), Mg (0.035±0.005) and P (0.158±0.017). The PIXE result for P in this work is 0.035±0.002. However, the P concentration reported above is much higher than the present result and those reported in literature. Na was not found this work. Tubers are associated with high P content and have enormous implications for many physical properties of starch such as viscosity, gelatinization temperature, transparency, among others. The variations in the qualitative/quantitative elemental compositions have been attributed to geographical and botanical origins, amongst others.

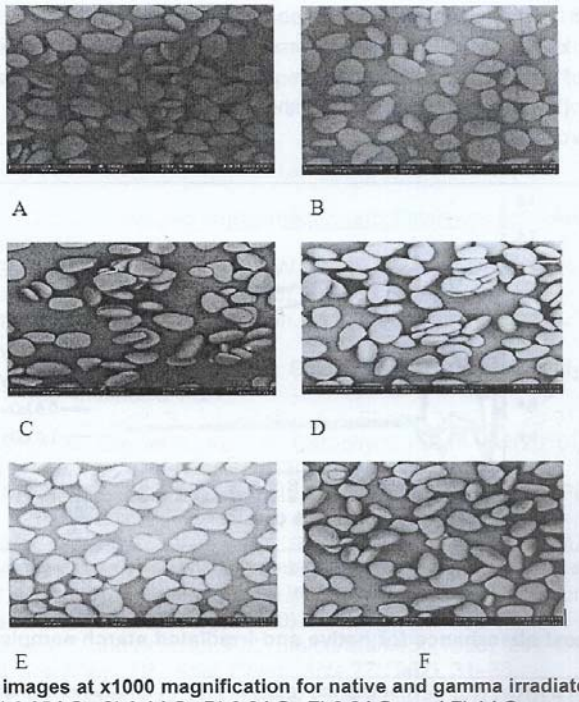


Figure5. SEM images at x1000 magnification for native and gamma irradiated starches A) native B) 0.05 kGy C) 0.1 kGy D) 0.2 kGy E) 0.6 kGy and F) 1 kGy, respectively.

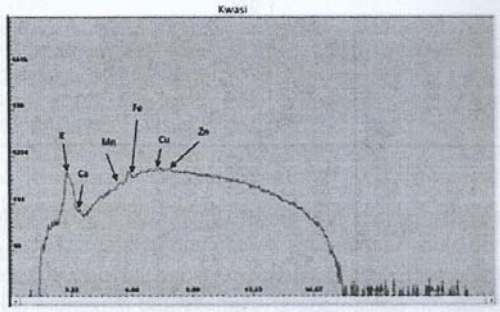


Figure 6. The elemental distribution in the native starch sample. The most predominant is K.

By comparison of the pattern of UV-Vis spectra of native *kwasi*and irradiated samples in the range 200-600 nm, it is possible to verify that the absorbance of *kwasi* starch is affected by the radiation dose as reported by some workers [7] (table 2). The result showsthat the highest absorbance is that of *kwasi* starch at 0.2 kGywhich has a value of 1.373at 290nm (fig.7). The result confirms that ionizing radiation altered the absorbance of *kwasi* starch. The doses absorbed are in the ultraviolet region (190-400 nm) and thus no indication of any light being absorbed making *Kwasi* starch colourless.Due to the lack of relevant literature about *dioscorea rotundata* starch, very little information was found on UV-Vis study of gamma-irradiated starch. However, a similar study[31] reported an increase in absorbance of starch due to the increase of UV-irradiation of starch from 0-13 h.Other workers [32,33] also reported increase in absorbance of chemically modified corn and potato starches, respectively, while another study [34] reported a variation in the absorbance of chemically modified corn and wheat starches.

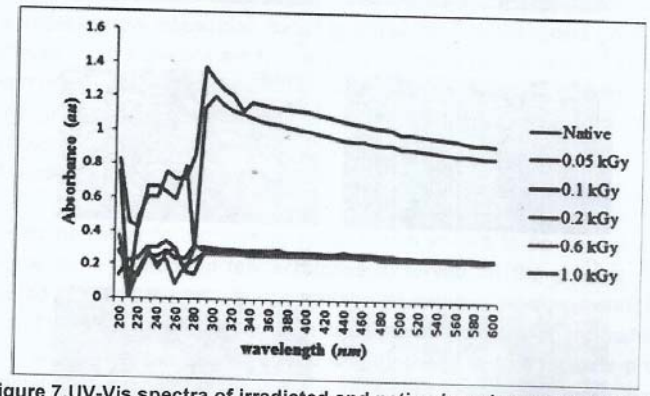


Figure 7.UV-Vis spectra of irradiated and native *kwasi* starch samples at different doses.

Table 2: Highest absorbance for native and irradiated starch samples at different doses

Sample (kGy)	Absorbance (a.u.)	Wavelength (nm)
Native	0.339	250
0.05	1.202	300
0.1	0.317	280
0.2	1.373	290
0.6	0.374	200
1.0	0.310	290

4. Conclusion

The XRD characterisation shows that *Kwasi* starch is semi-crystalline in nature. There was no trend in the values recoded for percentage crystallinity and crystallite sizes of both the irradiated and native starch samples whose values are approximately similar. The maximum decrease in percentage crystallinity and maximum crystal size occurred at 0.2 kGy. An oval shape of starch granules was observed by SEM coupled with a

strong intensity at 2θ of 17°indicating that it is B-type X-ray pattern characteristic of tubers. The DTA plot indicates phase transformations took place at temperatures of ~ 88 and 319°C. The TGA plot, on the other hand, shows that *kwasi* starch is stable from 699°Cwith weight loss of ~14.19%.These results are an indication that *kwasi* starch structure is stable to gamma irradiation to a maximum dose of 1 kGy and has high crystallinity compared to other starches, particularly within the *dioscorea* family. All the doses absorbed are in the ultra-violet region (190-400 nm), therefore no indication of any light being absorbed making *Kwasi* starch colourless.These properties make it promising for application in food preservation and radiation environment. This research shows that there is great diversity in composition, structure and morphology of starches; even from the same species, and thus comparison with other species is difficult unless some metrics are developed. Further, compared with starches from other sources such as maize and potato, it shows that there is more work to be done on *dioscorea rotundata*.

References

[1] Satin, M. (2006). www.fao.org/ag/magazine/pdf/starches.pdf. Accessed 20th October, 2012.
[2] Yoo, H.-S, J.-Y. YooJ.-H., Kim J.-W., Lee M.-W., Byun B.-K., BaikS.-T. Lim,*Carbohydrate Polymers* 8(2010),961-963.
[3] Fedelis, C.K. , OclooG. Otoo,Nkansah, M.R., Odonkor, S.,Enoch, T.Q.,*J. Agric Food Tech.* 1(2011),116-122.
[4] Bujang, K. B. Int. Seminar on Sago & Spices for Food Security,July 28-29,2010, Ambon, Rep. Indonesia.1-6.
[5] Neelam, K., Vijay, S., Lalit, S., *Int. Research J. Pharm.* 3(2012), 25-31.
[6] Singh, S., Singh N., Ezekiel R., Kaur A., *Carbohydr. Polym.* xxx(2010), xxx
[7] Desai, K.G., Park, H.J., *Drug Delivery* 13(2006),39–50.
[8] Lamanna, M., Morales, N.J., Garcia, N.L., Goyanes, S., *Carbohydr. Polym* 97(2013), 90-97.
[9] Bettaïeb, N.B., Jerbi, M.T., Ghorbel, D., *Radiat. Phys. Chem.* 103(2014), 1-8.
[10] Sockey A.S. and Hanna M.A.,*Food struct.*12(1993)397-410.
[11] A.O.A.C. Official Methods of Analysis, 17th ed. Association of Official AnalysisChemists (A.O.A.C.), (2000) Washington, DC, USA.
[12] Gebre-Mariam, T. and Schmidt, P.C.,*Starch/Stärke*50(1998), 241–246.
[13] Murphy, J. and Riley, J.P., *Anal. Chem. Acta,*27(1962), 31–36.
[14] Williams, P.C., Kuzina, F.D. and Hlynka, I.,*Cereal Chem*47(1970),411-421.
[15] Costa, F.J.O.G., Almeida, R.R., Lacerda, L.G., Carvalho-Filho, M.A.S.,*Alim Nutr.* 22 (2011), 7-15.
[16] Ayala, G., Agudelo, A., Vargas, R.,*Network Sci. J.*171(2011),138-147.
[17] Mano, J.F., Koniarova, D., Reis, R.L., *J. Materials Sci: Materials inMedicine* 14(2003), 27-135.
[18] Kumar, P., Ganure, A.L., Subudhi, B.B., Shukla, S., *Int. J. Pharm.Pharmaceutical Sci.*6 (2013), 868-880.
[19] Odeku, O. A., Picker-Freyer, K. M., *Starch/Starke* 59(2007), 430–444.
[20] Marimuthu, M., Sundaram, U., Gurumoorthi, P., *Indo American J.Pharmaceutical Research*,(2013), 4319-4329.
[21] Brunnschweiler, J., Luethi, D., Handschin, S., Fara, Z., Escher, F., Conde-Petit, B., *Starch/Stärke* 57(2005), 107-117.
[22] Napaporn, A., Sujin, S., Pavinee, C., Saiyavit, V.,*Starch/Stär* 53 (2001), 577–581.
[23] Riley, C.K., Wheatley, A.,O., Asemota, H.,N., *African J. Biotech.* 5(2006),1528-1536.
[24] Odeku, O.A., *Starch/Stärke* 00(2012), 1–18.

[25] Lee, J.-S., Ee M.-L., Chung, K.-H., Othman, Z., *Carbohydr. Polym.* 97(2013), 614-617.
[26] Lee Y.-J., Kim S.Y., Lim S.-T., Han S.-M., Kim H.-M., Kang I.-J., *J. Food Sci. Nutr.* 119(2006), 146-154.
[27] Polesi,L.F., Sarmento, S.B.S., Moraes, J., Franco,, C.M.L., Canniatti-Brazaca S.G., *Food Chem.* 191(2015),59-66.
[28] Otebayo, B., Aina, J., Asiedu, R., Bokang, M. , *Journal of Texture Studies* 36(2005), 324-332.
[29] Odebunmi, E.O., Oluwaniyi, O. O, Sanda, A. M. and Kolade, B. O., *International Jour. Chem.* 17(2007), 37-43.
[30] Polycarp, D., Afoakwa, E. O., Budu, A. S. and Otoo, E.,*International Food Research Journal*, 19(2012),985-992.
[31] Bajer, D., Kaczmarek, H., *Progress on Chemistry And Application of Chitin and its Derivatives*, 15(2010), 17-24.
[32] Abdel-Razik, E.A., Badawy, D.S., El Nahas, E.A.,*Int. J. Modern Organic Chem.* 4(2015), 1-17.
[33] Yu, X., Houtman, C., Atalla, R.H., The Complex Of Amylose And Iodine, *Carbohydr. Research*. 292(1996), pp129-141
[34] Gunasekaran, S., Devi, M.K., UV-Vis Spectroscopic Investigation On HydrolysisOf Starch And Starch-Drug Interaction,*Asian J. Chem.* 19 (2007), pp3363-3374