



Short communication

Formation of resistant corn starches induced by gamma-irradiation

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ARTICLE INFO

Article history:

Received 12 September 2012

Received in revised form 19 May 2013

Accepted 20 May 2013

Available online 27 May 2013

Keywords:

Apparent amylose content

Waxy corn starch

Normal corn starch

Hylon V

Hylon VII

Digestibility

ABSTRACT

The effect of gamma-irradiation on formation of resistant starch (RS) in corn starch with different amylose content was examined. Normal corn starch, waxy corn starch, and high amylose corn starch (Hylon V and Hylon VII) were irradiated at 5, 10, 25 and 50 kGy. Gamma-irradiation at 5 kGy increased the amylose-like molecules in starches and thus significantly enhanced the RS content ($p < 0.05$). Highest RS content was produced by 50 kGy irradiated in all the starch samples ($p < 0.05$). The irradiation-induced RS was more evident in waxy corn starch, followed by high amylose corn starch and normal corn starch.

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1. Introduction

Gamma-irradiation is a high-energy physical processing technology widely used for modifying polymers. Owing to its advantages over conventional methods – such as environmental friendly, economically viable, safe (Bhat & Karim, 2009), and fast processing rate, there is an increasing interest to study the effects of gamma-irradiation on starch of different botanical origin (Abu, Duodu, & Minnaar, 2006; Bao & Corke, 2002; Chung & Liu, 2009; Ciesla & Eliasson, 2007; Kong, Kasapis, Bao, & Corke, 2009; Lu, Donner, Yada, & Liu, 2012). Depending on the irradiation dosage and condition, the generation of free radicals during radiation treatment could possibly induce degradation and/or cross-linking of the starch chains (Bhat & Karim, 2009). These molecular changes bring about alteration in the structure, physicochemical properties as well as digestibility of starch.

Starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) according to the rate of glucose release and its absorption in the gastrointestinal tract (Englyst, Kingman, & Cummings, 1992). Available literature testified many health benefits of RS and its potential as functional ingredient in food products (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010;

Haralampu, 2000; Sajilata, Singhal, & Kulkarni, 2006). Gamma-irradiation has been known to modify the digestibility and RS content of starch. Chung and Liu (2009) indicated increase of RS in normal corn starch irradiated using 10 kGy and 50 kGy. Gamma irradiation was also found significantly increased the RS content in both normal and waxy corn starch at 5, 10 and 20 kGy (Yoon et al., 2010). It is also known that the amylose:amylopectin ratio affects the sensitivity of starch to gamma-irradiation (Chung & Liu, 2009; Kong et al., 2009). To the best of our knowledge, there is no report on the RS formation by gamma-irradiation on starch with a wider range of amylose–amylopectin ratio. Therefore, this study was designed to further elucidate the formation of RS in corn starch with a range of amylose:amylopectin ratios under 5, 10, 25 and 50 kGy irradiation.

2. Materials and methods

2.1. Materials and sample preparation

Waxy corn starch, Hylon V and Hylon VII were purchased from National Starch & Chemical (M) Sdn. Bhd., while the normal corn starch (S4126) was purchased from Sigma–Aldrich (M) Sdn. Bhd. The starches (300 g, approximately 11–14% moisture) were packed in a polyethylene bag and irradiated using a ⁶⁰Co gamma source (IR-174, MDS Nordin Inc., Ottawa, ON, Canada) at Malaysian Nuclear Agency using irradiation doses of 5, 10, 25 and 50 kGy (1 kGy/h) at room temperature.

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Table 1
Apparent amylose content (ACC) of corn starches irradiated at different doses.

Irradiation doses	Apparent amylose content (%)			
	Waxy	Normal	Hylon V	Hylon VII
0 (native)	2.2 ± 0.1 ^b	29.9 ± 0.9 ^b	51.8 ± 0.5 ^b	71.5 ± 1.1 ^b
5	3.0 ± 0.5 ^a	33.2 ± 0.5 ^a	60.6 ± 0.9 ^a	81.8 ± 0.7 ^a
10	1.2 ± 0.2 ^c	31.8 ± 0.5 ^a	39.1 ± 0.9 ^c	84.2 ± 0.4 ^a
25	0.9 ± 0.2 ^{cd}	23.2 ± 0.8 ^c	36.9 ± 0.3 ^d	59.3 ± 1.2 ^c
50	0.4 ± 0.1 ^d	23.1 ± 0.3 ^c	32.7 ± 0.7 ^e	51.1 ± 0.8 ^d

Mean values with different alphabet within the column differ significantly ($p < 0.05$).

2.2. Determination of apparent amylose content

The iodine spectrophotometric method by Hoover and Ratnayake (2001) was used to determine the apparent amylose content (AAC) of all samples. The samples were defatted using Soxhlet extraction prior to AAC determination. A calibration curve ($R^2 = 0.9878$) was derived using mixtures of pure potato amylose (Sigma, A0512) and amylopectin (Sigma, 10118).

2.3. Determination of resistant starch

Resistant starch (RS) content was measured using the Megazyme Resistant Starch Assay Kit (Megazyme Int. Ireland Ltd. Co., Wicklow, Ireland) according to the AACCI-approved method 32-40 (AACCI, 2000). Samples were incubated with α -amylase and amyloglucosidase (AMG) for 16 h at 37 °C to solubilize and hydrolyze non-RS to glucose. The RS is recovered by centrifugations and then dissolved in KOH and hydrolyzed to glucose with amyloglucosidase. The absorbance of the glucose was then measured with glucose oxidase/peroxidase reagent at 510 nm. Resistant starch was calculated as glucose $\times$ 0.9.

2.4. Scanning electron microscopy

The microstructure of starch granules was observed using SEISS EVO MA-10 scanning electron microscope. Prior to examination, the sample was placed onto an aluminium slide using electrically conductive tape and sputter coated with gold at 10 mbar for 90 s. Representative micrographs were taken at 3000 $\times$ magnification.

2.5. Statistical analysis

All experiment was carried out in triplicate. The statistical analysis was conducted with one way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) ver. 19.0 along with Tukey Multiple Comparison Test. The predetermined acceptable level of probability was fixed at 5%.

3. Results and discussion

Table 1 shows that all samples treated with 5 kGy contained higher AAC than their native counterparts, apart from that, normal corn starch and Hylon VII also indicated higher ACC after irradiated at 10 kGy ($p < 0.05$). It is postulated that at low irradiation dose, degradation of amylopectin may take place to produce short and amylose-like straight-chain molecules (Rombo, Taylor, & Minnaar, 2004) that were reflected as increase of AAC in this study. In contrast, high irradiation doses (25 kGy and 50 kGy) reduced the ACC of the samples ($p < 0.05$). Similar observation was reported by Yu and Wang (2007) for irradiated rice starch. Higher ionizing energy caused both amylose and amylopectin underwent more extensive degradation via radical oxidation (Lu et al., 2012), and thus substantial decrease in the iodine binding ability.

Table 2
Resistant starch (RS) content of gamma-irradiated corn starches at different radiation doses.

Irradiation doses	Resistant starch content (%)			
	Waxy	Normal	Hylon V	Hylon VII
0 (native)	4.8 ± 0.1 ^e	9.4 ± 0.1 ^d	29.0 ± 1.5 ^c	30.7 ± 0.5 ^d
5	16.5 ± 0.7 ^c	17.0 ± 0.2 ^a	39.0 ± 0.5 ^b	39.4 ± 1.6 ^b
10	8.7 ± 0.1 ^d	9.9 ± 0.1 ^d	30.7 ± 1.0 ^c	35.8 ± 0.4 ^c
25	17.5 ± 0.3 ^b	14.0 ± 0.2 ^c	40.0 ± 1.1 ^b	40.9 ± 1.4 ^{ab}
50	20.9 ± 0.3 ^a	15.1 ± 0.3 ^b	43.9 ± 0.2 ^a	43.1 ± 0.1 ^a

Mean values with different alphabet within the column differ significantly ($p < 0.05$).

The resistant starch (RS) contents of treated and non-treated starches are presented in Table 2. The RS content in native samples (Type II RS) was in accordance with the AAC in which Hylon VII > Hylon V > normal corn > waxy corn. A study by Cheetham and Tao (1998) evident the increasing amylose content in maize starch granules resulted transition of crystalline type from A through C to B. X-ray diffraction analysis in this study also found that Hylon V and Hylon VII demonstrated B-type crystallinity, whilst normal corn and waxy corn demonstrated A-type (data not shown). The resistance to digestibility of raw starch granules was ascribed to the type of crystal pattern, whereby B-type crystallinity which is normally found in potato and high amylose maize starch was reported to be more resistant than other crystalline order (Leszczyński, 2004; Fuentes-Zaragoza et al., 2010).

Irradiation increased the RS content in all starch samples ($p < 0.05$) except normal corn starch treated with 10 kGy ($p > 0.05$). In brief, RS content was observed to increase with increasing irradiation doses. A few researches noticed enhancement of starch digestibility at 2–3 kGy irradiation doses in various starches (Chung & Liu, 2009; Rombo, Taylor, & Minnaar, 2001; Zuleta, Dyner, Sambucetti, & de Francisco, 2006). It was reported that starch digestibility was increased when the irradiation doses were relatively low but decreased as the dose increased (Rombo et al., 2001; Yoon et al., 2010; Zuleta et al., 2006). Increased RS content at higher irradiation doses was also observed in normal corn starch and waxy corn starch (Chung & Liu, 2009; Chung et al., 2010; Yoon et al., 2010). The enhancement of starch indigestibility by irradiation was attributed to three main reasons (Rombo et al., 2004; Chung & Liu, 2009; Chung et al., 2010; Yoon et al., 2010): first, the cleavage of glycosidic linkages that encourages transglucosidation to produce β -bonded branched structures; second, the realignment of depolymerized starch chains to packed starch matrices; and lastly, increase of carboxyl and carbonyl groups through oxidation. These structural changes caused hindrance to enable effective enzyme attack on starch.

It is noteworthy that 5 kGy of irradiation induced extensive amount of RS in every starch sample, and significant reduction was observed at 10 kGy ($p < 0.05$). The trend detected was in line with the change of AAC in the samples, and thus suggested the association of amylose-like molecules produced by irradiation was largely accountable for the enhancement of RS content at low irradiation doses. In contrast, the increment of RS content at high irradiation doses (25 kGy and 50 kGy) was mainly ascribed to the structural modification by other mechanisms mentioned earlier. SEM micrographs (Fig. 1) indicate that gamma irradiation did not affect much the morphology of the starch granules; only marginal pin holes were observed on the surface of few granules (arrow). The rearrangement of the molecular structure might have occurred at the internal of the granule, which was not seen at the surface area as found by Chung and Liu (2009) and Chung et al. (2010).

The highest amount of RS was obtained at 50 kGy ($p < 0.05$), whereby the enhancement of RS content was found to be 16.1%, 5.7%, 14.9% and 12.4% in waxy corn starch, normal corn starch,

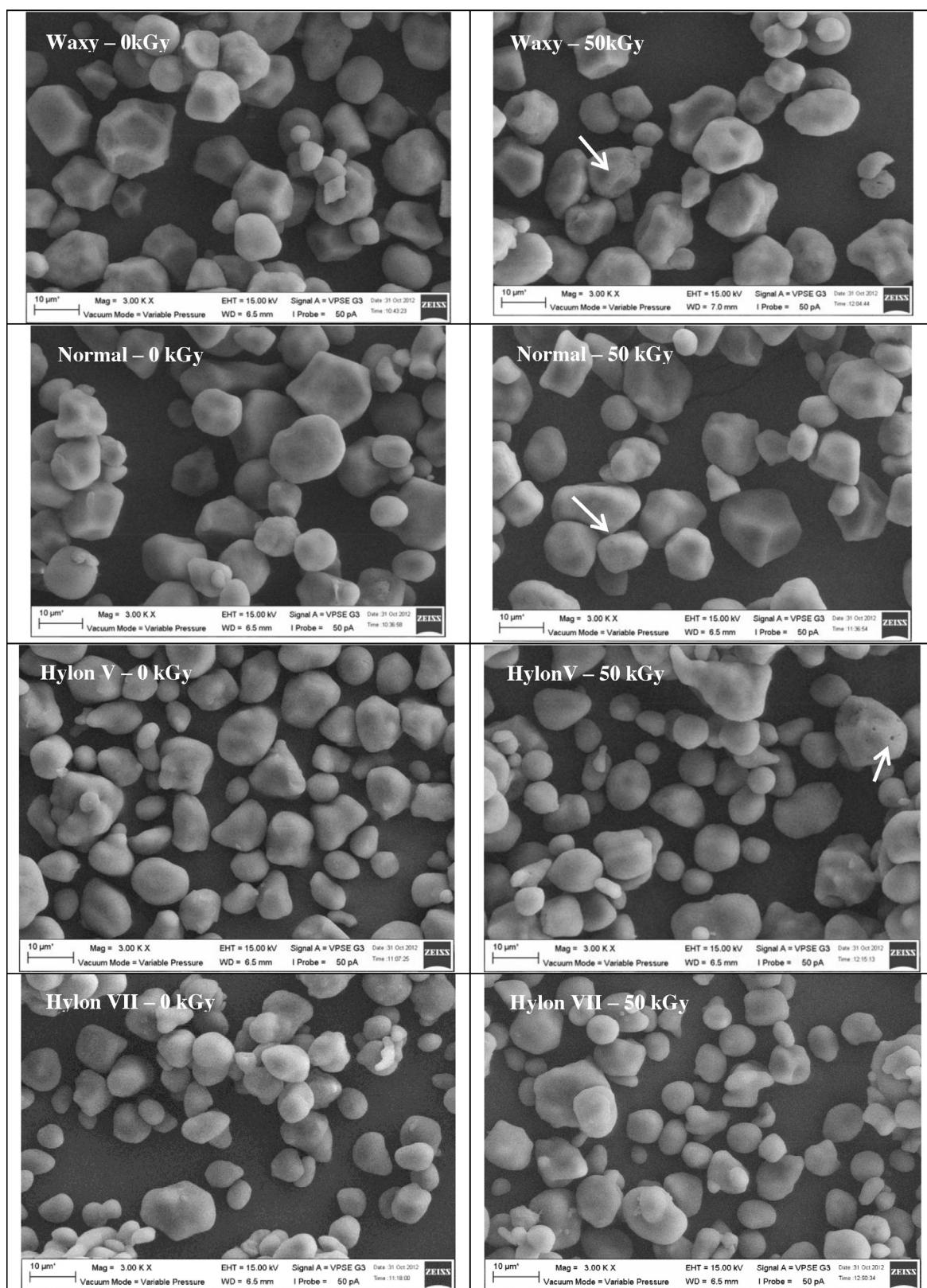


Fig. 1. Scanning electron micrographs of native and gamma-irradiated (50 kGy) corn starch at 3000 $\times$ magnification.

Hylon V, and Hylon VII respectively. The cross-linking and fragmentation of amylopectin by gamma-irradiation have been known to occur primarily in the amorphous region between cluster of the amylopectin (Bao, Ao, & Jane, 2005), consequently RS formation

was found highest in waxy starch. Yoon et al. (2010) also observed more evident increase of RS content in waxy starch than normal starch. Irrespective of the amylopectin content, results of this study revealed that gamma-irradiation induced more RS content

in high-amylose starch than normal starch. With the fact that food irradiated at any dose appropriate to achieve technological objectives is deemed wholesome (FAO/IAEA/WHO Study Group, 1999), results of this study evident the potential of gamma-irradiation as an effective physical means to raise RS content in starch for food application.

4. Conclusions

Depending on the dose, gamma irradiation could significantly increase the RS content in corn starch with different amylose content. The irradiation-induced RS was more evident in waxy corn starch, followed by high amylose corn starch and normal corn starch. RS induced at 5 kGy was essentially contributed by the degraded starch molecules, whilst enhancement of RS at higher doses was due to structural alteration by irradiation.

Acknowledgement

Financial support from the Ministry of Higher Learning, Malaysia (Project code: FRG0190-ST-1/2010) is gratefully acknowledged.

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