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Pasting Properties of γ-Irradiated Rice Starches as Affected by pH

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Changes in the viscosity properties of γ-irradiated rice starches (from 1 to 25% amylose content) from four genotypes (JY293, Jiayu 293; XS, Xiushui; ZF504, Zhefu 504; and ZXN, Zaoxiannuo) during pasting in water (pH 7) or in different pH solutions were studied using a rapid visco analyzer. Peak viscosity (PV) of all native rice starches was little affected at pH 4 and 10, while hot paste viscosity (HPV) and cool paste (final) viscosity (CPV) were generally lower at pH 4 and higher at pH 10 as compared with that at pH 7. The PV, HPV, and CPV of γ-irradiated starches were higher at pH 4 and lower at pH 10 than pH 7. All viscosity characteristics of native rice starches were reduced in stronger alkali (pH 11.5) or acidic (pH 2.5) solutions. However, the γ-irradiated starches were substantially higher at pH 2.5 but lower at pH 11.5, indicating that the effect of irradiation was highly pH dependent. The swelling volume of irradiated ZF504 and JY293 starch at all irradiation levels was higher at pH 4 than pH 7, while the values were lowest at pH 2.5. The irradiated ZXN and XS starches had higher swelling volumes at pH 4 and pH 2.5 than pH 7. Differential scanning calorimetry analysis showed that γ-irradiation caused progressively lower gelatinization peak temperature (T_p) and higher gelatinization range (T_r) at pH 7. T_p was higher and T_r was lower at a much stronger acidic condition (pH 1) for both native and irradiated starches. The possibility of using viscosity changes in low pH for the detection of irradiated starch was discussed.

KEYWORDS: Starch; pasting viscosity; γ-irradiation; pH

INTRODUCTION

Starch is often modified chemically, physically, or enzymatically to obtain the required functional properties to meet industrial needs hence widening its range of potential applications. Chemical modification such as cross-linking and/or acetylation is widely used (*1*). However, there is a growing interest in physical modification (such as irradiation, heat−moisture treatment, annealing, and shear) of starch, especially for food applications (*2*). In fact, such physically modified starches may be considered to be natural materials with high safety (*2*).

The usual purpose of γ-irradiation of grains and other foods is to protect them from insect infestation and microbial contamination during storage. γ-Irradiation also has important effects on various quality criteria of cereals. Experiments have been performed to study the effects of γ-irradiation on various aspects of wheat (*3*), rice (*4*, *5*), and maize (*6*) quality. Of interest is the depolymerization of starch by irradiation as these quality traits are mainly related to the starch properties.

Much research has been done on the effects of γ-irradiation on starch properties from various sources (*4*, *6*−*12*). There are some reviews available on the properties of irradiated starches (*13*, *14*). Free radicals produced by γ-irradiation modify the amount and structure of starch fractions (*6*, *15*). Granule structure remains visually undamaged at low doses of irradiation but may suffer severe damage at higher doses (100 kGy) (*13*, *14*). The crystallinity of irradiated starch becomes higher with increasing radiation doses in corn and rice starches (*7*, *16*). Increased radiation dose is invariably associated with lower viscosity and higher water solubility and acidity of starches among various studies (*4*, *7*−*11*, *13*). Swelling power is generally lowered (*7*, *9*), but may become higher under 20 kGy treatment, and then declines rapidly (*8*). Rayas-Duarte and Rupnow (*12*) showed that gelatinization enthalpy was increased gradually with increasing dose. Gelatinization peak temperature became higher only at 20 kGy irradiation of dry bean starch (*12*). Bachman et al. (*11*) reported that a dose of 2 kGy made retrogradation of Lasco triticale and Grana wheat starches lower and higher, respectively (*11*). The chain length of amylose and amylopectin shows a progressive reduction as the irradiation dose is raised (*6*, *8*, *12*). Gel permeation chromatographic separation showed that the fraction I (mostly amylopectin) is subject to more degradation due to irradiation (*6*).

Depolymerization of starches is higher in the presence of acid.

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Acids such HCl and H_2SO_4 cause scission of the glucosidic linkages, thereby altering the structure and properties of the native starch (*17*). Acid modification of oat starch granules caused a marked reduction in viscosity, degradation of amylopectin to smaller molecules, and formation of a more elastic gel structure based on amylose (*18*). Combined treatments of γ-irradiation and acidic conditions have been attempted. Raffi et al. (*19*) and Michel et al. (*20*) did both theoretical and experimental study of the depolymerization of starch under the combined action of protons and γ-radiation. Kang et al. (*21*) tried to modify maize starch by γ-irradiation and pH adjustment and found that the combination considerably affected viscosity. Low peak viscosity (PV) and high cooling stability were observed in γ-irradiated, pH 2-adjusted starch (*21*). γ-Irradiation significantly lowered starch intrinsic viscosity whether it was acidified or not (*22*).

Our earlier results showed that increasing levels of γ-irradiation significantly reduced rice flour viscosity and modified the pasting profile shape (*4*). In the present study, we report on the pasting viscosity properties of γ-irradiated rice starches of different amylose content as affected by low pH.

MATERIALS AND METHODS

Materials. Four rice cultivars (breeding lines) varying in apparent amylose content (Zaoxiannuo (ZXN), 1.2%; Zhefu 504 (ZF504), 12.0%; Xiushui (XS) 18.0%; and Jiayu 293 (JY293), 25.0%) were used. Milled rice samples (12% moisture) were treated with 5, 25, and 50 kGy γ-rays at room temperature. The treatment was performed in a ^{60}Co irradiator at Zhejiang University with a dosage rate of 2.5 kGy/hr. The rice samples were then ground to pass through a sieve of 100 mesh size on a Cyclone sample mill (UDY Corporation, Fort Collins, CO).

Pasting Viscosity. Rice pasting properties were determined using a rapid visco analyzer (RVA, model 3-D, Newport Scientific, Warriewood, Australia) according to the AACC Method 61−02 (*23*). Flour (3 g, 12% m.b.) was mixed with 25 g of double-distilled water (pH 6.86; hereafter, pH 7 as neutral solution) in the RVA sample can. Other solutions were adjusted with 0.5 N HCl and 0.5 N NaOH to give the desired pH. The RVA was run using Thermocline for Windows software (version 1.2). A programmed heating and cooling cycle was used where the samples were held at 50 °C for 1 min, heated to 95 °C in 3.8 min, and held at 95 °C for 2.5 min before they were cooled to 50 °C in 3.8 min and held at 50 °C in 1.4 min. The PV, hot (holding) paste viscosity (HPV), and cool paste (final) (CPV) viscosities were recorded. The viscosity parameters were measured in rapid visco units (RVU).

Swelling Volume. Flour swelling volume was determined according to the method of Crosbie et al. (*24*). Flour samples (0.4 g, d.b.) were mixed with 12.5 mL of distilled water in 125 × 16 mm Pyrex culture tubes, equilibrated at 25 °C for 5 min, heated to 92.5 °C, and then held at that temperature for 30 min. The samples were cooled in an ice water bath for 1 min, equilibrated at 25 °C for 5 min, and then centrifuged at 1000*g* for 15 min. The swelling volume was calculated by converting the height of the resultant gels to a volume basis, and the results were expressed as milliliters per gram of dry flour.

Thermal Properties. The thermal properties of milled rice flour were analyzed with a differential scanning calorimetry (DSC) 2920 thermal analyzer (TA Instruments, Newcastle, DE) equipped with DSC standard and dual sample cells. Rice flour (1.8 mg, d.b.) was weighed into an aluminum pan and 12 μL of distilled water was added. The pan was hermetically sealed and then heated at a rate of 10 °C/min from 30 to 110 °C. A sealed pan with 12 μL of distilled water was used as a reference. Onset (T_o), peak (T_p), and conclusion (T_c) temperature and enthalpy (ΔH) of gelatinization were calculated automatically by a Universal Analysis program, version 1.9D (TA Instruments, Newcastle, DE). The gelatinization range (T_r, that is, $T_c - T_o$) was calculated.

Statistical Analysis. All measurements were made in duplicates. Analysis of variance (ANOVA) was performed with the SAS program version 6.04 (SAS Institute Inc., Cary, NC). Least significant differences for comparison of means were computed at $p < 0.05$.

Table 1. Effects of pH on Pasting Viscosity Properties of Native and Irradiated Rice Starches from Four Genotypes[a]

		0 kGy			5 kGy		
		PV	HPV	CPV	PV	HPV	CPV
JY293	pH 7	249 a	196 b	342 a	127 ab	38 b	79 b
	pH 4	248 a	191 c	343 a	128 a	42 a	86 a
	pH 10	247 a	202 a	341 a	126 b	35 c	74 c
XS	pH 7	224 a	188 ab	277 ab	83 a	30 a	54 b
	pH 4	225 a	183 b	275 b	86 a	35 a	64 a
	pH 10	222 a	197 a	281 a	81 a	29 a	51 b
ZF504	pH 7	259 a	134 b	217 a	115 b	37 b	68 b
	pH 4	255 b	126 c	208 b	118 a	43 a	79 a
	pH 10	259 a	140 a	224 a	112 c	34 c	62 c
ZXN	pH 7	133 a	93 a	116 a	54 a	38 ab	48 b
	pH 4	130 a	85 b	112 ab	52 a	44 a	57 a
	pH 10	132 a	86 b	110 b	60 a	31 b	40 b

[a] The different small letter in the same column of the same variety was significant at 5%.

RESULTS

Pasting of Native and γ-Irradiated Rice Starch at Different pH Levels. Several literature reports have shown that increasing levels of γ-irradiation significantly reduced the starch viscosity beginning with the lowest treatment. Our previous (*4*) and present study also showed that the viscosity of rice flour was lowered dramatically at all levels of treatments (5, 25, and 50 kGy), and pasting curves were modified (Table 1, Figure 1). It can also be seen from Table 1 that the lowered viscosity between untreated and 5 kGy treated starches was independent of the amylose content. This can be explained by the equal probability of breakage of $\alpha(1\rightarrow4)$ and $\alpha(1\rightarrow6)$ linkages (*15*, *19*, *20*). For all four genotypes, PV in water, or at pH 4 and pH 10, were very similar except for ZF504 at pH 4. HPV and CPV at pH 4 were reduced or unchanged (for CPV of JY293) as compared to that at pH 7. At pH 10, HPV and CPV changed to some extent depending on genotype (Table 1). For 5 kGy γ-irradiated rice, PV, HPV, and CPV of all four genotypes were higher at pH 4 and lower at pH 10 as compared to pH 7 (Table 1). The viscosity of other rice irradiated at higher doses showed the same trend as for the 5 kGy treatment at both pH 4 and pH 10 (data not shown).

Native starches at stronger alkaline or acidic conditions (at pH 11.5 and pH 2.5, respectively) showed dramatically lower viscosity (Figure 1, JY293 and ZF504), except that the HPV of ZF504 was similar at pH 11.5 and pH 7 and the PV of ZXN was higher at pH 2.5 than at pH 7 (Figure 1). The pH 11.5 treatment on the γ-irradiated rices markedly reduced PV, HPV, and CPV, indicating that a stronger alkali treatment had the same effects on irradiated rice and untreated rice (Figure 1). However, the viscosity (especially PV) was significantly higher at pH 2.5 as compared with that at pH 7 (Figure 1). The PV at pH 2.5 of 5 kGy treated ZXN rice was increased up to that of native starch at pH 7 (Figure 1). The pasting curves at 25 and 50 kGy dosing treatments were apparently linear, while at pH 2.5, a dramatic peak appeared indicating that the PV at pH 2.5 was about 3-fold higher for ZXN and 2-fold for XS. To exclude any possible effects of hydrolysis of the aluminum can, cans with a protective coating were used. The pasting curves were found to be similar to those obtained with untreated cans (data not shown), showing that the aluminum can had no effect on viscosity at low pH.

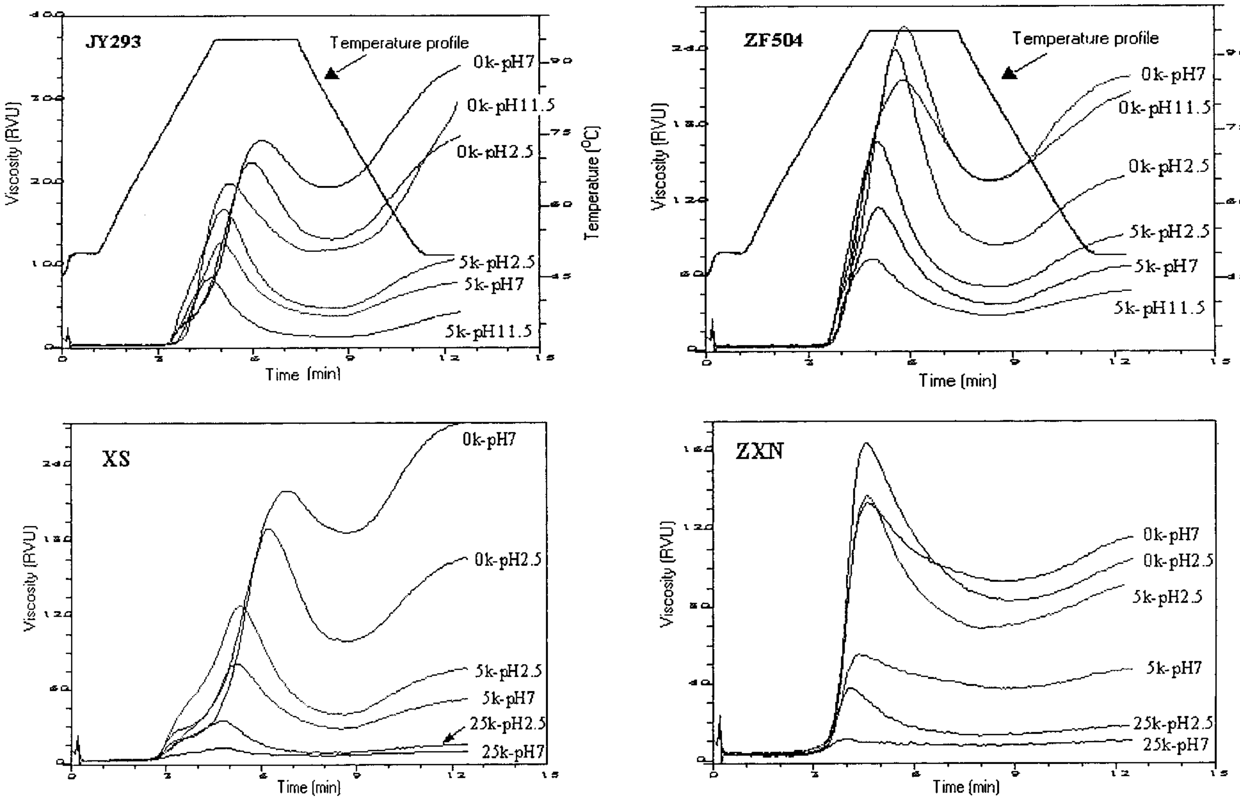

Figure 1. Pasting profiles of native and γ-irradiated rice starches at different pH values.

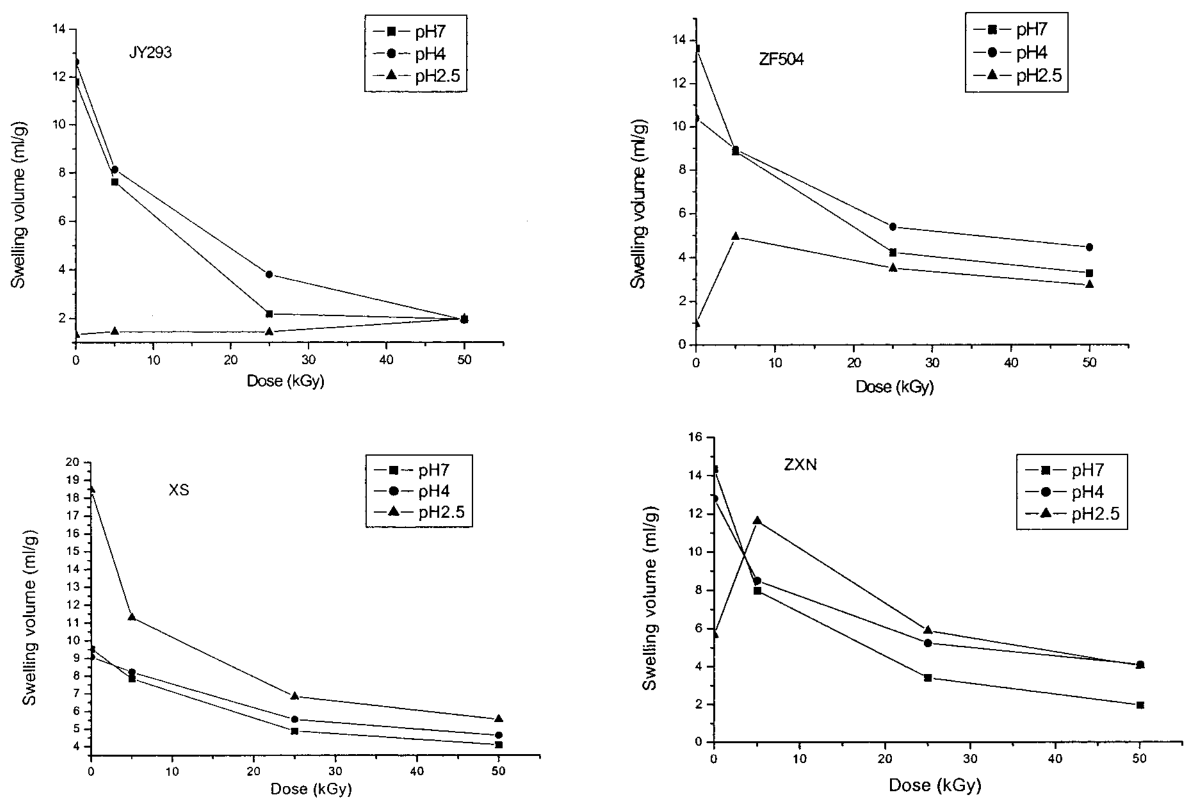

Figure 2. Swelling volume of native and γ-irradiated rice starches at different pH values.

Swelling Volume of Native and Irradiated Starches at Low pH. The significantly higher PV of the irradiated rice at pH 2.5 suggested that the swelling volume should also be higher at pH 2.5 or 4 than at pH 7, partly because PV reflects the ability of the starch granules to swell freely before breaking down. A progressive reduction of swelling volume with increasing dose was found at pH 7 solution for all genotypes (Figure 2). The swelling volume of the native ZF504 and ZXN at pH 7 was higher than at pH 4 and pH 2.5, but the native JY293 at pH 4 and XS at pH 2.5 were higher than at pH 7 (Figure 2). The swelling volume of irradiated starches from ZF504 and JY293 at all levels was greater at pH 4 than at pH 7, while the

Table 2. Effects of pH on Thermal Properties of Native and Irradiated Rice Starches from Four Genotypes[a]

		pH 7			pH 1		
	dose (kGy)	T_p	T_r	ΔH	T_p	T_r	ΔH
JY293	0	75.0 a	11.1 b	10.6 a	77.8 a (2.8)	10.9 a (−0.2)	10.5 b (−0.1)
	5	74.7 ab	11.8 ab	10.3 a	77.2 a (2.5)	10.6 b (−1.2)	11.1 a (0.8)
	25	74.6 bc	12.5 a	10.0 a	76.7 a (2.1)	10.9 a (−1.6)	10.5 b (0.5)
	50	74.2 c	12.5 a	9.9 a	75.2 a (1.0)	11.0 a (−1.5)	10.5 b (0.6)
XS	0	68.9 a	15.6 b	9.9 a	71.6 a (2.7)	15.4 a (−0.2)	10.0 a (0.1)
	5	68.1 ab	16.3 ab	9.4 a	71.3 a (3.2)	15.1 a (−1.2)	9.1 a (−0.3)
	25	67.4 bc	17.1 ab	9.3 a	69.2 b (1.8)	15.0 a (−2.1)	9.5 a (0.2)
	50	66.6 c	17.7 a	9.3 a	68.5 b (1.9)	15.4 a (−2.2)	9.5 a (0.2)
ZF504	0	78.6 a	10.8 a	11.2 ab	81.4 a (2.8)	9.2 b (−1.6)	10.3 b (−0.9)
	5	78.6 a	10.9 a	11.3 a	80.8 a (2.2)	9.5 b (−1.4)	12.0 a (0.7)
	25	77.4 b	11.3 a	10.8 b	79.4 ab (2.0)	10.1 a (−1.2)	11.9 a (1.1)
	50	76.6 c	11.4 a	10.8 b	78.1 b (1.5)	10.4 a (−1.0)	11.3 a (0.5)
ZXN	0	78.6 a	12.2 b	12.3 a	81.3 a (2.7)	11.8 b (−0.4)	12.1 a (−0.2)
	5	77.9 b	12.8 ab	11.2 b	80.6 b (2.7)	11.8 b (−1.0)	12.4 a (1.2)
	25	76.9 c	13.1 a	10.8 bc	79.6 c (2.7)	12.5 a (−0.6)	11.5 a (0.7)
	50	76.1 d	13.2 a	10.3 c	78.4 d (2.3)	12.8 a (−0.4)	11.4 a (1.1)

[a] Numbers in the same column followed by a letter in common were not significantly different ($p < 0.05$); the number in parentheses showed changes relative to that at pH 7.

values at pH 2.5 were lowest. Irradiated starches from ZXN and XS had higher swelling volumes at pH 4 and pH 2.5 than at pH 7 (Figure 2).

Gelatinization Temperature and Enthalpy of Native and Irradiated Starches at Low pH. A lowered gelatinization temperature was observed for the irradiated starches at pH 7 in all four genotypes (Table 2, Figure 3). The mean T_p of untreated JY293, XS, ZF504, and ZXN was 75.0, 60.9, 78.6, and 78.6 °C, respectively. These values decreased to 74.2, 66.6, 76.6, and 76.1 °C, respectively, at 50 kGy treatment (Table 2). The gelatinization range (T_r) became broader as the irradiation dose increased (Figure 3). The gelatinization enthalpy (ΔH) was not affected by γ-irradiation for XS and JY293, but a significant lowering was observed for ZF504 and ZXN (Table 2).

A small rise in gelatinization temperature was found at pH 2.5 (data not shown). A much stronger acid solution with pH 1 was used to see whether it had significant effects on gelatinization properties. T_p increased by 2.8 °C at pH 1 as compared with pH 7 for all four genotypes. The T_p of γ-treated starches was higher at pH 1 as compared to pH 7 but lower as compared to untreated starches at pH 1. It seemed that T_r became narrower at pH 1 for both treated and control samples (Table 2, Figure 3). ΔH of the untreated samples was a little smaller in pH 1 than pH 7, while for γ-irradiated starches, it was much larger in pH 1 than pH 7 (Table 2).

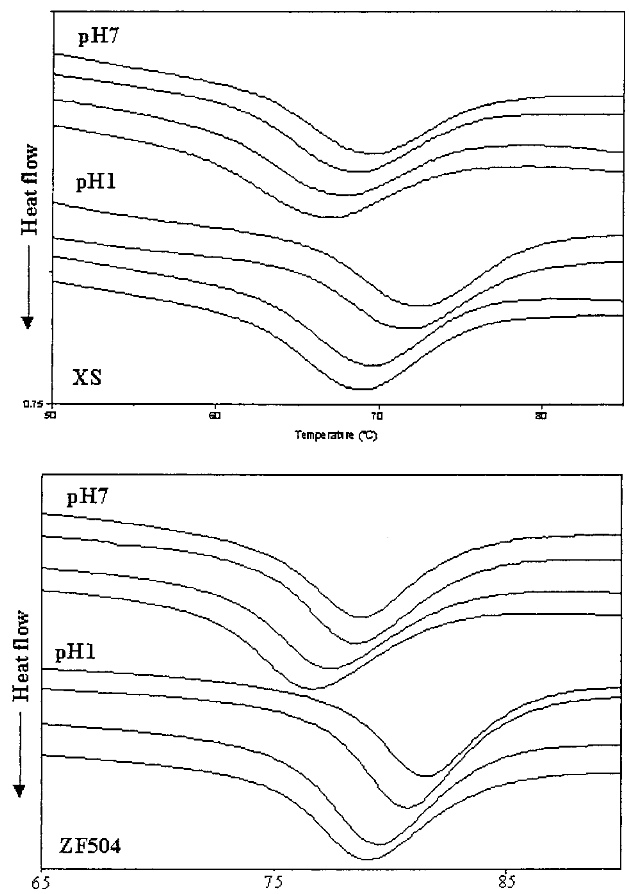

Figure 3. DSC thermal properties of native and γ-irradiated rice starches (XS and ZF504) at pH 7 and pH 1. The four lines under the given pH value represent the samples treated by 0, 5, 25, and 50 kGy irradiation, respectively.

DISCUSSION

The physicochemical properties of starches after γ-irradiation treatment have been investigated extensively for a long time. γ-Irradiation of starch has consistently been reported to reduce viscosity and increase water solubility and acidity (*4*, *7*−*11*, *13*). Varied effects were reported for properties such as retrogradation (*11*) and swelling power (*8*). The combination of γ-irradiation and acid treatment was found to reduce the viscosity of treated starch significantly. However, such reports were confined to the application of γ-irradiation to acidified (or pH-adjusted) starches. Literature on the reverse treatment (that is, acid to irradiated starches), as may apply in some food applications, is not available. In the present study, we found that γ-irradiated starches had a higher viscosity at pH 2.5 than at pH 7, while native starches had a lower viscosity at pH 2.5 than pH 7. We used irradiated potato starch in order to confirm that the present results were not of an isolated occurrence and found that the viscosity at pH 2.5 increased markedly in a 12.5 min program and a prolonged 23 min program (data not shown).

We assume that γ-irradiation might lead to the alteration of the stability of the most perfect crystallites or partial disruption of some of crystallites, giving rise to crystallites of poor quality. However, the crystallite quality would be improved when the irradiated starches are placed in acidic conditions. Consequently, the irradiated starch displays a higher viscosity and swelling volume at low pH. Because amylopectin content in rice starch is higher than amylose content and because the crystallinity is produced by ordering of the amylopectin chains, the crystalline regions of starch grain are much more easily attacked by γ-rays, especially the secondary structures such as double helices. Besides, the radicals produced by γ-irradiation are attributed to chain scission and hydrogen abstraction (*14*, *22*). Even though some reports showed that the crystallinity of irradiated starch increased with higher radiation doses in maize and rice (*7*, *16*), the quality of irradiated crystallites should become poorer. In acidic conditions, it is possible that protons are acting on some intra- and/or intermolecular linkages induced by irradiation between starch chains, giving rise to more perfect crystallites of the irradiated starch. However, radicals induced by γ-irradiation play minor roles in the rise in viscosity, because the radicals are destroyed by the reaction with water molecules (*22*). This assumption is also supported, at least in part, by the results of the swelling volume test and DSC test.

When starch is heated in excess water, the crystalline structure is disrupted due to the breakage of hydrogen bonds, and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin. The swelling volume of irradiated starch was reduced with γ-irradiation (Figure 2), indicating that some of the amylopectin side chains might be broken and the hydrogen bond destabilized by γ-irradiation. The irradiated ZXN and XS at all levels showed higher swelling volumes at pH 4 and pH 2.5 than at pH 7 (Figure 2). This was consistent with the viscosity data presented in Table 1 and Figure 1. The results could be explained that the increase of bond strength in low pH gives the starch a greater ability to hold water. The lowered swelling power of ZF504 and JY293 at pH 2.5 might be due to their lengthy (30 min) exposure to acid treatment at high temperature (92.5 °C). Therefore, γ-irradiation might cause starch to be more resistant to swelling in distilled water but not in the acid environment.

γ-Irradiation might decrease starch crystallite stability and cooperation in melting, and the stability would be increased in low pH. Thus, in the RVA system, starches could swell for a longer time in low pH than pH 7 before they went to breakdown, resulting in higher viscosity. DSC analysis at pH 7 showed that gelatinization temperatures (T_p) reduced gradually with increasing irradiation dose (Table 2), indicating the increased destabilization effect in the amorphous regions when the starch crystallites melt due to γ-irradiation, as in other DSC studies on heat−moisture treatment effects on starches (*26*−*28*). It has already been reported that irradiation of solid starch granules causes the granules to weaken, and their heterogenicity increases with applied dose (*14*). The broadening of the gelatinization range in the γ-irradiation treatment indicated a less cooperative melting between the amorphous and the crystalline domains of the granule. The reduction after γ-irradiation in ΔH found for ZF504 and ZXN indicated that some of the double helices might already be disrupted by γ-treatment (*26*). At pH 1, T_p was higher and T_r was lower as compared to pH 7 (Table 2, Figure 3). The results could be interpreted that acid treatment gave a more homogeneous population of crystallites and an increase in stability of the crystallites in the granule (*26*). ΔH of the untreated samples was a little smaller in pH 1 than pH 7, while for γ-irradiated starches, it was a little larger in pH 1 (Table 2), indicating that the more cooperative melting of irradiated starch existed in pH 1 rather than in pH 7.

Resistance to acid hydrolysis of heat−moisture-treated and annealed starches was found in various starches after 20 days of acid treatment (2.2 N HCl, 35 °C) (*2*, *17*, *27*). In this present study, acid hydrolysis might occur while starch is undergoing pasting in the RVA heating cycle due to high temperature (95 °C). However, the degree of hydrolysis might be very small as compared to that at 2.2 N HCl. Acid hydrolysis of the γ-irradiated starches at different temperature and duration of time should be the subject of further investigation.

A potential application of the present study might be in the detection of irradiation. The viscosity difference at pH 2.5 and pH 7 could be used to evaluate whether a starch sample has been exposed to irradiation. This method could be better than the proposed method, which was also based on viscosity (*29*). The previous method needs a reference (unirradiated) sample while the present method does not. Previous results showed that the viscosity of irradiated starch decreased after one month of storage (*29*). We found that the viscosity of stored irradiated starch in the present study was still larger in pH 2.5 than pH 7 (data not shown), which indicated that the present method is more practical. Even though the ΔH of the untreated sample was smaller at pH 1 than pH 7, while for irradiated samples ΔH is higher at pH 1 than pH 7, the use of ΔH to detect the irradiation of starches is not recommended because the changes are small. However, the present method is still limited to the detection of irradiation in raw starches but cannot be applied in the food made from them.

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