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Characterization of Physical Properties of Flour and Starch Obtained from Gamma-Irradiated White Rice

Physical and structural characteristics of rice flour and starch obtained from gamma-irradiated white rice were determined. Pasting viscosities of the rice flour and starch, analyzed by using a Rapid Visco Analyser, decreased continuously with the increase in irradiation dosage. Differential scanning calorimetry showed that gelatinization onset, peak and conclusion temperatures of rice flour and starch changed slightly but the enthalpy change decreased significantly with increase of irradiation dosage. All irradiated starch displayed an A-type X-ray diffraction pattern like the native starch. Gel permeation chromatography showed that the blue value ratio of the first peak (amylopectin) to the second one (amylose) decreased with the increase of the irradiation dosage. The weight-average molecular weight (M_w) and gyration radius (R_z) of amylopectin analyzed by using HPSEC-MALLS-RI (high-performance size-exclusion chromatography equipped with multiangle laser-light scattering and refractive index detector) decreased gradually from 1.48×10^9 (M_w) and 384.1 nm (R_z) of native rice starch to 2.36×10^8 (M_w) and 236.8 nm of 9 kGy-irradiated starch. The branch chain-length distribution of amylopectins determined by HPAEC-ENZ-PAD (high-performance anion-exchange chromatography with amyloglucosidase post-column on-line reactor and pulsed amperometric detector) showed that gamma irradiation had no significant effect on the amylopectin branch chains with $13 \leq DP \leq 24$ and $37 \leq DP$, but produced more branch chains with $6 \leq DP \leq 12$ when the irradiation dosage was less than 9 kGy. It might be deduced that gamma irradiation caused the breakage of the amylopectin chains at the amorphous regions, but had little effects on the crystalline regions of starch granules, especially at low dosage irradiation.

Keywords: Rice starch; Amylopectin; Gamma irradiation; Physical properties; Structural properties

Research Paper

1 Introduction

Gamma irradiation has long been used to protect foods from insect infestation and microbial contamination during storage. As a major ingredient, starch confers structure, texture, consistency and appeal to many food systems. The effects of gamma irradiation on chemical composition of food products have received wide attention because of the concern about food safety. Gamma irradiation was considered as one of the physical modification methods of starch [1, 2]. In comparison with other physical modification methods, such as microwave [3], UV [4, 5], ultrahigh hydrostatic pressure [6] and hydrothermal treatment [7], gamma irradiation treatment is rapid, convenient, and more extensive because ionizing energy penetrates through the starch granule rapidly and may cause greater damage to the starch structure. Gamma irradiation of food materials is more sanitary because the target material can be remained intact in a package or a container where the gamma rays can penetrate.

How gamma irradiation effects starch properties and food quality has been of great interests to food processors. Hence, work has been documented on irradiation of starches of rice [2, 8–10], wheat [11–13], maize [14], potato [15], and beans [16, 17]. *Tomasik* and *Zaranyika* [1] and *Sohkey* et al. [18] have reviewed the molecular changes in starches resulting from gamma irradiation. The starch granule structure remains visually undamaged at a low dosage of irradiation but suffers severe damage at a higher dosage (100 kGy) [2]. Crystallinity of irradiated starch increases with increasing radiation dosages in wheat and rice starches [10, 13],

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and decreased in potato starch [15]. Increase in irradiation dosage decreases viscosity and increases water solubility and acidity of starches [8, 13, 18–22]. The gelatinization enthalpy was reported to increase [16, 17] or decrease [2, 11, 15] gradually with increasing irradiation dosage. *Bachman* et al. [22] reported that a dosage of 2 kGy irradiation resulted in decreased and increased retrogradation of *Lasco triticale* and Grana wheat starches, respectively. The chain length of amylose and amylopectin displayed a progressive reduction as the irradiation dosage increased [14, 16, 21].

Although some studies have been carried out on irradiation of rice starches, few reports have been on the effects of gamma irradiation on amylopectin structure, molecular weight, and gyration radius of rice starch. In this study, we investigated how gamma irradiation affects the structures and physical properties of rice flour and starch in order to understand the mechanisms of gamma irradiation affecting starch granule structures.

2 Materials and Methods

2.1 Materials

The white japonica rice sample was bought from a local market. The moisture content of the white rice was 11.7%. The white rice was divided into seven parts, each part (100 g) was treated with 0, 0.5, 1.0, 3.0, 5.0, 7.0, and 9.0 kGy γ-radiation at room temperature, respectively. The treatment was performed in a ^{60}Co irradiator located at Zhejiang University with a dosage rate of 0.5 kGy/h. After treatment, 30 g of white rice were ground to pass through a 100-mesh sieve on a Cyclone Sample Mill (UDY Corporation, Fort Collins, Colorado, USA), and another 50 g of white rice were used for starch isolation.

2.2 Starch isolation

White rice (50 g) was dispersed in 200 mL 0.05% aqueous NaOH at room temperature for 24 h. After draining off the supernatant and washing with distilled water several times, the grains were wet milled and filtered through a nylon screen (53 μm). The slurry was centrifuged and the top yellow layer was removed. The solids obtained by centrifugation were sequentially purified five times by the toluene-salt solution shaking procedure [23]. The clean white layer of isolated starch was washed with water and ethanol before drying in a convection oven at 32°C for 48 h.

2.3 Pasting properties

Rice flour and starch pasting properties were determined by using a Rapid Visco Analyser (RVA, model 4, Newport Scientific, Sydney, Australia). Each rice sample (flour or starch, 3 g, 12% moisture basis) was mixed with 25 g of deionized water in an RVA sample canister. The idle temperature was set at 50°C, and the following 12.5-min test profile was run: (1) 50°C held for 1.0 min, (2) the temperature was linearly ramped up to 95°C at 4.8 min, (3) the temperature was held at 95°C until 7.3 min, (4) the temperature was linearly ramped down to 50°C at 11.1 min and (5) held at 50°C until 12.5 min.

2.4 Thermal properties

Thermal properties of the flour and starch samples were determined by using a differential scanning calorimeter (DSC-7, Perkin-Elmer, Norwalk, CT) equipped with an intracooling II system. Approximately 3 mg (dry basis) of samples was precisely weighed in an aluminum pan, mixed with 9 mg of deionized water and sealed. The sample was allowed to equilibrate for 1 h and scanned at a rate of 10°C /min over a temperature range of 30–110°C. An empty pan was used as reference. Gelatinization onset (T_o), peak (T_p), conclusion (T_c) and enthalpy change (ΔH) were determined.

2.5 X-ray diffraction pattern of starch

X-ray diffraction patterns of the flour and starch were obtained with copper K_α radiation in a diffractometer (D-500, Siemens, Madison, WI). The analysis was conducted by following the procedure of *Yoo* and *Jane* [24].

2.6 Gel-permeation chromatography of starch

Starch molecular-weight distribution profiles were determined by using a gel-permeation chromatographic (GPC) column (2.6 cm i.d. $\times$ 90 cm) packed with Sepharose CL-2B gel (Pharmacia Inc., Piscataway, NJ) [25]. Starch was dispersed following the method reported by *Song* and *Jane* [26]. A starch dispersion (5 mL) containing 15 mg starch and glucose (0.75 mg, as a marker) was injected into the column. The column was run in the ascending mode. A solution made of distilled water containing 25 mM NaCl and 1 mM NaOH was used as eluent at a flow rate of 30 mL/h. Fractions of 4.8 mL per cup were collected and analyzed for total carbohydrate [27] and blue value [28] at 490 and 630 nm, respectively. The amylopectin fractions (fraction no. 20 to 35) were collected for analyzing branch chain-length distribution.

2.7 Molecular weight determination of amylopectin and amylose

The absolute molecular weights of amylopectin and amylose were determined by using high-performance size-exclusion chromatography equipped with a multi-angle laser-light scattering detector (model Dawn-F, Wyatt Technology, Santa Barbara, CA) with He-Ne laser light at 632 nm and a refractive index detector (RI, HP1047A, Hewlett Packard) (HPSEC-MALLS-RI) [29]. A starch dispersion (0.4 mg/mL) was prepared by the same procedure used for GPC and filtered through a nylon filter (5 μm) before injection. The weight-average molecular weight (M_w) and the gyration radius (R_z) were calculated by using ASTRA 4.7 software (Wyatt Technology, Santa Barbara, CA). Molecular weights of amylose and degraded amylopectin from gamma-irradiated starch were calculated by using a calibration curve constructed from a series of pullulan molecular weight standards (0.58, 1.22, 2.37, 4.80, 10.0 and 18.6×10^4) (Showa Denko K. K., Tokyo, Japan).

2.8 Amylopectin branch chain-length analysis

The collected amylopectin fractions from the GPC analysis were combined and used for analyzing branch chain-length distribution. Amylopectin was debranched by isoamylase following the method of *Jane* and *Chen* [25]. The branch chain-length distribution of amylopectin was determined by using a high-performance anion-exchange chromatography system (Dionex-300, Sunnyvale, CA) equipped with an amyloglucosidase post-column on-line reactor and a pulsed amperometric detector (HPAEC-ENZ-PAD) [30].

2.9 Statistical analysis

All measurements were made in duplicates. Analysis of variance (ANOVA) was performed with the SAS program version 8.0 (SAS Institute Inc., Cary, NC). Least significant differences for comparison of means were computed at $p < 0.05$.

3 Results and Discussion

3.1 Pasting viscosity

The pasting viscosity of the gamma-irradiated rice flour decreased continuously with the increase in irradiation dosage, especially the peak viscosities and final viscosities (Fig. 1A). Effects of gamma irradiation on pasting viscosity of rice starch (Fig. 1B) were similar to that of the flour. The result was in agreement with the previous reports of the effects of irradiation on pasting viscosities of various starches [2, 8, 13, 18–22]. The reduction in viscosity indicated that gamma irradiation caused molecular degradation to starch granules. It was found that the RVA pasting viscosity profiles of starch samples (Fig. 1B) were similar to those of their flour counterparts (Fig. 1A), except that the breakdown viscosities (the difference between peak viscosity and hot paste viscosity) of starch samples were smaller than that of their flour counterparts. These differences could be attributed to the absence of proteins in rice starch [31].

3.2 Thermal properties

Thermal analysis of the gamma-irradiated rice flour showed that the gamma irradiation caused an increase in the onset temperature (T_o) and decrease in conclusion temperature (T_c) and enthalpy change of gelatinization (ΔH) of rice flour, while the peak temperature (T_p) remained unchanged (Tab. 1). For irradiated rice starch, T_o remained unchanged, whereas T_p, T_c and ΔH displayed a slight decrease (Tab. 1). Our previous results also showed that gamma irradiation decreased the T_p (2°C decrease) and ΔH of rice flour, after being irradiated at 50 kGy [2]. Increases in T_o and T_p after irradiation were reported in wheat and potato starches [11, 15] and bean flour [17]. However, the T_p of wheat starch decreased after being irradiated with 446 kGy [11]. *Rombo* et al. [17] also reported that the T_o decreased in maize flour after gamma irradiation. The ΔH was reported to decrease in potato and wheat starches [11, 15] and bean flour [17], but to increase in maize flours [17] after irradiation. As DSC thermal properties reflect gelatinization of the crystalline part of starch, our results indicated a small decrease in the crystalline ordering in rice starches after irradiation. It was also found that the T_o, T_p and T_c of rice starch were lower than that of the flour, whereas the enthalpy change of rice starch were higher than that of the flour counterparts (Tab. 1).

3.3 Crystalline structure

The X-ray diffraction patterns of the irradiated rice flour and starch showed that they all displayed the A-type X-ray diffraction patterns. The native rice starch and flour had a crystallinity of 36.1% and 31.5%, respectively (Fig. 2). Starch irradiated at 0.5 kGy had a crystallinity of 35.6%, whereas all other starches irradiated at larger dosage had greater crystallinities than the normal starch (Fig. 2). The 1 kGy treatment gave the highest crystallinity (40%), further increase in irradiation dosage decreased the crystallinity. On the other hand, all irradiated flours

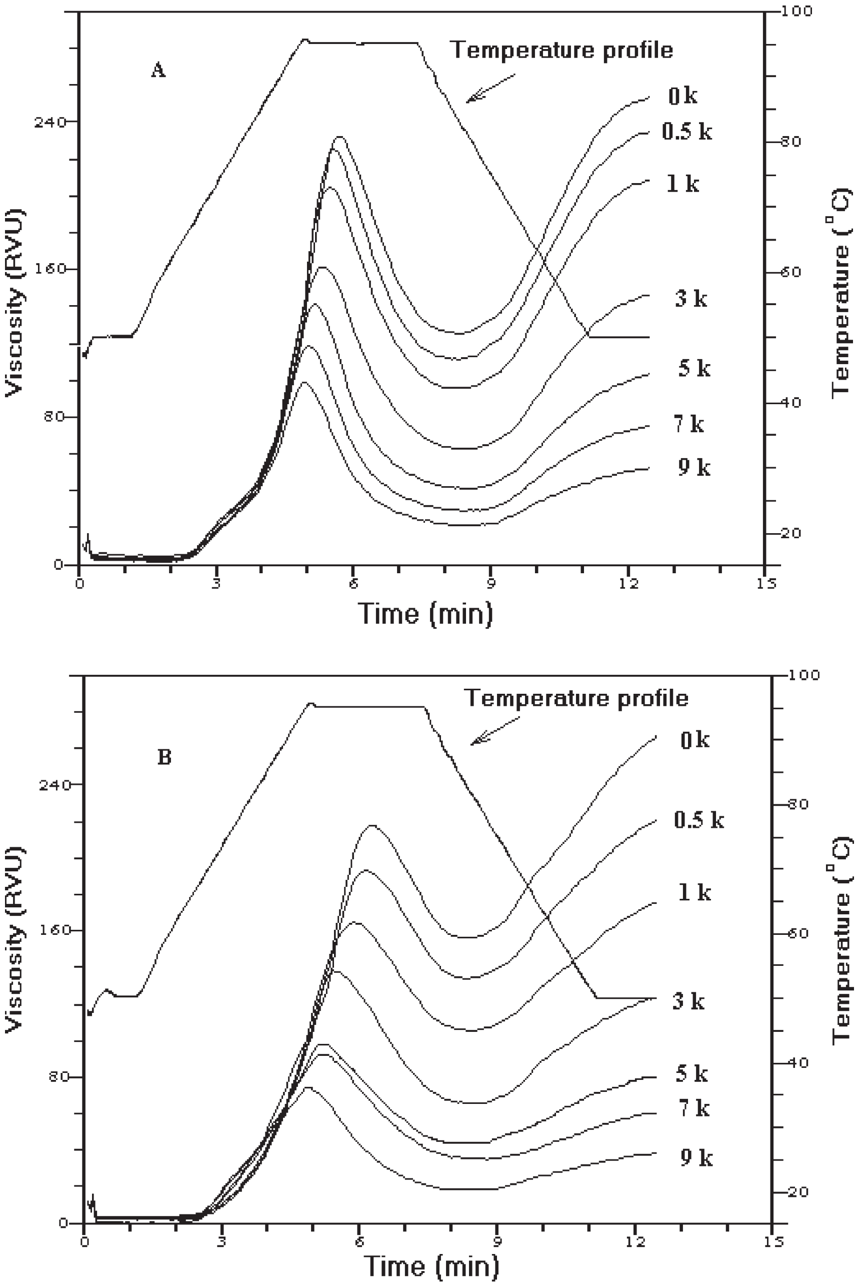


Fig. 1. Rapid Visco Analyser pasting profiles of rice flour (A) starches (B) obtained from gamma-irradiated white rice.

had a decreased crystallinity as compared with that of the native flour (Fig. 2). Increased crystallinity of starches, determined by X-ray diffraction techniques, has been reported in wheat [13] and rice [10] starches. The decrease in ΔH of irradiated potato starch was reported to be in agreement with the decrease in crystallinity [15]. In the present study, the decrease in ΔH of irradiated rice flour and starch were not in agreement with the decrease in crystallinity (Tab. 1, Fig. 2), indicating the crystallinity of irradiated starch samples was not always correlated with their ΔH.

3.4 Molecular weight distribution

The molecular weight distribution of rice starch was determined by using gel permeation chromatography. The results showed that rice starch displayed two peaks, representing amylopectin (first peak, fractions 20 to 35) and amylose (second peak, fractions 56 to 82) (Fig. 3). The percent of total carbohydrate content and the blue value of peak 1 (amylopectin part) decreased while that of the peak 2 increased. The total carbohydrate contents of fractions 36 to 56 increased for samples with higher irra-

Tab. 1. Thermal properties of rice flour and starch obtained from gamma-irradiated white rice.

Irradiation dosage [kGy]	Flour				Starch			
	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J/g]	T_o [°C]	T_p [°C]	T_c [°C]	ΔH [J/g]
0	57.7 c	64.9 a	71.6 a	7.5 a	56.4 a	63.6 a	69.6 a	12.1 a
0.5	57.0 c	64.5 a	71.3 ab	7.6 a	56.3 a	62.8 bc	69.2 ab	11.5 ab
1	58.1 bc	64.8 a	71.2 abc	7.1 ab	56.3 a	62.5 c	69.2 ab	10.5 ab
3	57.5 c	64.4 a	70.7 bcd	7.0 ab	56.4 a	62.4 c	68.7 b	9.7 b
5	58.2 bc	64.7 a	70.9 abc	5.5 bc	56.7 a	63.1 b	69.2 ab	10.3 ab
7	59.9 a	64.3 a	70.0 d	3.9 cd	56.7 a	62.8 bc	69.3 ab	10.3 ab
9	59.0 ab	64.5 a	70.4 cd	3.8 d	56.4 a	62.7 bc	69.0 ab	10.8 ab

Different letters in each column indicate significant difference at the 0.05 level.
T_o: onset temperature, T_p: peak temperature, T_c: conclusion temperature, and ΔH: enthalpy of gelatinization.

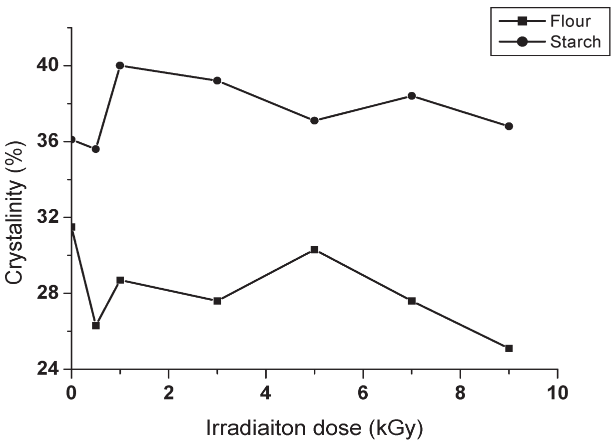


Fig. 2. Crystallinity of rice flours and starch obtained from gamma-irradiated white rice.

diation dosage treatments such as 5 kGy, 7 kGy and 9 kGy. Similarly, the blue value ratio of the first peak (amylopectin) to the second peak (amylose) decreased with the increase of the irradiation dosage. The results indicated that the amylopectin molecules were degraded by increasing dosage irradiation. These results agreed with that reported previously [14, 17].

3.5 Amylopectin and amylose molecular weight and size

The molecular weights of amylopectin and amylose were determined by using high-performance size-exclusion chromatography equipped with multiangle laser-light scattering and refractive index detectors. The weight-average molecular weight (M_w) of untreated rice amylopectin and amylose was 1.48×10^9 and 3.85×10^5, respectively, which were similar to that previously reported [29]. The M_w of amylopectin and amylose decreased with the increase in irradiation dosage, and the M_w of amylopectin and amylose decreased to 2.36×10^8 and 3.66×10^5 at 9 kGy, respectively (Tab. 2). The *Z*-average radius of gyration (R_z) of amylopectin was decreased gradually from 289.0 nm (R_z) of the untreated starch to 204.2 nm (R_z) of 9 kGy irradiated rice starch (Tab. 2), indicating the decrease in the molecular size of amylopectin due to irradiation. *Sokhey* and *Chinnaswamy* [14] reported that the molecular weight of fraction I decreased with increasing irradiation dosage, they found that the molecular weights of fractions II and III were increased at lower irradiation dosage and then decreased at higher dosage.

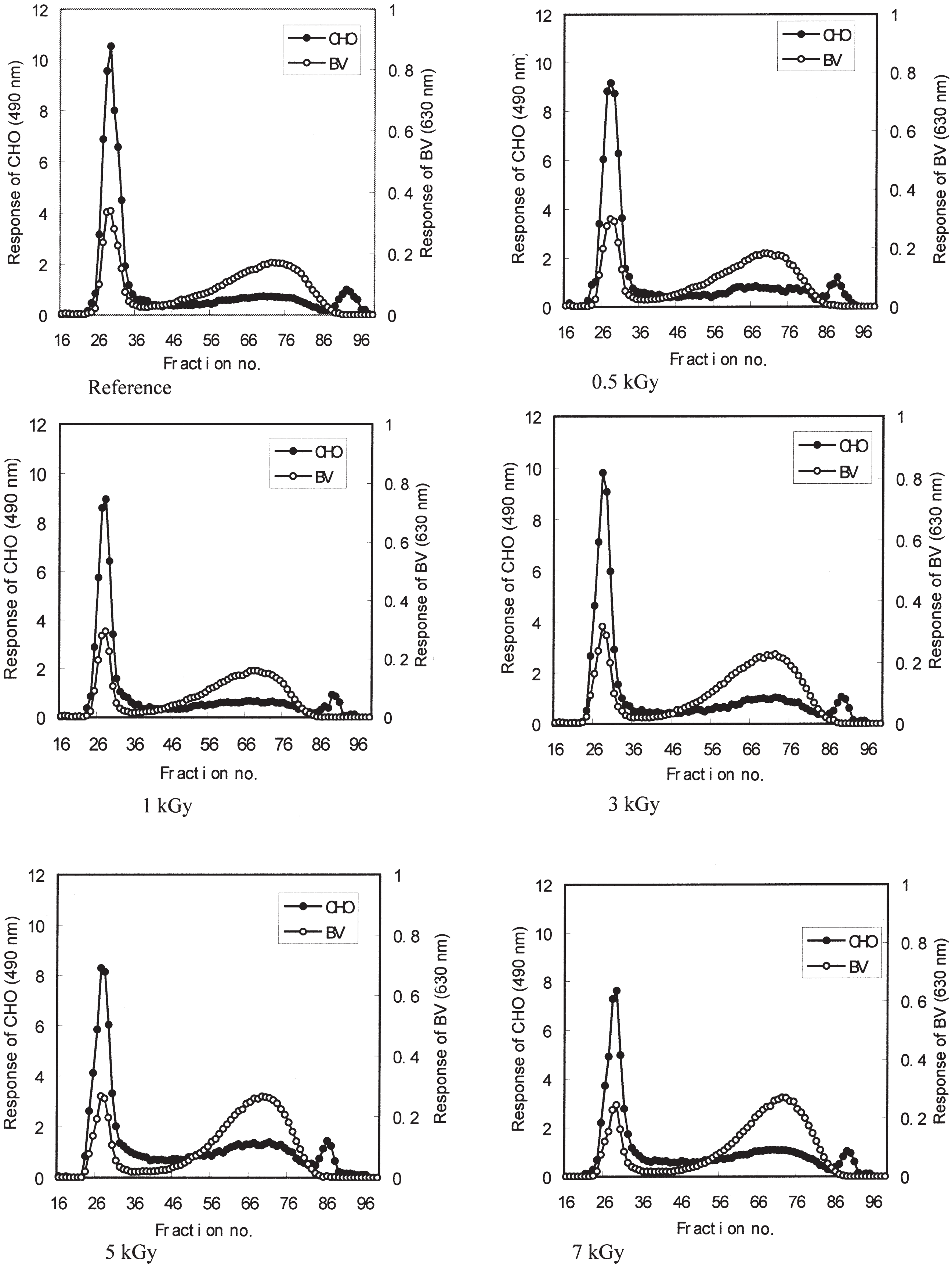
Tab. 2. Weight average molecular weight (M_w) of amylopectin and peak two (amylose and degraded amylopectin) and gyration radii of amylopectin (R_z) of the rice starch obtained from gamma-irradiated white rice.

Irradiation dosage [kGy]	Amylopectin M_w ($M_w \times 10^8$)	R_z [nm]	Peak two M_w ($M_w \times 10^5$)
0	14.77 a	384.1 a	3.85 a
0.5	10.75 b	361.2 a	3.81 ab
1	9.80 b	345.2 a	3.81 ab
3	4.67 c	262.4 b	3.80 b
5	2.78 d	254.5 b	3.74 c
7	2.38 d	234.7 b	3.73 c
9	2.36 d	236.8 b	3.66 d

Different letters in each column indicate significant difference at the 0.05 level.

3.6 Amylopectin branch chain-length distribution

The amylopectin collected from GPC was used for analyzing branch chain-length distribution. It seemed that the gamma irradiation had no significant effect on the amylo-


CHO
BV
Response of CHO (490 nm)
Response of BV (630 nm)
Fraction no.
Reference
0.5 kGy
1 kGy
3 kGy
5 kGy
7 kGy

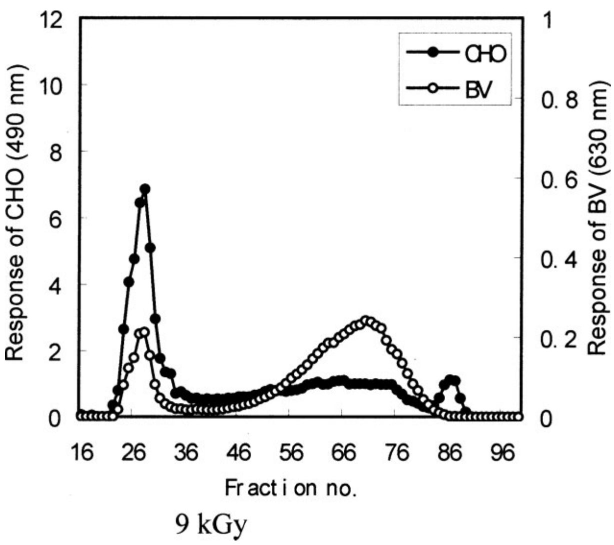


Fig. 3. Sepharose CL-2B gel permeation chromatography profiles of rice starches obtained from gamma-irradiated white rice, CHO: total carbohydrate content, BV: blue value.

pectin branch chains with 13≤DP≤24 and 37≥DP (Tab. 3). More branch chains with 6≤DP≤12 were produced when the irradiation dosage was less than 9 kGy. Branch chains with 25≤DP≤36 decreased when the irradiation dosage was less than 9 kGy. However, less chains with 6≤DP≤12 but more chains with 25≤DP≤36 were produced under 9 kGy treatment (Tab. 3).

The results indicated that gamma irradiation fragmented amylopectin and decreased the molecular weight. The fragmentation mostly resulted from the cleavage at the amorphous regions, instead of at crystallite regions, because there was significant decrease in the gyration radius of the amylopectin molecule (Tab. 2) but little change in the amylopectin branch chain length (Tab. 3). This mechanism was similar to that of the acid hydrolysis of starch [32]. Another mechanism was that gamma irradiation might cause both cleavage and cross-linking of starch chains. The increase in the branch chains > DP 25 and decrease in the branch chain ≤ DP 24 at 9 kGy indicated that cross-linking might occur between branch chains. Gamma irradiation produced free radicals on the starch molecules [4], which might induce cross-linking, but this mechanism awaits further study. On the other hand, gamma irradiation could also damage amylose molecules, causing a decrease in their molecular weights (Tab. 2). However, the possibility to attack amylose was considered lower than to attack amylopectin because the amylose content in normal rice starch is smaller than that of amylopectin.

4 Conclusion

Gamma irradiation caused significant reduction in the RVA viscosity of rice flour and starch, in the ΔH of irradiated rice flour, and in the molecular weight and gyration radius of amylopectin, but caused little changes of T_o, T_p and T_c of both rice flour and starch. The crystallinity increased in irradiated starch but decreased in the irradiated flour as compared with the native samples. It can be concluded from the present study that gamma irradiation causes cleavage of amylopectin molecules at the amorphous regions.

Tab. 3. The amylopectin branch chain-length of rice starches obtained from gamma-irradiated white rice.

Irradiation dosage [kGy]	Distribution [%]			
	DP 6–12	DP 13–24	DP 25–36	DP ≥ 37
0	25.9 ± 0.08 c	50.0 ± 0.19 a	10.9 ± 0.13 bc	13.3 ± 0.14 a
0.5	25.4 ± 0.88 c	49.0 ± 1.48 a	11.3 ± 0.64 b	14.3 ± 1.73 a
1	26.0 ± 0.10 a-c	50.0 ± 0.11 a	10.7 ± 0.04 c	13.3 ± 0.05 a
3	26.3 ± 0.09 ab	49.5 ± 0.10 a	10.9 ±0.28 bc	13.3 ± 0.29 a
5	26.7 ± 0.24 a	49.6 ± 0.25 a	10.7 ± 0.16 c	13.0 ± 0.34 a
7	26.0 ± 0.08 a-c	49.5 ±0.40 a	11.2 ± 0.13 bc	13.3 ± 0.61 a
9	24.5 ± 0.00 d	49.2 ± 0.29 a	11.9 ± 0.13 a	14.4 ± 0.16 a

Data were expressed by mean ± SD (n = 2). Different letters in each column indicate significant difference at the 0.05 level.

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