Effects of gamma irradiation, stearic acid alone and in combination on functional, structural and molecular characteristics of high amylose maize starch

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Abstract

The effects of gamma irradiation, stearic acid alone and in combination on functional, structural and molecular characteristics of high amylose maize starch (Hylon VII) were studied. Stearic acid (0, 1.5 & 5 %) was added to Hylon VII starch, and then irradiated at 0, 30 and 60 kGy. Gamma irradiation significantly ($P \leq 0.05$) increased solubility, water absorption capacity and oil absorption capacity as well as decreased swelling power (at 90 and 95 °C) of Hylon VII starch. These changes related well with increased amylose contents and decreased amylopectin, molecular weight and transition endotherms of the starches due to gamma irradiation. Stearic acid addition also, significantly increased ($P \leq 0.05$) water and oil absorption capacities, relative crystallinity as well as decreased swelling power of Hylon VII. Addition of stearic acid to Hylon VII seemed to reduce the effect of gamma irradiation on the solubility and transition endotherms of Hylon VII, even though not statistically significant ($P \geq 0.05$). Gamma irradiation, stearic acid addition and in combination did not change the X-ray diffraction pattern and microstructure of Hylon VII. Gamma irradiation had more effect on the molecular structure of Hylon VII compared with stearic acid. Both irradiation and stearic acid alone and in combination did not have any effects on the microstructure of Hylon VII.

Key words:

DSC, gamma irradiation, Hylon VII, size exclusion, solubility, stearic acid

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Abbreviations: **LM,** light microscopy; **SEM,** scanning electron microscopy; **DSC,** differential scanning calorimetry; **WAXS,** wide-angle x-ray scattering; **DS,** damaged starch; **MWD,** molecular weight distribution; **kGy,** kilogray; **AU,** arbitrary unit; **kDa,** kilodaltons; **WAC,** water absorption capacity; **OAC,** oil absorption capacity; **To,** onset temperature; **Tp,** peak temperature, **Tc,** conclusion/endset temperature, **ΔH,** melting enthalpy, **PCA,** principal component analysis

1. Introduction

Starch, the major storage polysaccharide of higher plants, is a polymeric mixture of essentially linear and branched α-D-glucan molecules [1]. High amylose maize starches are obtained from maize containing the *amylose-extender* (*ae*) genotype as a result of genetic modification/hybridization [2]. These starches contain about 40 to 70 % amylose [3]. High amylose starches are often used in confectionery industry due to excellent gelling and film – forming characteristics [4]. They are also good source of raw material for production of resistant starch ingredients [5].

However, high amylose maize starches are unattractive for commercial use due to difficulties in pasting and solubilising them at normal processing conditions (i.e. 60-90 °C). To fully gelatinize and disperse high amylose maize starch, temperatures higher than 130 °C are required [4]. The use of high temperatures (above 130 °C) could result in high utilization of energy, hence high cost of production by industry. In this regard, several modification methods have been used to improve upon the functional properties and utilization of high amylose maize starches. These include, addition of fatty acids [6], microwave radiation [7], extrusion [8], chemical treatment [9], freeze- thawing [9], enzyme treatment [10] and gamma irradiation [11].

The addition of fatty acids to starch has been found to form amylose - lipid complexes in the starch [12]. Starch-lipid complexes have been obtained from high amylose maize starches [13-15]. These complexes have been reported to reduce stickiness of starch, improve freeze-thaw stability and have an anti-staling effect in bread [16]. They can also be used to produce amylose-lipid nanomaterials [17] as well as as fat replacers [13].

Gamma irradiation has been found to provide cost effective and environment-friendly alternative to change the physical, chemical, and/or biological characteristics of a product [18]. Gamma irradiation involves the use of a radioactive isotope, either in the form of cobalt-60 or cesium-137, which emits high-energy gamma rays or photons capable of intruding in-depth into the target product, up to several meters. It produces free radicals that are capable of inducing molecular changes and fragmentation of starch in isolated starch or starch based products [19, 20]. Irradiation has been reported to decrease relative crystallinity (RC) (degree of crystallinity) of potato and wheat starches and flours [19, 21, 22] as well as maize starch irradiated at 10 and

50 kGy [23]. Decreases in gelatinization temperatures and enthalpy for maize starch [23] and starch from irradiated rice have been reported [24]. Decreases in degree of polymerization and molecular weight of maize starch have also been reported [8]. These changes in the molecular structure of starch due to irradiation have been reported to increase starch solubility, reduced viscosity, pasting temperature, viscosity and swelling power [23]. However, there is limited literature on gamma irradiation of Hylon VII.

Changes in the molecular and structural characteristics of Hylon VII starch due to gamma irradiation and stearic acid addition could result in modification of its functional properties. Therefore the objective of this study was to determine the effects of gamma irradiation, stearic acid alone and in combination on functional, structural and molecular characteristics of Hylon VII.

2. Materials and method

2.1. Materials

High amylose maize starch (Hylon VII) was obtained from Ingredion Incorporated, Westchester-USA (formally National Starch and Chemical Company). The moisture content was 9.6 %. The protein content as determined using Dumatherm was 1.07 % (N × 6.25). The ash and fat contents were 0.11 % and 0.13 %, respectively. Analytical grade stearic acid was obtained from Sigma–Aldrich Company (St. Louis, MO, USA). Amylose/amylopectin assay kit was obtained from Megazymes (Megazyme International, Bray, Ireland). All other reagents used were analytical grade.

2.2. Methods

2.2.1. *Incorporation of stearic acid into starch*

Stearic acid was incorporated into Hylon VII in concentrations 0, 1.5 & 5 % (dry weight basis of the starch). The method described by D'Silva et al. [25] was used. Briefly, stearic acid was first dissolved in absolute ethanol. The starch was then added to this solution and covered with Parafilm and aluminium foil and placed in a shaking water bath at 50 °C for 30 min at a speed of 120 rpm. The ethanol was evaporated in a forced draught oven at 40 °C.

2.2.2. *Gamma irradiation*

Twenty grams (20 g) of Hylon VII starch samples (stearic acid treated & non-stearic acid treated) were packaged in low -density polyethylene plastic containers (47mm diameter, 95mm in height and 1mm thick), placed in boxes and then irradiated at a commercial gamma irradiator facility operated by Synergy Sterilisation, SA (Pty), Isando, South Africa using ^{60}Co source. Samples were exposed to target irradiation doses of 0, 30 and 60 kGy (the actual doses delivered were 0, 31.5 and 60.4 kGy) with an average dose rate of 0.02 kGy/min. Irradiation was done in triplicate. All the Hylon VII samples (irradiated and un-irradiated) were stored at 8 °C prior to analysis.

2.2.3. *Starch solubility and swelling power*

Starch solubility was determined using the method described by Adebowale et al. [26] and Liu et al. [27] with some modifications. Starch samples (0.125, dry basis) were heated in 10 mL of distilled water at 50, 70 and 90 °C in shaking water bath (rpm of 150) and 95 °C in boiling water bath (Water boils at 95 °C in Pretoria, South Africa due to high altitude) . Samples were cooled

and centrifuged at 3000 x *g* for 15 min at 25 °C. The supernatant was decanted and evaporated at 105 °C for 16 h in an air-oven. The solubility was determined as the ratio in weight of dried supernatant to the weight of the dry starch and expressed as percent (%). The residue obtained after centrifugation was then weighed to obtain the swelling power. Swelling power was expressed based on the weight of starch used.

2.2.4. *Oil and water absorption capacities*

The oil and water absorption capacities were determined using the method described by Beuchat [28] with some modifications. About 1 g (dry basis) was weighed into 10 ml centrifuge tube and 5 ml of oil (sunflower oil, SPAR, South Africa) and water added for oil and water absorption capacities respectively. The mixture was shaken in shaking water bath for 30 min at 26 ± 2 °C using rpm of 150 and then centrifuged at 3500 x *g* for 30 min. The volume of decanted supernatant fluid (oil/water) was measured and milliliters of oil or water retained per gram of sample were calculated.

2.2.5. *Light Microscopy (LM)*

The general size and shape of the starch granules were observed using light microscopy. About 0.005g (dry basis) of the Hylon VII starch was dissolved in 1 ml of 30 % glycerol solution. Three (3) drops of the suspension were placed on a slide and cover slip applied. Samples were observed using a Nikon Optiphot Transmitted Light Microscope (Tokyo, Japan) with phase contrast optics. Polarised light was used to detect birefringence in the granules and the images captured. Observations were done at 100 x magnifications.

2.2.6. *Scanning Electron Microscopy (SEM)*

The shape, size and surface features of the treated and untreated Hylon VII starch samples were observed using scanning electron microscopy. The method described by Liu et al. [27] was used with some modifications. Starch samples were mounted on aluminium stub covered with double–sided adhesive tape and sputter coated with a thin gold film (about 20nm in thickness). Scanning Electron Microscopy (SEM) micrographs were obtained using a JEOL JSM-5800 LV SEM (Tokyo, Japan) at an accelerating potential of 5kV. Observations were done at 2500 x magnifications.

2.2.7. *Differential Scanning Calorimetry (DSC)*

The thermal properties of the Hylon VII samples (treated and untreated) were measured using a high pressure Differential Scanning Calorimetry (DSC) system with STARe® software (HPDSC-827, Mettler Toledo, Greifensee, Switzerland). The method described by Wokadala et al. [6] was used. Indium (T_o = 156.0 ◦C, Heat flow = -28.6 J/g) was used to calibrate the instrument as regards temperature and enthalpy. The starch sample (10 mg, db) was placed in a crucible and then distilled water (30 mg) added. The pan was sealed, allowed to equilibrate for at least 2 h at ambient temperature. Scanning was done from 30 °C to 180 °C at a rate of 10 °C/min with pressure level of 40.00 ± 0.01bar. An empty pan was used as a reference.

2.2.8. *Wide Angle X-Ray Scattering (WAXS)*

The crystallinity of the Hylon VII samples (treated and untreated) was determined using the X-ray diffraction (XRD) of powders. The WAXS measurements were carried out using an X'Pert-Pro PANanalytical diffractometer (Eindhoven, Netherlands). The method described by

Wokadala et al. [6], with modifications of the operation conditions, was used. The starch samples were equilibrated at 95 % relative humidity (using a saturated salt solution of potassium nitrate) for 7 days at about 25°C. The XRD operating conditions were: 35 kV, 50 mA and Co $K_{\alpha}1$ (1.78901 Å). Scanning was done from 5° to 30 ° (2θ) with a step size of 0.0170 ° and a scan step time of 40.5s. Relative intensities were then plotted against 2 theta peaks identified. The relative crystallinity was determined as the percent integrated area of crystalline peaks to the total integrated area above straight baseline [29].

2.2.9. *Molecular weight distribution (MWD)*

Size exclusion chromatography of the starch samples was done using the method described by Han & Lim [30] with some modifications. In brief, 10 mg starch sample (dry basis) was weighed into a 2 ml Eppendof tube and 500 µL of 1 M NaOH solution added [30]. The mixture was incubated at 35 °C in shaking water bath for 15 min at 200 rpm to solubilise the starch. The solubilised starch was centrifuged to ensure there was no precipitate. About 250 µL of the solution was diluted with ultrapure water to obtain 5 mL (5000 µL) of 50 mM NaOH solution (1 mg/ml starch solution). The solution was shaken in a shaking water bath at 30 °C for 1h at 150 rpm. The solution was then filtered through syringe filter of 0.45 µm pore size (Pallmall; cat 150 597/0). The filtrate with dissolved starch (40 µL) was injected onto Ultrahydrogel™ Linear column (7.8 x 300 mm, Waters) which was protected by Ultrahydrogel™ guard column. The mobile phase was 50 mM NaOH at a flow rate of 0.3 ml/min pumped by a Shimazu Prominence Ultra-Fast Liquid Chromatography pump (Shimazu Corporation, Kyoto, Japan) at 0. 4MPa. The column oven (CTO-10ASvp) temperature was set at 45°C and Differential Refractive Index (DRI) detector (RID – 10A) was used. The retention times for the peaks of the irradiated and

non-irradiated starch fractions were used to calculate their average molecular weights from Log_{10} of molecular weights of pullulan standards (Mw 805 000, 113 000, 21 700, 6 000 and 342 Da).

2.2.10. *Amylose/Amylopectin ratio*

The amylose/amylopectin ratio was determined for native Hylon VII and gamma irradiated Hylon VII using a commercial amylose/amylopectin assay kit from Megazyme (K-AMYL 07/11) (Megazyme International, Bray, Ireland). The procedure described by Megazyme was a modification of concanavalin A (Con A) method developed by Yun and Matheson [31]. The principle of this method involves removal of lipids in starch by precipitating the DMSO starch solution in ethanol and recovering the precipitated starch. Amylopectin was precipitated using Concanavalin A solution and removed by centrifugation. The amylose in an aliquot of the supernatant was enzymatically hydrolysed to D-glucose, which is analysed using glucose oxidase/peroxidase reagent (GOPOD). The total starch in a separate aliquot of acetate/salt solution was also hydrolysed to D-glucose and analysed. The amylose concentration in starch was estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of Con A precipitated sample to that of the total starch sample.

2.2.11. *Statistical analysis*

All the experiments were done in triplicate. Multifactor Analysis of Variance (ANOVA) was performed on the data and compared at $P \leq 0.05$ using Fisher's least significant difference (LSD) test. Multivariate statistical method using principal component analysis (PCA) was applied on the measured variables. Statistica for Windows version 11 (Statsoft, Tulsa, USA) software was

used. Independent variables were irradiation doses (0, 30 and 60 kGy) and stearic concentrations (0, 1.5 and 5 %). Dependent variables were the measured values.

3. Results and Discussion

3.1. Solubility and swelling power

The effects of gamma irradiation, stearic acid alone and in combination on solubility and swelling power of Hylon VII are presented in Table 1. Solubility of native Hylon VII starch increased significantly ($P \leq 0.05$) with increasing temperature (Table 1). Solubility values of the native Hylon VII starch were lower in the presence of stearic acid compared to irradiated Hylon VII starches. However, these decreases were only significant at 5 % stearic acid concentration (Table 1). Irradiation increased the solubility of the native Hylon VII starch and these values significantly increased ($P \leq 0.05$) with increasing temperature, especially at 90 and 95 °C (Table 1). Colonna and Mercier [32] reported solubility value of 4.2 % for Hylon V starches at 96 °C. Decreased solubility has also been reported for normal potato starch with stearic acid at 85 °C compared to those without stearic acid [33]. Increased solubility due to irradiation has also been reported for other normal starches such as maize [34], potato [35], wheat [36], octenyl succinylated waxy and high amylose rice [37] starches.

Swelling power of native Hylon VII starch significantly increased ($P \leq 0.05$) with increasing temperature (Table 1). Addition of stearic acid to Hylon VII starch significantly ($P \leq 0.05$) decreased swelling power at 90 and 95 °C compared with the control (Table 1). Irradiation of Hylon VII starch significantly ($P \leq 0.05$) increased the swelling power at 50 and 70 °C. At 90

and 95 °C significant decrease was observed compared to the control. When Hylon VII starch-stearic acid mixture was irradiated, swelling power at 50 and 70 °C increased and then decreased at 90 and 95 °C compared with native Hylon VII, Hylon VII-stearic acid mixture and irradiated Hylon VII starch alone (Table 1). Colonna and Mercier [32] reported swelling power value of 7 g/g for Hylon V starches at 96 °C. Decreases in swelling power due to the presence of stearic acid has been reported for normal potato starch at 85 °C compared to those without stearic acid [33]. Also, decreases in swelling power due to irradiation has been reported for other normal starches, for example maize [23], potato [38], beans [38] and wheat [36] starches.

Solubility and swelling power provide indication of interaction between starch chains within the amorphous and crystalline regions [39]. The results for solubility and swelling power of native Hylon VII at 95 °C (Tables 1and 2) were comparable to values of 4.2 % and 7g/g reported for high amylose maize (V) starches at 96 °C for solubility and swelling power respectively [32]. However, these values are lower than reported solubility and swelling power values of 9.7 – 15.0 % and 13.7 – 20.7 % respectively for normal maize starch at 90 °C [40]. The low solubility and swelling power of Hylon VII could be attributed to the low level of amylopectin in Hylon VII, which is principally responsible for granular structure of starch and the likely restraint of the high amylose content to water absorption. Swelling behaviour has been attributed to the amylopectin fraction of starches [41].

The decrease in solubility of Hylon VII with stearic acid (Table 1) could be attributed to the interaction of amylose with the stearic acid at gelatinization temperatures. This interaction could

have inhibited the swelling of starch granules and exposure of hydroxyl groups of amylose and amylopectin to water molecules for solubility to occur [42, 43] . Also, the decrease in swelling power of Hylon VII - stearic acid mixture (Table 1) might be due to the formation of hydrophobic layer on the surface of the granules by stearic acid as well as possible complex formation between the stearic acid and starch.

The increase in solubility and decrease in swelling power of Hylon VII after gamma irradiation could be attributed to partial depolymerisation of the branched amylopectin component of the starch. This partial depolymerisation resulted in weak starch granules and formation of low molecular weight fractions which were not able to bind to water for swelling and gel formation due to their solubility in water [36].

3.2. Oil and water absorption capacities

The effects of gamma irradiation, stearic acid alone and in combination on both water and oil absorption capacities of the Hylon VII starch are presented in Table 2. Water absorption capacity (WAC) and oil absorption capacity (OAC) for the native Hylon VII (control) were 1.2 and 1.0 g/ml respectively. WAC of Hylon VII-stearic acid mixture significantly ($P \leq 0.05$) increased at 5 % stearic acid concentration (Table 2). Significant ($P \leq 0.05$) increase in WAC was recorded for irradiated Hylon VII starch when compared with the control. WAC for Hylon VII starch irradiated at 30 kGy was however not significantly ($P \geq 0.05$) different from 60 kGy irradiated starch. Irradiation of Hylon VII starch in the presence of stearic acid significantly ($P \leq 0.05$) increased WAC (Table 2). Similarly, Oil absorption capacity (OAC) of irradiated Hylon VII

starch significantly ($P \leq 0.05$) increased compared with the control. OAC for Hylon VII-stearic acid mixture significantly ($P \leq 0.05$) increased at 5 % stearic acid concentration. Irradiation of Hylon VII-stearic acid mixture significantly ($P \leq 0.05$) increased OAC (Table 3). Increase in WAC and OAC due to irradiation has been reported for wheat starch [36] and cowpea flour and pastes [44] respectively.

The increase observed in water and oil absorption capacities of Hylon VII in the presence of stearic acid could be due to amphillic nature of stearic acid. Also, competition for available water/oil by the production of soluble, low molecular weight compounds by gamma irradiation could have contributed to increased water and oil absorption capacities compared to the control [36].

Table 1: Effects of gamma irradiation, stearic acid alone and in combination on solubility and swelling power of Hylon VII starch

Table 2: Effects of gamma irradiation, stearic acid alone and in combination on water absorption capacity (WAC), oil absorption capacity (OAC), amylose and amylopectin contents of Hylon VII starch

3.3. Microscopy

The granule morphology and surface features of Hylon VII starch (without irradiation and without stearic acid) showed variations in size and shape as seen with light and scanning electron

microscopy (Fig. 1). Hylon VII starch granules were round or spherical, angular polyhedral, and irregular (granules with appendages) in shape with smooth and few porous surfaces. The diameter of these granules ranged from 5 μm to about 15 μm and exhibited maltese cross or birefringence (Fig. 1). Some granules were observed to have overlapped maltese crosses and weak to no maltese cross (birefringence) along the periphery. Irradiation and stearic acid did not seem to cause any structural change in Hylon VII starch at the granular or microscopic level (Fig. 1). There was no granular cracking or roughness on the surface of the irradiated Hylon VII starch with or without stearic acid. High amylose starches have been reported to exhibit the above size and shapes [15, 45, 46]. Irradiation has been reported not to cause any significant change at the microscopic level for other normal starches, such as maize [23, 27, 34], potato [38, 47], beans and cowpea [38, 48] starches. Chung and Liu [23, 38] reported that granule structure and birefringence remains unchanged up to 10 kGy irradiation dose for normal maize, potato and bean starches, but became fractured at 50 kGy.

The morphology exhibited by native Hylon VII was consistent with literature. Elongated and irregular shaped granules have been reported for high amylose maize starches [42, 49]. The observed morphology of Hylon VII could be attributed to low level of ordered amylopectin in the lamellar structure [50].

Figure 1: Selected micrographs of Hylon VII starch irradiated with and without stearic acid using Optical light **(1)**, Polarised light **(2)** and Scanning Electron **(3)** Microscopy. Bar = 10 μm.

I = 0 % stearic acid + 0 kGy irradiation; **II** = 0 % stearic acid + 60 kGy irradiation; **III** = 5 % stearic acid + 0 kGy irradiation and **IV** = 5 % stearic acid + 60 kGy irradiation. Magnifications: 100x for optical and polarized light microscopy; 2500x for scanning electron microscopy.

Arrows in polarized light micrographs **(2)** indicate weak or no birefringence along periphery (**A**), overlap maltese crosses (**B**) and single bright maltese cross (**C**) of starch granules.

Arrows scanning electron micrographs **(3)** indicate angular polyhedral (**A**), filamentous (**F**), irregular (**I**), rod (**R**) and spherical (**S**) starch granules.

3.4. Differential Scanning Calorimetry (DSC)

The effects of gamma irradiation, stearic acid alone and in combination on thermal transition endotherms of Hylon VII starch are shown in Table 3. Native Hylon VII starch exhibited 2 thermal transition endotherms (Peaks II & III). The endotherms for the native Hylon VII were 84.31 to 103.96 °C and melting enthalpy (ΔH) of 3.52 J/g for peak II and 138.99 to 147.06 °C and ΔH of 0.21 J/g for peak III. Also, Hylon VII irradiated (30 kGy and 60 kGy) without stearic acid showed transition endotherms at Peaks II & III. Hylon VII starch with stearic acid (irradiated and un-irradiated) exhibited 3 thermal transition endotherms (Peaks I, II & III). Peak I is attributed to the thermal transition of the stearic acid, which has peak temperature (T_p) of about 69 °C [6]. Addition of stearic acid to Hylon VII starch resulted in delay in onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) of Hylon VII for both peaks II and III, though these effects were not significant ($P \geq 0.05$)(Table 3). Irradiation significantly decreased ($P \leq 0.05$) the transition endotherms (T_o, T_p, T_c and ΔH) of Hylon VII starch with or without stearic acid for both peaks II and III (Table 3). Peak temperature (T_p) for peaks II and III of Hylon VII starch-stearic acid significantly ($P \leq 0.05$) decreased with irradiation (Table 3).

Zhang *et al*. [51] reported broad endotherms from 76.5 to 109.4 °C and ΔH of 3.5 J/g for Hylon V and Chang et al. [42] reported transition endotherm with onset (T_o), peak (T_p) and conclusion

(T_c) temperatures of 77.6, 86.2 and 107.5 °C respectively as well as enthalpy (ΔH) of 15.8 J/g for Hylon V. Decreased gelatinization temperature and melting enthalpy due to irradiation has been reported for normal maize starch [23, 34, 43]. Delay in starch gelatinization temperature due to the addition of lauric acid has been reported by Chang et al. [42] .

The differential scanning calorimetry (DSC), which measures the thermal stability of starch exhibited characteristic transition endotherms for high amylose maize starches. Broad endotherm recorded for Hylon VII starch (Table 3) could be attributed to gelatinization of amylopectin crystallites and melting of amylose-lipid complexes [15, 42]. High amylose maize starches have been reported to have longer inner and outer branch amylopectin compared with normal maize starches [52]. Longer branch lengths of high amylose starches are proposed to have gelatinization temperatures of 80-110 °C [53]. Amylose-lipid complexes are formed when the amylose component of the starch entraps lipid as a "guest" [2]. Three endotherms have been reported for potato amylose with peak temperatures below 80 °C, 80 – 104 °C and above 104 °C representing non-complexed lipids, Type I and Type II complexes respectively [54].

The increase in onset (T_o), peak (T_p) and conclusion (T_c) temperatures (Table 3) for transition endotherms (peak II and III) as a result of the addition of stearic acid to Hylon VII could be attributed to formation of amylose-lipid complexes with the stearic acid. This consequently reduced swelling as discussed earlier and hence increased transition temperatures. Increased T_o, T_p and T_c have been reported to reflect more ordered crystallinities [42].

Gamma irradiation decreased T_o, T_p, T_c and melting enthalpy with increasing irradiation doses (Table 3). This decrease could be attributed to structural changes resulting in decrease in crystallite stability. Defective or weak crystalline structure of the Hylon VII starch due to irradiation [34] may have accounted for the decrease observed in the thermal properties and consequently resulted in increases observed in solubility, water and oil absorption capacities.

Table 3: Effects of gamma irradiation, stearic acid alone and in combination on thermal transitions of Hylon VII starch

3.5. Wide Angle X-Ray Scattering (WAXS)

X-ray diffraction patterns of the irradiated Hylon VII starch with or without stearic acid are shown in Fig. 2. Native Hylon VII had diffraction peaks at 6.39, 16.55, 17.2, 22.83, 25.77 and 27.94 ° 2θ, which are attributed to B-type crystalline structure. It also showed some evidence of V-amylose crystal with a peak at 19.91 (≈ 20) 2θ. The presence of stearic acid however, resulted in additional peaks at approximately 7.60, 12.77, 25.00 and 28.00° 2θ (Fig. 2). These peaks were similar to that obtained for pure stearic acid (Fig. 2), hence could be attributed to stearic acid peaks. Irradiation did not change the type of X-ray diffraction pattern of the Hylon VII starch. Irradiated Hylon VII-stearic acid mixture showed similar X-ray diffraction pattern as the Hylon VII-mixture. Relative crystallinity of the native Hylon VII was 19.5 %. The presence of stearic acid contributed to increased levels of relative crystallinity of the Hylon VII samples (Fig. 2). Irradiation generally decreased the relative crystallinity of the native Hylon VII starch with or without stearic acid.

Similar X-ray diffractive peaks were reported for high amylose maize starch by Cheetham and Tao [29], Zhang et al. [15] and Chang et al. [42]. Other researchers reported no change in X-ray diffraction pattern of normal maize [23, 27], octenyl succinylated high amylose rice [37] and potato and beans [23, 38] starches when exposed to irradiation. Decrease in relative crystallinity due irradiation has been reported for maize [27], octenyl succinylated high amylose rice[37] and potato [47, 55] starches.

The native Hylon VII showed characteristic X-ray diffractogram (i.e B and V crystal-types) for high amylose maize starches. The V-type polymorph was assigned to closely packed single helices complexed with different polar and non-polar compounds, such as fatty acids [42]. According to Gernat et al. [56], high amylose starches in addition to B-type may also have Vh-type, which is a crystalline structure of amylose-fatty acid complexes formed by single helices with six anhydroglucose monomer residues per helical turn.

The addition of stearic acid to Hylon VII contributed additional peaks to the X-ray diffraction pattern of Hylon VII. This can be explain by the fact that stearic acid is crystalline in nature. Gamma irradiation did not affect the X-ray diffraction pattern of Hylon VII. The similarity in the XRD pattern of the Hylon VII starches (with or without irradiation) (Fig. 2) indicates that irradiation affected mostly the amorphous structure rather than the crystalline structure [34]. The reduction of relative crystallinity due to irradiation could be attributed to starch depolymerization and weakening of the amylopectin crystallites [27, 37, 47]. This suggests that gamma irradiation depolymerise amylopectin in the amorphous region of Hylon VII starch granules [43].

Figure 2: Effects of gamma irradiation, stearic acid alone and in combination on X-Ray crystallinity of Hylon VII starch

3.6. Molecular weight distribution (MWD)

The effects of irradiation, stearic acid alone and in combination on molecular weight distributions of Hylon VII starch obtained by size exclusion chromatography (SEC) are illustrated in Table 4 and Fig. 3. Three peaks namely shoulder peak **Ia**, peak **II**a and a shoulder peak **III** were observed for native Hylon VII starch (Fig. 3). Addition of stearic acid did not significantly ($P \geq 0.05$) change the molecular weight distribution pattern of Hylon VII (Table 4). However, the shoulder peak **Ia** was shifted to relatively low molecular weight component (shoulder peak **Ib**) when Hylon VII starch was irradiated at 30 and 60 kGy (Fig. 3). This component (**Ib**) lies between shoulder peak **Ia** and peak **IIa** of native Hylon VII. The proportion of shoulder peak **Ib** of irradiated Hylon VII starch samples was also higher than that for shoulder peak **Ia** of Native Hylon VII as seen in Fig. 3. Similarly, peak **IIa** of the native Hylon VII shifted to low molecular weight component (peak **IIb**) when Hylon VII starch was irradiated at 30 and 60 kGy (Fig. 3). High proportion of peak **IIb** was recorded compared to peak **IIa** for native Hylon VII. The shoulder peak **III** for the native Hylon VII starch was however incorporated into peaks **II b** upon gamma irradiation (Fig. 3). There were no significant ($P \geq 0.05$) differences between molecular weight distribution of gamma irradiated Hylon VII with and without stearic acid.

Native Hylon VII starch had weight-average molecular weight (M_w) values of ≈722 kDa, 49 kDa and 8 kDa for shoulder peak **Ia**, peak **IIa** and shoulder peak **III** respectively. Based on the

molecular weight, shoulder peak **Ia**, peak **IIa** and shoulder peak **III** could be suspected to be amylopectin, amylose and other low molecular weight starch products of Hylon VII respectively (Table 4). The shift in shoulder peak **Ia** and peak **IIa** of Hylon VII starch corresponded well with decreased molecular weight of shoulder peak **Ib** and peak **IIb** of the irradiated Hylon VII starches (Table 4). Average molecular weights of 1.40×10^7 (1400 kDa) and 5.28×10^4 (52.8 kDa) were reported for amylopectin and amylose respectively in high amylose corn starch [57]. Blaszczak et al. [58] reported 788 kDa for amylopectin and 404 kDa for amylose in Hylon VII. Decrease in molecular weight due to irradiation has been reported for other starches such as maize [8, 23], potato and beans [38] starches.

Molecular weights of amylopectin and amylose components of the native Hylon VII were lower than reported values (Table 4). This disparity could be attributed to the method as well as the solvent used in the dissolution of the starch. The shoulder peak **III** recorded for the native Hylon VII (Fig. 3 and Table 4) might have been produced due to sample preparation and shear degradation during SEC. Gidley et al. [59] stated in their report on characterizing the size and molecular weight distribution of starch that, there is a problem of degradation of amylopectin component through shear scission during SEC. The shift in peaks of the native Hylon VII due to gamma irradiation could be attributed to partial depolymerisation of amylopectin branched chain.

Figure 3: A representative graph showing the effects gamma irradiation on molecular weight distribution of Hylon VII starch obtained by size exclusion chromatography (**Ia** = Amylopectin peak of native Hylon VII, **IIa** = Amylose peak for native Hylon VII, **III** = low molecular weight peak for native Hylon VII, **Ib and II b** are peaks for irradiated Hylon VII)

Table 4: Effects of gamma irradiation, stearic acid alone and in combination on the molecular weight distribution of Hylon VII starch

3.7. Amylose/Amylopectin ratio

The effect of gamma irradiation on amylose and amylopectin ratio of Hylon VII is shown in Table 2. The amylose and amylopectin contents of native Hylon VII were 57.67 and 42.33 % respectively. The amylopectin component of Hylon VII decreased with increasing gamma irradiation doses, whereas the amylose component increased correspondently (Table 2). Apparent amylose content of 56 % has been reported for amylomaize starch type ZBB VII [60]. Shi et al [61] reported amylose content of 71% for Hylon VII using potentiometric iodine method. Sokhey & Chinnaswamy [8] reported decreased amylopectin fraction with increasing irradiation doses and corresponding increase in amylose fraction for maize starch samples with varied amylose contents. Similar findings were also reported for normal starches, such as maize [23], beans and potato [38] starches as well as maize and beans flours [62].

The amylose content of the native Hylon VII was lower (Table 2) than that reported by Shi et al [61]. This disparity could be attributed to the method used in the estimation of the amylose content as well as purity of the starch. The decrease observed in the amylopectin component of the Hylon VII with increasing irradiation was due to the partial depolymerisation of the amylopectin branched chain as discussed earlier. The partial depolymerisation of amylopectin component significantly contributed to the increased amylose content measured for the irradiated Hylon VII. The method used in estimating the amylose content could have also contributed to the high level of the measured amylose. Concanavalin A (Con A) specifically complexes branched polysaccharides, which is amylopectin (Megazymes International, 2011). However,

due to the partial depolymerisation of the amylopectin branched chain by gamma irradiation; the less branched amylopectin chains could have escaped the reaction with Con A and hence contributing to increased amylose content of the irradiated Hylon VII. This phenomenon therefore partially explained the molecular weight distribution of the irradiated Hylon VII (Fig. 3) as well as the increases recorded for solubility, water and oil absorption capacities.

3.8. Principal Component Analysis (PCA)

Principal component analysis (PCA) was used to understand the relationship between the treatments (irradiation and stearic acid) and the measured variables and to explain the relationship between the variables (Fig. 4). The first two principal components (PCs), PC1 and PC2 contributed about 87.10 % of the total variation. PC1 accounted for 72.77 %, whereas PC2 accounted for about 14.33 % (Fig. 4). PC 1 separated Hylon VII (0 % stearic acid + 0 kGy, 1.5 % stearic acid + 0 kGy and 5 % stearic acid + 0 kGy irradiation) (clusters **A** and **C**) with high molecular weight distribution (MWD pks 1 and 2), reduced swelling power (at 90 and 95 °C), high peak temperatures (DSC peaks II and III) and high relative crystallinity to the left of the loading from the right (0 % stearic acid + 30 kGy, 0 % stearic acid + 60 kGy, 1.5 % stearic acid + 30 kGy, 1.5 % stearic acid + 30 kGy, 5 % stearic acid + 30 kGy and 5 % stearic acid + 60 kGy irradiation) (clusters **B** and **D**). The Hylon VII starch samples on the right of the loading plot were associated with increasing solubility (cluster **B**), water and oil absorption capacities (cluster **D**) (Fig. 4**b**). Irradiation of Hylon VII in the presence of stearic acid contributed to high water and oil absorption capacities. Irradiation alone mostly contributed to high solubility (at 50, 70, 90 and 95 °C) values. Stearic acid concentration of 5 % contributed mainly to high crystallinity and high peak temperatures at transition endotherms II and III. High molecular weight

distributions (MWD pks 1 and 2) and reduced swelling power (at 90 and 95 °C) were mainly associated with the control Hylon VII starch and that incorporated with 1.5 % stearic acid. PC 2 separated clusters **A** and **B** which contain no or low stearic concentrations from clusters **C** and **D** which have Hylon VII starch with 5 % stearic acid. Clusters **C** and **D** with 5 % stearic contributed mostly to high relative crystallinity of Hylon VII starch- stearic acid mixture (Fig. 4**b**).

WAC, OAC and solubility (at 50, 70, 90 and 95 °C) were close to each other and were positively correlated (Fig. 4**a**). These results agreed with the increases observed in the aforementioned variable with increased in irradiation dose. Similarly, variables such as T_p for peaks II and III, molecular weights of peaks (1 and 2) and swelling power (at 90 and 95 °C) were close to each and were positively correlated. These parameters decreased with irradiation. Relative crystallinity positively correlated well with T_p for peak II endotherm (DSC) (Fig. 4**a**). Molecular weights also negatively correlated with WAC and OAC. Peak temperatures (T_p) of peaks II and III equally correlated negatively with solubility (at 50, 70 and 90 °C) (Fig. 4**a**).

Figure 4: Principal component analysis (PC1 and PC2) plots for loading of the measured variables (**a**) and score (**b**) of irradiated Hylon VII with and without stearic acid.

Hylon VII starch structure – functional relationship following gamma irradiation

The changes in the functional properties (solubility, water and oil absorption capacities as well as swelling power) due to gamma irradiation (30 and 60 kGy) were to be expected. These changes confirmed the hypothesis that gamma irradiation partially depolymerise/degrade starch molecules via scission of glycosidic bonds [8, 19, 20]. Gamma irradiation would have greater

effect on the longer branched amylopectin component of Hylon VII compared to short linear amylose component. Chung and Liu [23] suggested destruction of normal maize starch structure in the crystalline and amorphous regions due to reduction in proportion of longer amylopectin branch chains and average chain length.

The observed increases in solubility, water and oil absorption capacities as well as decrease in swelling power (at 90 and 95 °C) could partially be attributed to degradation or depolymerisation of Hylon VII starch amylopectin by gamma irradiation. The results from DSC seem to support the hypothesis that gamma irradiation causes degradation of amylopectin and amylose of starch. Significant decreases in the transition endotherms occurred with gamma irradiation of Hylon VII starch. Similar decreases in transition endotherms (especially peak gelatinization temperature) due to irradiation have been reported for normal maize starch [23, 34, 43]. A decrease in peak gelatinization temperature is said to be an indicative of defective or weak crystalline structure and decrease in starch crystallinity [34] since amylopectin is mainly responsible for starch crystallinity. Gamma irradiation depolymerises amylopectin component of starch to smaller fractions in other starches [8, 23, 38, 43, 44, 62]. The depolymerisation of amylopectin in turns decreases starch crystallinity, hence the observed decrease in peak gelatinization temperature. The significant correlations recorded (Fig.4a) between the functional properties suggest that they directly or indirectly measure starch gelatinization characteristics.

Even though gamma irradiation caused a significant effect on the functional properties of Hylon VII starch, the morphological properties such as granule size, shape and surface features studied

using light and scanning electron microscopy (LM and SEM) seemed not to be affected. This suggests that the degradation of effect of gamma irradiation on starch occurs mostly at the molecular level in the form of changes to starch molecules [43].

The results from the WAXS or XRD suggest that gamma irradiation did not change the X-ray diffractive pattern of Hylon VII starch. However, relative crystallinity of Hylon VII decreased with gamma irradiation. Starch crystallinity is mainly attributed to amylopectin component of starch. This suggests that changes to starch molecules due to gamma irradiation mostly occur in the amorphous region which is made up of branch amylopectin and amylose [34]. Similar results for XRD and relative crystallinity have been reported for other starches. The decrease in relative crystallinity due to partial depolymerisation of amylopectin by gamma irradiation may in part contributed to increases in solubility, water and oil absorption capacities as well as decrease in swelling power (at 90 and 95 °C).

Increases in solubility, water and oil absorption capacities correlated indirectly with molecular weight distribution of the Hylon VII as measured with size exclusion chromatography (SEC). The SEC data also confirm the hypothesis that gamma irradiation causes degradation of amylopectin and amylose of starch. Significant shift in both amylopectin and amylose components to higher retention time due to gamma irradiation was observed. Also, the molecular weights of these components decrease with gamma irradiation. Similar reductions in molecular weights due gamma irradiation have been reported for other starches [8, 23, 38]. The partial depolymerisation of the long branched amylopectin and possibly branches in the amylose chain

may have resulted in formation of lower molecular weight molecules, hence the observed changes in the functional properties as stated above.

The amylose/amylopectin ratio data also support the above submissions regarding the changes caused by gamma irradiation in the starch molecules. The decrease in amylopectin proportion with corresponding increase in amylose proportion as determined using Con A precipitation procedure shows that gamma irradiation degrade the amylopectin component of starch. However, the increase in the amylose component suggests that other lower molecular weight fractions of the Hylon VII starch have been measured as amylose. Similar decrease in amylopectin fraction with increasing irradiation doses and corresponding increase in amylose fraction have been reported other starches [8, 23, 38, 62]. The production of these lower molecular weight fractions as well as reduction in amylopectin could have resulted in the increases in solubility, water and oil absorption capacities as well as decrease in swelling power (at 90 and 95 °C).

4. Conclusions

Gamma irradiation, stearic acid addition and in combination did not cause any observable structural change in Hylon VII starch at the granular or microscopic level but cause changes at the molecular level. Gamma irradiation alone had more effect on the molecular structure of Hylon VII compared with stearic acid. These molecular changes in turn influence the functional properties of the Hylon VII. Increased amylose and decreased amylopectin, molecular weight and peak temperatures resulted in increased solubility, water and oil absorption capacities as well

as decreased swelling power of Hylon VII starch. Gamma irradiation and stearic acid have potential in Hylon VII modification for various industrial applications.

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Effects of gamma irradiation, stearic acid and in combination on molecular, structural and functional characteristics of high amylose maize starch

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Tables

Table 1: Effects of gamma irradiation, stearic acid alone and in combination on solubility and swelling power of Hylon VII starch

Dose (kGy)	Stearic acid conc. (%)	Solubility (%)				Swelling Power (g/g)			
		50 °C	70 °C	90 °C	95 °C	50 °C	70 °C	90 °C	95 °C
0	0	$0.16^{a} \pm 0.00$	$0.52^{ab} \pm 0.06$	$2.64^{ef} \pm 0.68$	$5.92^{h} \pm 0.00$	$3.06^{a} \pm 0.11$	$3.68^{efg} \pm 0.07$	$6.03^{rstuv} \pm 0.03$	$6.33^{wx} \pm 0.17$
	1.5	$0.08^{a} \pm 0.00$	$0.12^{a} \pm 0.06$	$1.32^{abcd} \pm 0.17$	$5.04^{gh} \pm 0.00$	$3.19^{ab} \pm 0.06$	$4.05^{ij} \pm 0.01$	$5.98^{rstu} \pm 0.03$	$6.17^{uvw} \pm 0.04$
	5	$0.08^{a} \pm 0.00$	$0.44^{ab} \pm 0.06$	$0.44^{ab} \pm 0.28$	$5.48^{gh} \pm 0.06$	$3.32^{bc} \pm 0.08$	$3.61^{efg} \pm 0.01$	$5.69^{opq} \pm 0.06$	$6.09^{stuv} \pm 0.22$
30	0	$1.00^{abc} \pm 0.06$	$3.16^{f} \pm 0.28$	$10.76^{jk} \pm 0.17$	$26.28^{o} \pm 1.87$	$3.33^{bcd} \pm 0.07$	$3.81^{gh} \pm 0.02$	$6.12^{tuvw} \pm 0.19$	$5.72^{opq} \pm 0.05$
	1.5	$0.28^{a} \pm 0.17$	$2.32^{def} \pm 0.23$	$9.52^{ij} \pm 0.68$	$25.48^{o} \pm 1.19$	$3.31^{bc} \pm 0.02$	$3.91^{hi} \pm 0.02$	$5.87^{qrs} \pm 0.03$	$5.29^{m} \pm 0.09$
	5	$0.48^{ab} \pm 0.00$	$2.00^{cdef} \pm 0.11$	$10.68^{jk} \pm 0.06$	$23.96^{n} \pm 0.28$	$3.55^{def} \pm 0.13$	$3.77^{fgh} \pm 0.04$	$5.59^{n} \pm 0.01$	$5.52^{n} \pm 0.18$
60	0	$1.64^{bcde} \pm 0.06$	$5.80^{gh} \pm 0.17$	$20.40^{m} \pm 1.36$	$35.40^{q} \pm 0.62$	$3.55^{def} \pm 0.16$	$4.23^{jk} \pm 0.08$	$5.16^{lm} \pm 0.02$	$5.01^{l} \pm 0.02$
	1.5	$1.04^{abcd} \pm 0.00$	$5.64^{gh} \pm 0.28$	$19.16^{lm} \pm 1.30$	$35.00^{q} \pm 0.28$	$3.53^{cde} \pm 0.08$	$4.28^{k} \pm 0.04$	$5.22^{lm} \pm 0.02$	$4.99^{l} \pm 0.02$
	5	$0.96^{abc} \pm 0.00$	$4.52^{g} \pm 0.06$	$18.72^{l} \pm 0.45$	$33.20^{p} \pm 0.91$	$3.65^{efg} \pm 0.10$	$4.15^{jk} \pm 0.05$	$5.37^{mn} \pm 0.05$	$5.04^{l} \pm 0.07$

Values are mean ± standard deviation of 3 experimental replicates. Values of different letters a-q in the cells are significantly different at $P \leq 0.05$

Table 2: Effects of gamma irradiation, stearic acid alone and in combination on water absorption capacity (WAC), oil absorption capacity (OAC), amylose and amylopectin contents of Hylon VII starch

Dose (kGy)	Stearic acid concentration (%)	WAC* (g/ml)	OAC* (g/ml)	Amylose (%)	Amylopectin (%)
0	0	$1.20^{a} \pm 0.00$	$1.00^{a} \pm 0.00$	$57.67^{a} \pm 1.65$	$42.33^{c} \pm 1.65$
	1.5	$1.21^{a} \pm 0.01$	$1.00^{a} \pm 0.00$	ND	ND
	5	$1.30^{b} \pm 0.00$	$1.20^{b} \pm 0.00$	ND	ND
30	0	$1.41^{c} \pm 0.01$	$1.20^{b} \pm 0.00$	$66.11^{b} \pm 1.37$	$33.89^{b} \pm 1.37$
	1.5	$1.41^{c} \pm 0.01$	$1.20^{b} \pm 0.00$	ND	ND
	5	$1.50^{d} \pm 0.00$	$1.27^{c} \pm 0.06$	ND	ND
60	0	$1.41^{c} \pm 0.01$	$1.20^{b} \pm 0.00$	$73.44^{c} \pm 1.33$	$26.56^{a} \pm 1.33$
	1.5	$1.47^{d} \pm 0.05$	$1.23^{bc} \pm 0.06$	ND	ND
	5	$1.50^{d} \pm 0.00$	$1.27^{c} \pm 0.06$	ND	ND

Values are mean ± standard deviation of 3 experimental replicates. Values of different letters a-d in column are significantly different at $P \leq 0.05$.

*WAC and OAC are water absorption capacity and oil absorption capacity, respectively.

ND = Not determined

Table 3: Effects of gamma irradiation, stearic acid alone and in combination on thermal transitions of Hylon VII starch

Dose (kGy)	Stearic acid conc (%)	Indices*	Peak I	Peak II	Peak III
0	0	T_o	ND	$84.31^{cde} \pm 2.11$	$138.99^{c} \pm 0.84$
		T_p	ND	$94.10^{cd} \pm 0.33$	$142.52^{c} \pm 0.28$
		T_c	ND	$103.96^{d} \pm 2.39$	$147.06^{d} \pm 0.91$
		ΔH (J/g)	ND	$3.52^{c} \pm 1.20$	$0.21^{ab} \pm 0.05$
	1.5	T_o	$65.30^{b} \pm 0.42$	$86.69^{e} \pm 0.21$	$139.40^{c} \pm 0.51$
		T_p	$67.92^{b} \pm 0.08$	$97.82^{e} \pm 2.74$	$142.96^{c} \pm 0.60$
		T_c	$70.29^{a} \pm 0.11$	$102.26^{d} \pm 0.33$	$147.78^{d} \pm 0.78$
		ΔH (J/g)	$1.08^{a} \pm 0.04$	$1.82^{ab} \pm 0.33$	$0.23^{ab} \pm 0.06$
	5	T_o	$65.85^{c} \pm 0.46$	$86.32^{e} \pm 1.31$	$125.87^{b} \pm 0.24$
		T_p	$68.78^{c} \pm 0.17$	$95.95^{de} \pm 0.93$	$131.81^{b} \pm 2.11$
		T_c	$72.14^{c} \pm 0.21$	$101.71^{d} \pm 1.69$	$137.19^{c} \pm 1.62$
		ΔH (J/g)	$7.47^{c} \pm 1.18$	$1.56^{ab} \pm 0.37$	$0.30^{b} \pm 0.01$
30	0	T_o	ND	$81.76^{bcd} \pm 2.34$	$126.44^{b} \pm 0.60$
		T_p	ND	$93.11^{bcd} \pm 0.69$	$131.45^{b} \pm 0.90$
		T_c	ND	$101.07^{cd} \pm 0.13$	$136.12^{c} \pm 2.33$
		ΔH (J/g)	ND	$2.33^{b} \pm 0.16$	$0.33^{b} \pm 0.12$
	1.5	T_o	$65.84^{c} \pm 0.08$	$80.76^{bc} \pm 0.18$	$127.58^{b} \pm 0.02$
		T_p	$68.75^{c} \pm 0.11$	$95.56^{de} \pm 0.11$	$130.95^{b} \pm 0.00$
		T_c	$72.21^{c} \pm 0.04$	$103.85^{d} \pm 0.99$	$135.02^{c} \pm 0.47$
		ΔH (J/g)	$0.77^{a} \pm 0.01$	$2.13^{b} \pm 0.35$	$0.14^{a} \pm 0.01$
	5	T_o	$66.00^{c} \pm 0.16$	$85.16^{de} \pm 3.28$	$137.57^{c} \pm 2.40$
		T_p	$68.79^{c} \pm 0.27$	$94.93^{cde} \pm 2.37$	$142.57^{c} \pm 1.15$
		T_c	$72.04^{c} \pm 0.03$	$101.48^{d} \pm 2.61$	$147.43^{d} \pm 0.95$
		ΔH (J/g)	$7.6^{c} \pm 0.28$	$1.79^{ab} \pm 0.02$	$0.22^{ab} \pm 0.05$
60	0	T_o	ND	$73.93^{a} \pm 0.32$	$118.45^{a} \pm 0.33$
		T_p	ND	$83.77^{a} \pm 0.00$	$122.73^{a} \pm 0.11$
		T_c	ND	$84.22^{a} \pm 0.00$	$128.59^{b} \pm 0.63$
		ΔH (J/g)	ND	$2.04^{b} \pm 0.03$	$0.13^{a} \pm 0.02$
	1.5	T_o	$64.18^{a} \pm 0.18$	$80.10^{b} \pm 0.41$	$118.05^{a} \pm 1.69$
		T_p	$66.95^{a} \pm 0.13$	$91.08^{b} \pm 0.09$	$122.27^{a} \pm 1.55$
		T_c	$69.99^{a} \pm 0.04$	$97.92^{b} \pm 0.24$	$125.60^{a} \pm 1.34$
		ΔH (J/g)	$2.86^{b} \pm 0.36$	$1.33^{ab} \pm 0.14$	$0.13^{a} \pm 0.02$
	5	T_o	$65.00^{b} \pm 0.08$	$82.97^{bcde} \pm 1.87$	$128.49^{b} \pm 1.84$
		T_p	$67.98^{b} \pm 0.25$	$92.45^{bc} \pm 0.71$	$134.43^{c} \pm 1.99$
		T_c	$71.17^{b} \pm 0.48$	$97.95^{bc} \pm 0.82$	$139.62^{c} \pm 1.57$
		ΔH (J/g)	$7.11^{c} \pm 0.04$	$0.95^{a} \pm 0.19$	$0.20^{ab} \pm 0.03$

Values are mean ± standard deviation of 3 experimental replicates. Values for T_o, T_p, T_c and ΔH in columns with different letters a-e are significantly different at $P \leq 0.05$.

*T_o, T_p, T_c and ΔH are onset temperature, peak temperature, conclusion temperature and enthalpy change respectively.

ND = No transition detected, Peak I= stearic acid endotherm, Peak II = endotherms for amylopectin crystallites and amylose-lipid complexes, Peak III = endotherm for amylose crystallites

Table 4: Effects of gamma irradiation, stearic acid alone and in combination on the molecular weight distribution of Hylon VII starch

Dose (kGy)	Stearic acid concentration (%)	Molecular weight, M_w (kDa)*				
		Ia	Ib	IIa	IIb	III
0	0	$721.67^{b} \pm 25.17$	ND	$49.21^{b} \pm 2.23$	ND	$8.25^{b} \pm 0.01$
	1.5	$714.03^{b} \pm 21.35$	ND	$48.37^{b} \pm 3.42$	ND	$7.25^{a} \pm 0.09$
	5	$656.12^{a} \pm 19.62$	ND	$37.59^{a} \pm 0.26$	ND	$7.15^{a} \pm 0.28$
30	0	ND	$106.39^{a} \pm 14.54$	ND	$13.31^{ab} \pm 0.90$	ND
	1.5	ND	$101.69^{a} \pm 1.52$	ND	$14.57^{b} \pm 1.71$	ND
	5	ND	$105.03^{a} \pm 5.23$	ND	$13.70^{b} \pm 0.55$	ND
60	0	ND	$97.22^{a} \pm 5.81$	ND	$10.98^{ab} \pm 1.28$	ND
	1.5	ND	$94.06^{a} \pm 3.19$	ND	$9.70^{a} \pm 0.28$	ND
	5	ND	$87.20^{a} \pm 6.52$	ND	$10.02^{a} \pm 0.85$	ND

Values are mean ± standard deviation of 3 experimental replicates. Values of different letters a-c in column are significantly different at $P \leq 0.05$

* Ia = Amylopectin peak (shoulder peak) of native Hylon VII, IIa = Amylose peak for native Hylon VII, III = low molecular weight peak (shoulder peak) for native Hylon VII, Ib and II b are peaks for irradiated Hylon VII)

ND = Not detected

Effects of gamma irradiation, stearic acid and in combination on functional, structural and molecular characteristics of high amylose maize starch

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Graphs

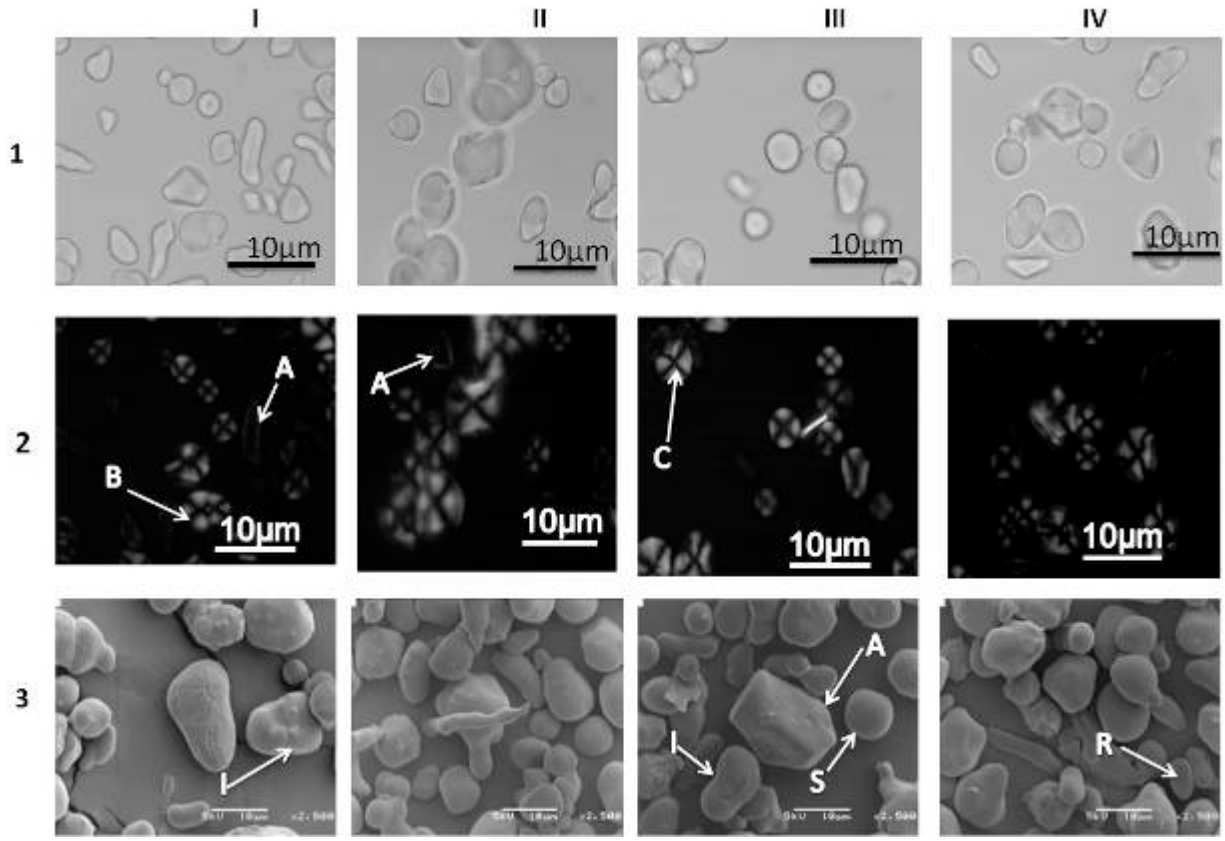

Figure 1: Selected micrographs of Hylon VII starch irradiated with and without stearic acid using Optical light **(1)**, Polarised light **(2)** and Scanning Electron **(3)** Microscopy. Bar = 10 µm.

I = 0 % stearic acid + 0 kGy irradiation; **II** = 0 % stearic acid + 60 kGy irradiation; **III** = 5 % stearic acid + 0 kGy irradiation and **IV** = 5 % stearic acid + 60 kGy irradiation. Magnifications: 100x for optical and polarized light microscopy; 2500x for scanning electron microscopy.

Arrows in polarized light micrographs **(2)** indicate weak or no birefringence along periphery (**A**), overlap maltese crosses (**B**) and single bright maltese cross (**C**) of starch granules.

Arrows scanning electron micrographs **(3)** indicate angular polyhedral (**A**), irregular (**I**), rod (**R**) and spherical (**S**) starch granules.

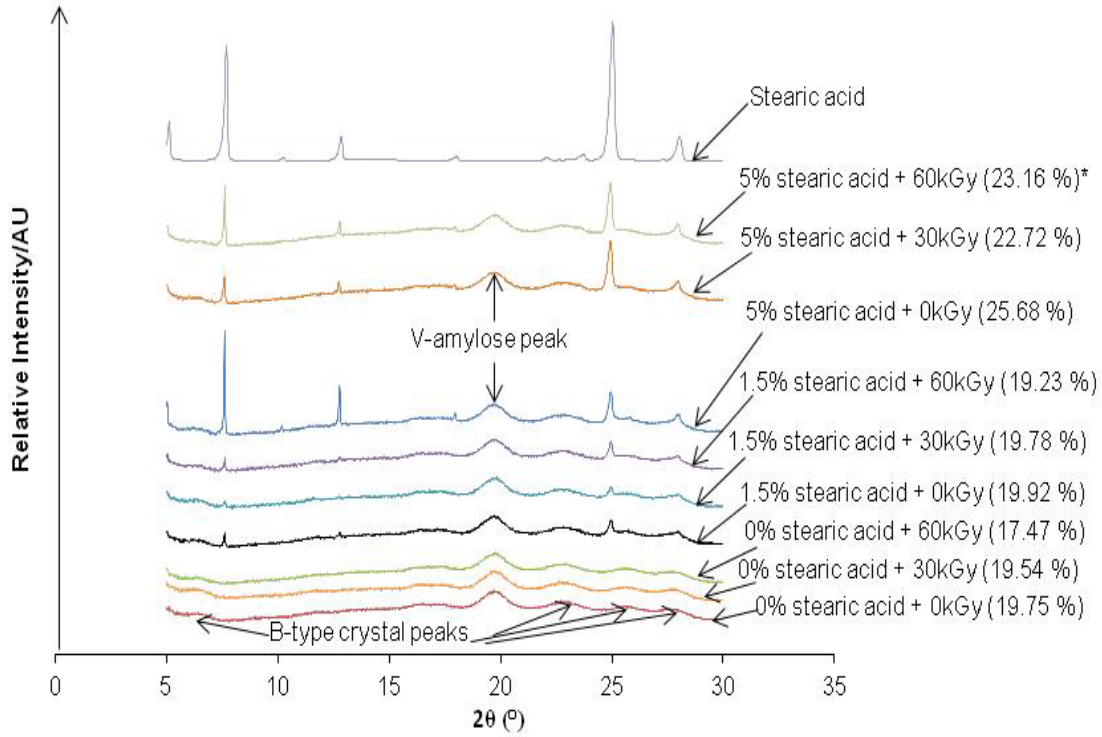

Figure 2: Effects of gamma irradiation, stearic acid alone and in combination on X-Ray crystallinity of Hylon VII starch

*Values in the parenthesis represent relative crystallinity (%)

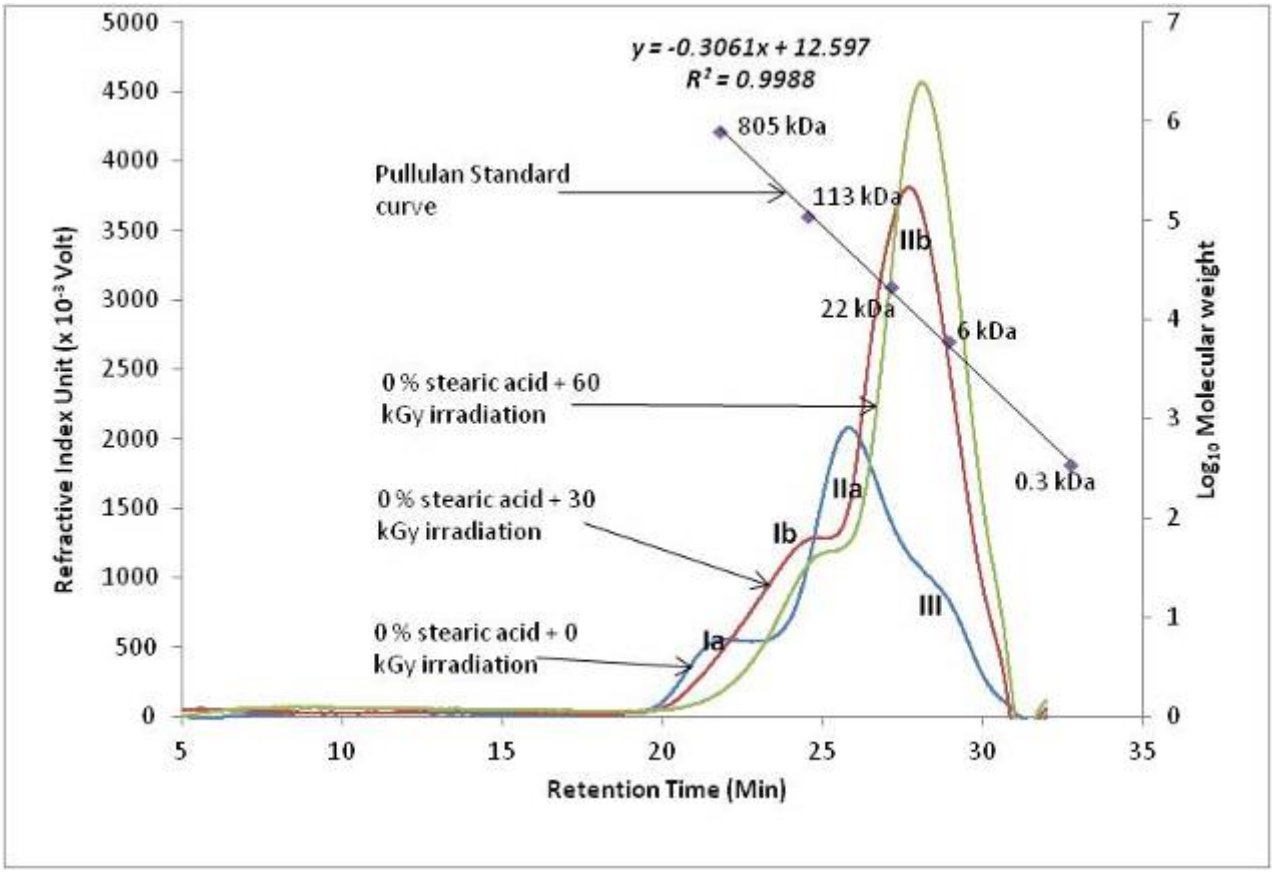

Figure 3: A representative graph showing the effects of gamma irradiation on molecular weight distribution of Hylon VII starch obtained by size exclusion chromatography (**Ia** = Amylopectin peak (shoulder peak) of native Hylon VII, **IIa** = Amylose peak for native Hylon VII, **III** = low molecular weight peak (shoulder peak) for native Hylon VII, **Ib** and **II b** are peaks for irradiated Hylon VII)

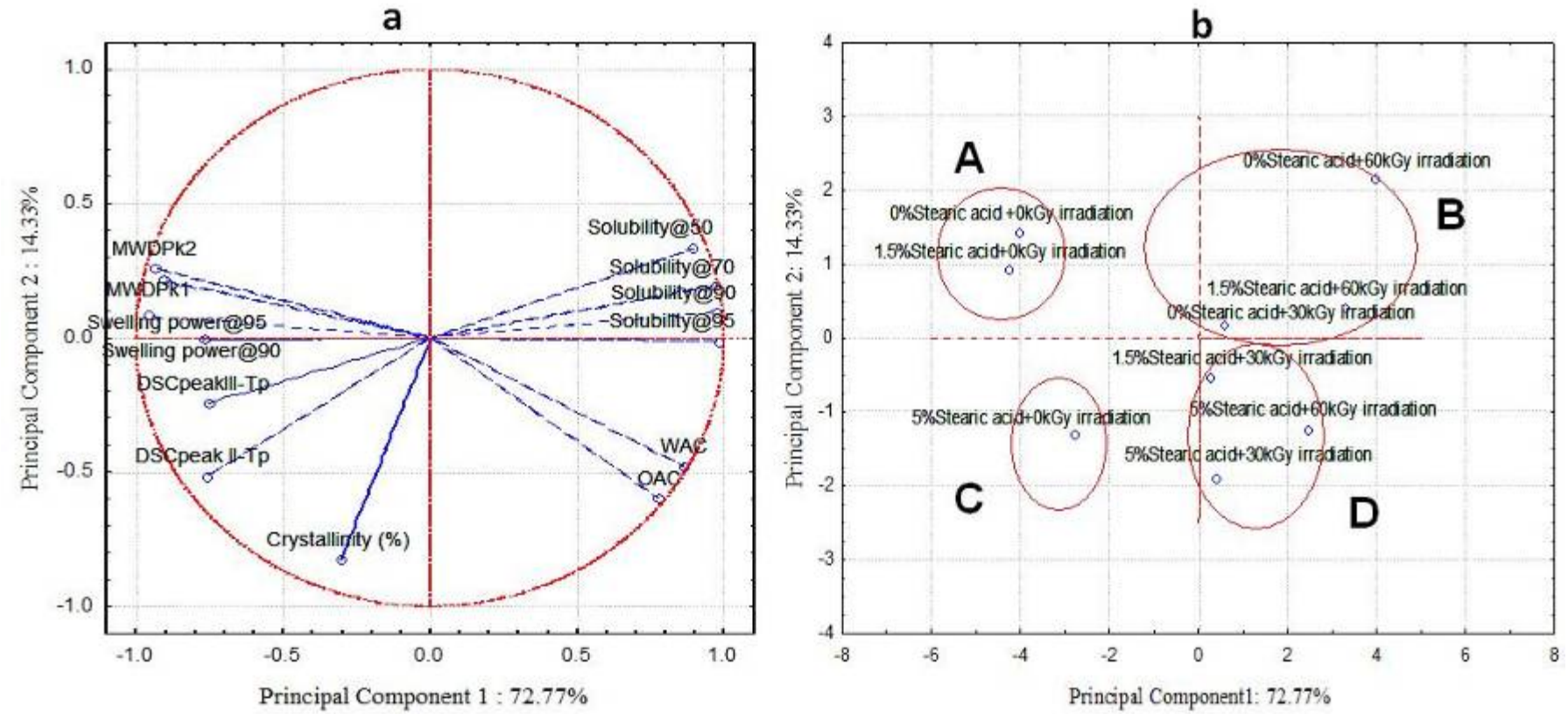

Figure 4: Principal component analysis (PC1 and PC2) plots for loading of the measured variables (**a**) and score (**b**) of irradiated Hylon VII with or without stearic acid.

DSC peak II- Tp = Peak temperature endotherm for Peak II; DSC peak III- Tp = Peak temperature endotherm for Peak III; MWDPk 1& 2 = molecular weight distribution for peaks 1 and 2 respectively; WAC = water absorption capacity; OAC = oil absorption capacity.