Gamma irradiation of corn starches with different amylose-to-amylopectin ratio

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Abstract Corn starches with different amylose-to-amylopectin ratio (waxy, normal, Hylon V, and Hylon VII) were treated with five doses of gamma irradiation (1, 5, 10, 25, and 50 kGy). The effects of gamma irradiation on the physicochemical properties of starch samples were investigated. Waxy samples showed an increase of amylose-like fractions when irradiated at 10 kGy. The reduction in apparent amylose content increased with amylose content when underwent irradiation at 25 and 50 kGy. Low amylose starches lost their pasting ability when irradiated at 25 and 50 kGy. Results from thermal behavior and pasting profile suggested that low level of cross-linking occurred in Hylon VII samples irradiated at 5 kGy. Severe reduction in pasting properties, gelatinization temperatures and relative crystallinity with increasing irradiation intensity revealed that waxy samples were affected more by gamma irradiation; this also indicated amylopectin was the starch fraction most affected by gamma irradiation. Alteration level was portrayed differently when different kind of physicochemical properties were investigated, in which the pasting properties and crystallinity of starches were more immensely influenced by gamma irradiation while thermal behavior was less affected. Despite the irradiation level, the morphology and crystal pattern of starch granules were found remain unchanged by irradiation.

Keywords Gamma irradiation · Thermal behavior · Crystallinity · Pasting properties · Morphology

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Introduction

As the major component in foods derived from plant sources, starch contributes widely to the textural properties of many foods and has many industrial applications as it can imparts and heightens structure, consistency, texture, and appeal to many food systems (Lee et al. 2006; Chung and Liu 2009). Nevertheless, due to the lack of wider functionalities such as low shear resistance, low thermal resistance, thermal decomposition, and high retrogradation tendency, starch is not ideal and optimal in some industrial food applications (Chung and Liu 2009; Yoon et al. 2010). For this reason, starches from different sources have been modified chemically, physically, or enzymatically to suit and obtain the desired functional traits at industrial levels.

Gamma irradiation of starch, being a physical process, has recently gained increasing interest among researchers as a safer, faster and more environmentally friendly starch modification method over conventional chemical modification (Bhat and Karim 2009). Starches have been modified extensively using gamma irradiation to induce physicochemical transformations in starch granules, for example in—wheat (MacArthur and D'Appolonia 1984), rice (Bao and Corke 2002), maize (Lee et al. 2006; Chung and Liu 2009; Liu et al. 2012), potato (Ciesla and Eliasson 2007), bean (Rayas-Duarte and Rupnow 1993), and cowpea (Abu et al. 2006). Desired functional and processing traits like reduction of viscosity, high water solubility, reduction of gelatinization enthalpy and others can be attained through gamma irradiation of starch (Yu and Wang 2007; Chung and Liu 2009; Bhat and Karim 2009; Liu et al. 2012). Kang et al. (1999) was able to produce modified corn starch with low viscosity and sufficient viscosity stability using gamma irradiation and addition of ammonium persulfate (APS). Our past research also showed that gamma irradiation could significantly increase the resistant starch content in corn starches depending on the irradiation dose

applied and the total amount of amylose in the starch (Lee et al. 2013).

Extensive studies on various starches reported different alterations by irradiation on the physicochemical properties. For example, the gelatinisation enthalpy was found to decrease for gamma irradiated rice, corn, and potato starch up to 40 kGy (Ciesla and Eliasson 2002; Lee et al. 2006), but no significant effect of gamma irradiation were recorded for grain amaranth starch irradiated up to 10 kGy by Kong et al. (2009) and even on corn starch irradiated up to 50 kGy by Chung and Liu (2009). Also, Chung and Liu (2009) discovered that the crystallinity of corn starch remained unchanged at 2 kGy and reduced at 10 kGy, but an increase of crystallinity in wheat (at 1 and 3 kGy) and rice starches (up to 9 kGy) was spotted in other study (MacArthur and D'Appolonia 1984).

It has also been found that the amylose-to-amylopectin ratio affects the sensitivity of starch toward gamma irradiation (Bhat and Karim 2009; Chung and Liu 2009). Various studies have been carried out on single type of starch with different amylose content to investigate the aforementioned relationship. For instance, Wu et al. (2002) and Zuleta et al. (2006) studied the effects of gamma irradiation (3 kGy) on physicochemical properties of various types of rice cultivars (with amylose content up to 26 %). They discovered that amylopectin was the starch fraction most affected by gamma irradiation in rice cultivars and the apparent amylose content (AAC) of high amylose rice starches was not significantly affected by gamma irradiation. The rheological and physical properties of two cultivars of gamma irradiated grain amaranth starch with different amylose content (6.0 and 10.3 %) irradiated up to 10 kGy have also been reported by Kong et al. (2009). However, all the studies mentioned above only covered narrower range of amylose content (less than 30 %) and with irradiation dosage up to 10 kGy only.

A lot of studies have been carried out on gamma irradiation of corn or maize starches (Lee et al. 2006; Chung and Liu 2009; Liu et al. 2012), yet there is still controversy in the reported physicochemical properties. For instance, corn starch granules structure was found visually unchanged when irradiated at 40 kGy (Lee et al. 2006) and even up to 500 kGy (Liu et al. 2012); on the other hand, Kamal et al. (2007) discovered that the shape of corn starch granule was deformed by both gamma and electron beams at doses between 5 and 100 kGy. However, all of these studies only examined the effects of various irradiation doses on normal corn starch, without revealing the influence of amylose and amylopectin content towards irradiation. This study was therefore designed to provide unprecedented insight into the relationship between amylose-to-amylopectin ratio of corn starch with the dosage of gamma irradiation. With the acknowledgement of FAO/IAEA/WHO Study Group (1999) that food irradiated at any dosage is confirmed to be safe and wholesome, it is therefore important to understand the effect of gamma irradiation on

starches at higher irradiation intensity. Since amylose and amylopectin also characterize the physicochemical properties of starches, thus it is crucial to find out the interaction between amylose and amylopectin in gamma irradiated starches when expose to higher irradiation dosages.

In addition, studies have shown that other than amylose-to-amylopectin ratio, factors like botanical origin of starches and irradiation conditions would critically influence the effect of gamma irradiation on starches. In their review on radiation processing on starches, Bhat and Karim (2009) pointed out that future research on gamma irradiation of starch should focus on setting standardized doses for each of the starch molecules, based on their origin. In relation to that, this research served as a fundamental and important study to provide better insight into starch modification by gamma irradiation as well as to fill in the knowledge gap on physicochemical changes of starches with different amylose content as induced by gamma irradiation. Thus this study reported is part of the attempt to elucidate how gamma irradiation would affect the physicochemical and morphological properties of corn starches with different amylose-to-amylopectin ratio.

Materials and methods

Materials

Corn starches (Waxy native starch, unmodified normal corn starch, Hylon V, and Hylon VII) with different amylose-to-amylopectin ratios were used in the experimental work as model materials. All the starches are commercially available and the waxy native starch, Hylon V and Hylon VII were purchased from N-Starch Sdn. Bhd. while the unmodified normal corn starch was purchased from Sigma-Aldrich (S4126).

The starches were packed in a polyethylene bag (300 g for each kind of starches) and irradiated using a ^{60}Co gamma source at the Nuclear Research Institution of Malaysia. The starch samples were subjected to five doses of irradiation (1, 5, 10, 25 and 50 kGy) with the dose rate of 1 kGy/h at room temperature.

Apparent amylose content and starch-iodine complex absorption spectrum

Apparent amylose content was estimated by a colorimetric method as described by Knutson (1985). The absorbance of the sample and each of the standard mixtures were measured at 600 nm against a reagent blank as the reference. A standard curve for mixtures of pure potato amylose and amylopectin was plotted and the regression equation for the standard curve was determined and the equation obtained was used to

calculate the total amylose content of the sample ($y = 0.0016x + 0.1233$; $R^2 = 0.9787$).

Thermal analysis

Thermal analysis was performed using PYRISTM Diamond Differential Scanning Colorimeter (Perkin-Elmer, Norwalk, U.S.A) which comes with a thermal analysis data station. Indium was selected as the calibration standard ($T_o = 156.6 \pm 2.0$ °C, $\Delta H = 28.45 \pm 2.0$ J/g). The reference cell contained a sealed, empty, aluminium pan. 3 mg (dry basis) of starch sample was weighed into an aluminium pan, into which distilled water of ratio 1:3 was added. The sample pans were all hermetically sealed and kept at room temperature for 24 h to equilibrate. The samples were scanned at temperature between 30 and 110 °C at a rate of 10 °C/min. Thermodynamic parameters of gelatinization i.e. onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy (ΔH) were generated from DSC endotherms. The qualitative index of crystalline structure was measured by gelatinization temperatures (T_o , T_p and T_c), whereas, ΔH quantifies the crystalline order quantitatively.

Pasting profile

Pasting profile was studied using the Rapid Visco Analyzer, RVA-4 (Newport Scientific, Australia). For waxy and normal starch samples, the starch slurries (10 % dry basis, 30 g total weight) were held at 50 °C for 1 min, heated to 95 °C at 6 °C/min, held at 95 °C for 5 min, cooled to 50 °C at 6 °C/min, and held at 50 °C for 2 min (Chung and Liu 2009). For high amylose starches (Hylon V and VII), a 4-g sample and 25 g distilled water were placed in an aluminum sample canister. The RVA pasting curve was obtained by using a 38-min test profile: initial equilibrium at 30 °C for 6 min, heating to 95 °C over 5 min, holding at 95 °C for 20 min, cooling to 40 °C over 5 min and holding at 40 °C for 2 min. A long profile was created to be able to observe for differences in pasting properties of high-amylose corn starches (Ozturk et al. 2009). The pasting parameters such as peak time, pasting temperature, peak viscosity, breakdown, setback and final viscosity were determined (Thermocline for Windows, Newport Scientific, Australia).

X-ray diffraction

X-ray diffraction patterns of gamma-irradiated corn starches were obtained with a PANalytical X'Pert-Pro MPD X-ray diffractometer (PANalytical B.V., Almelo, the Netherlands). The X-ray beam was adjusted to 40 kV and 30 mA, and the scanning rate was 2.0°/min over the diffracted angle of 4 to 40°. The relative crystallinity was calculated according to the

method of Nara and Komiya (1983) using Origin 9.0 Pro software (Microcal Inc., Northampton, Mass., U.S.A.).

Scanning electron microscopy

SEM analysis was carried out using ZEISS EVO MA-10 scanning electron microscope. Prior to examination, the sample was placed onto an aluminum slide using electrically conductive tape, and sputter coated with gold at 10 mbar for 90 s. Representative micrographs were taken for samples at 3000× magnification.

Statistical analysis

All data were analysed by one way ANOVA and the means were separated by Tukey's test (SPSS, Version 16.0). All analyses were performed at least in triplicate and the experimental results were expressed as mean ± standard deviation.

Results and discussion

Apparent amylose content and starch-iodine complex absorption spectrum

The potential degradation of amylose by gamma irradiation in this study was determined using colorimetric methods (based on the amylose-iodine complex formation). The principle of the test lies in the blue colour developed by the addition of the iodine reagent to a solution containing the amylose under standardized conditions. The ability of amylose to form this complex is due to its particular conformation, the polymer has a 3-D helicoidal structure. When helicoidal chains pack two by two to form a double helix, a central hydrophobic cavity is formed. This is where molecules like iodine can fix in and form a complex (Knutson 1999).

The apparent amylose content (AAC) of four types of corn starches was found to be approximately equal to the manufacturer specifications (Table 1). At irradiation dose up to 10 kGy, only gradual decrease is recorded and no significant difference ($P > 0.05$) was observed in term of AAC for all samples except for waxy starch. Instead of decrease, it is very interesting to find that the AAC for waxy samples increased at 10 kGy. The increase of AAC at 10 kGy for waxy starch may suggest that cleavage of amylopectin chains would have taken place to form new short and amylose-like straight-chain macromolecules. The high susceptibility of waxy samples towards gamma irradiation may be explained by the degradation of starch polymers which resulted in the progressive decrease of amylopectin branch chain length (Ciesla and Eliasson 2007). According to Ciesla and Eliasson (2007), this would normally be accompanied by an essential increase in the amylose

Table 1 Apparent amylose content of gamma irradiated corn starches

Sample	Radiation dose (kGy)	Apparent amylose content (%)	Manufacturer specification (%)
Waxy	0	4.1±0.6 ^a	Approx. 5
	1	4.1±0.3 ^a	—
	5	4.0±0.4 ^a	—
	10	6.4±0.2 ^c	—
	25	5.5±0.3 ^b	—
	50	5.2±0.4 ^b	—
Normal	0	28.6±0.8 ^a	Approx. 27
	1	28.1±0.4 ^{ab}	—
	5	27.9±0.3 ^{ab}	—
	10	27.5±0.6 ^{ab}	—
	25	25.6±0.3 ^b	—
	50	22.2±0.7 ^c	—
Hylon V	0	56.5±0.5 ^a	Approx. 55
	1	56.5±0.4 ^a	—
	5	55.6±0.3 ^a	—
	10	55.8±0.6 ^a	—
	25	51.9±0.5 ^b	—
	50	49.7±0.8 ^b	—
Hylon VII	0	73.1±0.5 ^a	Approx. 70
	1	73.6±0.7 ^a	—
	5	71.3±0.8 ^{ab}	—
	10	69.9±0.3 ^{ab}	—
	25	67.6±0.2 ^b	—
	50	64.2±0.6 ^c	—

Values with different superscripts within the same sample are significantly different ($P<0.05$)

content and this may further support the results we obtained for waxy starch whereby there is a surge in amylose content when irradiated up to 10 kGy. Rayas-Duarte and Rupnow (1993) also highlighted that the AAC results for bean starch obtained by gel permeation chromatography increased with increasing radiation dose, since the amylopectin molecules were degraded during irradiation, causing a surge in low molecular weight fractions (amylose-like) which closely resemble the current study.

When the irradiation dose was increased to 25 kGy, the AAC of all samples decreased significantly ($P<0.05$); further drop in AAC was seen in Hylon VII and normal samples when irradiated at 50 kGy. Various works have been conducted and discovered that gamma irradiation may bring about degradation of starch macromolecules with increasing dose of irradiation through the free radicals generated (Bhat and Karim 2009). These free radicals are capable of inducing molecular changes and fragmentation of starch and subsequently cause a decrease in amylose content of gamma irradiated starches (Rayas-Duarte and Rupnow 1993; Lee et al. 2006). The application of ionizing radiation is reported to break the

glycoside bonds (at chain ending) in starch granules, which is later followed by the decomposition of macromolecules and the creation of macromolecules with shorter chains (Yu and Wang 2007). Chung and Liu (2009) reported similar results with normal corn starch; in which the AAC of the starch were not affected when irradiated up to 10 kGy but reduced significantly when treated with irradiation dose of 50 kGy.

The observations on the starch-iodine complex absorption spectrum analysis for all starch samples displayed similar trend of changes as in AAC (Fig. 1). According to Knutson (1999), the spectra obtained through this analysis can be used to make preliminary molecular weight estimations for starches. The absorbance peaks of starch samples irradiated with low doses (less than 25 kGy) are skewed towards higher wavelength and thus not symmetrical around the maximum (Fig. 1). However, as the gamma irradiation dose increased on all samples, the $\lambda_{\max}$ reduced and the slope after the $\lambda_{\max}$ ('d' slope) became more negative indicating that the reduction of the molecular size of the samples has taken place (Knutson 1999). These changes were attributed to shortening of polysaccharide chains, depending on the irradiation dose and the shift in starch-iodine complex towards shorter wavelengths with increasing irradiation dose also supports this view (Lee et al. 2006).

Thermal analysis

Generally, starch granules would swell and the crystalline organization in starch would decompose to form amorphous regions when starch is heated in the presence of water. Gelatinization is a semi-cooperative process occurs when the amorphous regions take up water and swell to a gel phase which later generates strain on the crystalline regions. This action stresses the crystallites so that they cooperatively melt at a lower temperature compared to those not associated with the gel phase (Wang et al. 1992). This molecular disordering is called gelatinization and is often observed as an endothermic phenomenon and quantified using differential scanning calorimeter (DSC).

The thermograms for four native corn starches (Hylon V, Hylon VII, normal and waxy) used in this study is outlined in Fig. 2. It can be observed that starches with high amylose content (Hylon V and Hylon VII) showed thermograms with lower peak and broader gelatinization temperature range ($T_C - T_O$) whereas the starches with higher amylopectin content (normal and waxy) demonstrated sharper peak and narrow gelatinization temperature range. These results were in agreement with those observed for normal corn starch as well as for high amylose and waxy corn starches (Schirmer et al. 2013). The structural relationship between amorphous regions and the crystallites in a starch granule is responsible for the shape and onset temperature (T_O) of the endotherms (Wang et al. 1992). The waxy starch, being primarily amylopectin,

Fig. 1 The absorption spectra of starch-iodine complex for all starches

possesses a different amorphous-crystalline structure than the normal starch granule does. Narrow gelatinization temperature range of waxy starch suggests that the melting of starch is highly cooperative and that more energy is needed for the initiation of gelatinization. This is due to the absence of the amorphous regions arising from the limited or no amylose

content of waxy starch itself (Wang et al. 1992). Wang et al. (1992) also mentioned that the presence of amylose may dilute the crystalline regions, causing the cooperative melting to be less feasible as the crystallites might be separated far apart. Furthermore, Chung and Liu (2009) reported that amylose alone does not exhibit a peak near the gelatinization

Fig. 2 DSC thermograms of native untreated corn starches (waxy, normal, Hylon V, and Hylon VII)

temperature in a heating thermogram (broad endotherm due to the heterogeneity of amorphous regions) and the peak area for starch gelatinization generally increases with increase in amylopectin content (Liu et al. 2006).

From the results obtained (Table 2), the higher the amylose content of the starch, the higher the gelatinization onset temperature (T_O), peak temperature (T_P) and conclusion temperature (T_C) with Hylon VII exhibited highest value among all untreated samples ($P<0.05$). The changes of melting parameters of semicrystalline synthetic polymers were shown to be related to the changes of the melting co-operative unit of a polymer and the size distribution of the crystalline lamellae (Bershtein and Egorov 1994). It is already known that an increase in amylose content in starches would lead to an increase in the amylopectin A-chain length as well as the crystalline lamellae (Jenkins and Donald 1995). These structural changes in starch structure induced by increasing amylose content would contribute to an increase in thermodynamic parameters. Yuryev et al. (1998) recorded similar observation on barley starches with different amylose content in which starches with

higher amylose content recorded higher gelatinization temperatures.

A significant decrease ($P<0.05$) in the gelatinization temperature (T_O , T_P and T_C) was observed for all samples that underwent 25 and 50 kGy of gamma irradiation. Generally, the overall endotherms for all starches shifted to lower temperatures as an effect of gamma irradiation and their range remained not much of difference in relative to the irradiation dose applied. The T_O for waxy starch treated with 50 kGy marked the greatest reduction among all samples ($P<0.05$). The decrease in gelatinization temperature of irradiated waxy samples indicates that severe irradiation conditions caused granule structure imperfection. The studies carried out by MacArthur and D'Appolonia (1984) on wheat starch showed that gamma irradiation caused the granule to weaken, which might be related to the decreasing of temperature of gelatinization. Chung and Liu (2009) on normal corn starch irradiated at 50 kGy also discovered that the gelatinization temperatures of the irradiated starch decreased significantly with increasing intensity of radiation. A significant increase in peak temperature (T_P) of Hylon VII sample irradiated at 5 kGy was

Table 2 Thermal properties of native and gamma irradiated corn starches

Sample	Radiation dose (kGy)	Gelatinization				
		$T_O(^{\circ}\text{C})$	$T_P(^{\circ}\text{C})$	$T_C(^{\circ}\text{C})$	$T_C - T_O(^{\circ}\text{C})$	$\Delta H(\text{J/g})$
Waxy	0	67.1±0.4 ^{de}	71.8±0.3 ^{de}	77.0±0.6 ^{cd}	9.9±0.2 ^a	3.3±0.1 ^b
	1	66.6±0.1 ^{cd}	71.3±0.2 ^{cd}	76.5±0.3 ^c	9.9±0.2 ^a	3.4±0.1 ^b
	5	67.7±0.4 ^c	72.5±0.5 ^c	78.1±0.7 ^d	10.4±0.3 ^a	3.4±0.1 ^b
	10	66.0±0.4 ^c	70.7±0.2 ^c	75.8±0.5 ^c	9.8±0.3 ^a	3.5±0.1 ^b
	25	63.9±0.4 ^b	69.0±0.4 ^b	74.2±0.7 ^b	10.3±0.5 ^a	3.0±0.1 ^a
	50	61.2±0.4 ^a	66.8±0.1 ^a	72.6±0.3 ^a	11.3±0.1 ^b	2.7±0.1 ^a
Normal	0	66.5±0.3 ^{ab}	72.1±0.2 ^c	76.5±0.3 ^{bc}	10.0±0.3 ^b	2.8±0.1 ^{ab}
	1	67.0±0.2 ^b	72.0±0.2 ^c	76.9±0.3 ^c	9.9±0.2 ^b	3.0±0.0 ^b
	5	66.9±0.6 ^b	72.2±0.4 ^c	76.9±0.7 ^c	9.9±0.6 ^b	2.7±0.1 ^a
	10	66.5±0.2 ^{ab}	71.5±0.3 ^{bc}	75.7±0.4 ^{abc}	9.2±0.2 ^{ab}	3.0±0.1 ^b
	25	65.8±0.3 ^a	71.0±0.2 ^{ab}	75.4±0.5 ^{ab}	9.7±0.3 ^{ab}	2.9±0.1 ^{ab}
	50	65.8±0.6 ^a	70.3±0.5 ^a	74.6±0.7 ^a	8.8±0.6 ^a	2.7±0.1 ^a
Hylon V	0	71.9±0.2 ^{cd}	79.3±0.5 ^{bc}	93.4±1.1 ^a	21.5±1.0 ^a	3.2±0.2 ^c
	1	72.8±1.3 ^c	80.2±2.2 ^c	93.4±1.6 ^a	20.6±0.9 ^a	2.5±0.1 ^b
	5	72.3±0.3 ^{cd}	80.4±0.7 ^c	100.0±0.9 ^b	27.6±0.7 ^b	3.6±0.2 ^c
	10	71.4±0.5 ^c	78.3±0.4 ^{bc}	93.3±1.1 ^a	21.9±0.9 ^a	1.8±0.2 ^a
	25	69.8±0.5 ^b	77.5±1.3 ^{ab}	90.4±1.5 ^a	20.6±1.8 ^a	1.7±0.1 ^a
	50	67.6±0.6 ^a	75.4±1.1 ^a	88.9±1.6 ^c	21.3±5.9 ^a	2.4±0.0 ^b
Hylon VII	0	72.2±0.4 ^c	84.3±0.5 ^{ab}	106.0±0.4 ^{bc}	33.7±0.6 ^{ab}	3.4±0.1 ^{abc}
	1	72.2±0.3 ^c	84.2±0.1 ^{ab}	104.4±1.5 ^b	32.2±1.7 ^a	3.3±0.4 ^{abc}
	5	71.3±0.0 ^{bc}	91.2±1.5 ^c	104.9±1.2 ^{bc}	33.6±1.2 ^{ab}	4.0±1.1 ^c
	10	70.6±0.5 ^{bc}	88.3±4.4 ^{bc}	107.6±1.7 ^c	37.0±1.7 ^b	3.8±0.2 ^{bc}
	25	69.6±0.6 ^{ab}	80.8±0.9 ^a	104.6±0.2 ^{bc}	35.0±0.8 ^{ab}	2.5±0.1 ^{ab}
	50	67.7±1.7 ^a	82.0±1.8 ^a	101.1±0.5 ^a	33.4±2.0 ^{ab}	2.1±0.2 ^a

Values within each column and within same sample with different superscripts are significantly different ($P<0.05$)

observed and will be further discussed in pasting properties. It could be presumed from Table 2 also that gamma irradiation has only marginal effect on enthalpy changes, since ΔH of all samples exhibited certain level of fluctuation in which the same phenomenon was also being reported by Kong et al. (2009).

Pasting profile

A characteristic pasting curve is generated reflecting the viscosity changes observed when starches are heated and cooled in excess water. Generally, pasting is talking about the changes in viscosity right before, during and after the gelatinisation of starches. Determining this viscosity profiling of starches provides us with helpful information regarding starch behaviour under various industrial application conditions and for comparing relative differences between starches (Liu 2005). During the initial stage of heating, no viscosity change is observed as hydration occurs at the molecular level within granule. This is known as the gelatinization stage which, in the absence of a detectable viscosity change, is better gauged under microscopy observations. However, once the hydration has reached the critical pasting stage, a rapid onset in the development of viscosity is seen and the temperature at this point is known as the pasting temperature. At this point, the structural changes in the granules are irreversible, and the swollen granules are enriched in amylopectin while polymers with lower molecular weight, amylose molecules in particular, start to leach from the granules and make up the continuous phase (Liu 2005).

Figure 3 outlines representative RVA pasting profiles of the native and gamma irradiated corn starches and the pasting profiles were significantly altered by gamma irradiation. For native starches, significant difference in the pasting profiles between low and high amylose samples was observed. Low amylose samples (waxy and normal) displayed a sharper, more uniform pasting peak. They reached peak viscosity in shorter pasting time and have a lower pasting time and temperature as compared to their high amylose counterparts. The characteristic difference between low and high amylose native samples can be attributed to their amylose content. With little amylose and being primarily amylopectin (>90 % for waxy and >70 % for normal), low amylose starches could swell more freely and develop large peak viscosity at a low pasting time and temperature (Song and Jane 2000). Amylopectin is primarily accountable for granule swelling and amylose is found to suppress swelling and maintain the integrity of swollen granules (Hermansson and Svegmark 1996).

Gamma irradiated samples indicated increased granule destruction with increasing dosage of irradiation (Fig. 3). Peak, final, and setback viscosities for all samples except Hylon VII were all significantly decreased with the increase in radiation dose (data not shown). It was likely that the degradation of

amylopectin and amylose in starch by gamma irradiation could cause decreased swelling ability of starch granules, which would lead to a decrease in peak viscosity. Peak viscosity is an indication of water holding capacity. Gamma irradiated waxy (25 kGy and above) and normal (50 kGy) did not even show viscosity after about 7 min (Fig. 3). Similar results have been reported by Yu and Wang (2007) for rice starch and Chung and Liu (2009) as well as Liu et al. (2012) for corn starch. The decrease in peak viscosity at high irradiation dosage suggested that the degradation of amylose and amylopectin in starch by gamma irradiation could cause decreased swelling ability of starch granules when being heated. This would eventually cause a substantial reduction in water binding ability which would lead to a significant decrease in peak viscosity (Abu et al. 2006). Low or negligible peak viscosities of gamma irradiated starches at 50 kGy also reflect the fragility of the swollen starch granules, which subject to breakdown during the continuous mechanical stirring (Lee et al. 2006). High-dose gamma irradiation may induce severe cleavage of glycosidic linkages, causing a reduction in water holding capacity, and allow granules to readily rupture under shear, resulting in very low or no viscosity during holding at 95 °C.

Hylon VII showed increased peak and final viscosities when irradiated at 1 and 5 kGy. A significant reduction in breakdown is even observed for Hylon VII irradiated at 5 kGy. This suggests that a certain degree of cross-linking may have taken place. This finding is further supported by the observation in thermal behavior in which a significant increase in peak temperature (T_p) is noted for Hylon VII sample irradiated at 5 kGy. When low degree of cross-linking occurs, most of the starch granules swelled to a larger size and partially disintegrated, resulting in increase of peak and final viscosities. Due to the proper integrity of the cross-linked starch granules, more water can be absorbed, contributing to the development of swollen starch granules that are larger in size (Wongsagonsup et al. 2014). It has been reported that cross-linking depressed the disintegration of starch granules, which would cause high peak viscosities and setback (Kurakake et al. 2009); and the persistence of the swollen granules after cooling resulted in higher final viscosity (Liu et al. 1999).

X-ray diffraction

The X-ray diffractograms of native corn starch samples with different amylose-to-amylopectin ratios are presented in Fig. 4. The corresponding crystallinity level calculated from the ratio of diffraction peak area and total diffraction area and the crystal pattern type are given in Table 3. The scattering angle, at which the diffraction intensities can be observed, was 2θ , and the spectra were compared to those presented by Gernat et al. (1993) and found to be equally or better resolved.

Fig. 3 Pasting profiles of starches irradiated using different doses

Fig. 4 X-ray diffraction spectra for corn starches with different amylose-to-amylopectin ratio

Table 3 Degree of crystallinity and crystal pattern of gamma irradiated corn starches with different amylose-to-amylopectin ratio

Samples	Relative crystallinity (%)	Crystal pattern
Waxy		
0	34.5±0.1 ^a	A
1	33.4±0.1 ^b	A
5	31.2±0.2 ^c	A
10	30.9±0.3 ^c	A
25	28.6±0.2 ^d	A
50	28.3±0.2 ^d	A
Normal		
0	33.6±0.1 ^a	A
1	31.7±0.3 ^b	A
5	31.8±0.1 ^b	A
10	30.9±0.2 ^b	A
25	27.7±0.3 ^c	A
50	26.3±0.1 ^d	A
Hylon V		
0	27.9±0.3 ^a	B
1	27.3±0.3 ^a	B
5	26.3±0.2 ^b	B
10	25.3±0.1 ^c	B
25	25.0±0.2 ^c	B
50	24.0±0.4 ^d	B
Hylon VII		
0	25.5±0.2 ^a	B
1	24.9±0.1 ^a	B
5	22.5±0.2 ^b	B
10	22.8±0.3 ^b	B
25	22.2±0.2 ^b	B
50	22.9±0.3 ^b	B

Values with different superscripts within the same sample are significantly different ($P < 0.05$)

The X-ray diffraction patterns were compared with reported standard diffraction patterns of different crystalline types (Zobel 1964). X-ray diffraction patterns of waxy, normal maize and high amylose maize starch have been previously reported (Zobel 1964; Gernat et al. 1993), in which waxy corn starch and normal corn starch showed a typical A-type pattern, with strong reflections at 2θ about 15° and 23° , and an unresolved doublet at 17° , 18° 2θ . High amylose starches, Hylon V and Hylon VII gave the strongest diffraction peak at around 17° 2θ and a few small peaks at around 2θ values of 23° , 22° and 20° . An additional peak was found to appear at about 5° 2θ for high amylose samples (Zobel 1964; Gernat et al. 1993). These latter spectra of high amylose starches are basically the same as that of potato starch, which is a characteristic of B type crystal.

Overall, with an increase in amylose content, the peak at 15° 2θ became progressively weaker and broader, while the peaks at 17° and 18° 2θ merged to a large peak, and line-widths were significantly attenuated. Similarly, the peak at 22° decreased in intensity and split into two peaks. With continuing increase in amylose content, the peak at $2\theta = 22^\circ$ undergone further enlargement of line-width and split down. These results clearly indicate that a transition of crystallinity types in maize starch granules from A to B crystal pattern.

It has been discovered that the granular structure of B-type starches were less susceptible to gamma irradiation than A-type starches and C-type starches showed intermediate susceptibility (Chen et al. 2006). In current study, the reported waxy and normal corn starches showed typical A-type patterns, while amylose-rich starches (Hylon V & VII) showed B-type patterns. Gamma irradiation had no significant effect on the crystal pattern of all the corn starches tested. This observation was in good agreement with previous study carried

Fig. 5 **a** Morphology of low amylose starch granules irradiated using different doses. **b** Morphology of high amylose starch granules irradiated using different doses

out by Chung and Liu (2009) with irradiation intensity up to 50 kGy on corn starch. Lee et al. (2006) also discovered that corn starch irradiated at 500 kGy also showed unaltered crystal pattern.

The degree of crystallinity of the samples was measured by the method of Nara and Komiya (1983). The samples showed decrease in the degree of crystallinity with increasing amylose content of the starch samples. Waxy starch with lowest amylose content has the highest crystallinity (34.5 %), while Hylon VII content has the lowest crystallinity (25.5 %). All samples showed significant decrease in the degree of crystallinity with increasing irradiation dosage ($p < 0.05$) with normal corn starch marked the greatest reduction of 7.3 %. The decrease of crystallinity for normal and waxy samples (low amylose content) was observed to occur even at low irradiation of 1 kGy. Significant decrease in the degree of crystallinity for high amylose corn starch samples (Hylon V & VII) were only occurred at higher irradiation dosage (beyond 5 kGy). Hylon VII samples showed remarkable resistance toward irradiation in which the degree of crystallinity remained unchanged at irradiation dosage of 5 kGy and beyond. Similar report has also been observed by Chung and Liu (2009).

Scanning electron microscopy

In nature, starch exists in the form of granules, which can differ in size and shape. The origin of starch granules can be inferred from their size, shape, and, the hilum position of the original growing point of granule. High amylose maize starch exhibits filamentous granules (budlike protrusions) while waxy starch granules are found to have oval indentations (Liu 2005). Figure 5a and b showed the granule morphology of the samples observed under the scanning electron microscope (SEM). In the SEM photograph, the amylopectin-rich starches (waxy and normal) contained more angular granules and they are more regular in shape as compared to the amylose-rich starches (Hylon V and VII). It can be noticed from the micrograph also that the surfaces of the amylose-rich starches were smoother than those of the amylopectin-rich starches. This observation is found to be similar with Chen et al. (2006) who studied the morphology of corn starches with different amylose content. On top of that, the micrographs obtained showed that both native and irradiated samples remained intact and visually unchanged up to 50 kGy, indicating the starch granules were apparently unaffected by irradiation. Similar finding has been reported by Lee et al. (2006).

Conclusions

This study documented the effect of gamma irradiation on corn starches with different amylose-to-amylopectin ratio. In

general, gamma-irradiation induced the degradation of amylose and amylopectin at the amorphous region as well as the crystalline region and consequently altered the physicochemical properties of the samples tested. It is noteworthy that gamma irradiation of waxy corn starch at 10 kGy yielded amylose-like molecules without significant alteration in physicochemical properties that may be beneficial for certain applications. Thermal endotherms obtained suggested that waxy samples showed increased vulnerability towards depolymerization with increasing irradiation intensity. In line with the degree of crystallinity in high amylopectin sample, the degradation of granule crystallinity with gamma irradiation was also found higher than starches with higher amylose content. On the other hand, in agreement with the result showed in thermal behaviour, lower level of irradiation doses (5 kGy) on high amylose corn starch (Hylon VII) was able to induce lower level of cross-linking that enhanced the peak and final viscosities.

This study also found that severe reduction in pasting properties, gelatinization temperatures and relative crystallinity happened on waxy samples with increasing irradiation intensity. This revealed that waxy samples were affected more by gamma irradiation, suggesting amylopectin was the starch fraction most affected by gamma irradiation. Generally, the degree of modification and changes caused by gamma irradiation were reflected differently in different kind of physicochemical properties investigated. Comparatively the pasting properties and crystallinity of starches were more drastically affected by gamma irradiation, while thermal behavior was not that much affected. In spite of the irradiation intensity, the morphology and crystal pattern of starch granules remained unchanged by irradiation.

Since starch functionality is greatly based on the molecular weight and size of amylose and amylopectin, future studies should focus on the characterization of the starch molecular-weight distribution profiles for gamma irradiated starches with different amylose content. Other than that, the results obtained could also be useful in providing more in-depth evidences to further support the intriguing findings of current study especially on the formation of amylose-like molecules and cross-linking effect of Hylon VII samples documented.

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