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Physicochemical Properties of Gamma-Irradiated Corn Starch

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Abstract

Structural modification of corn starch by gamma irradiation was evaluated for under dry conditions at varied intensities from 0 to 40 kGy. Under scanning electron microscopy, the granule shape of corn starch was not significantly affected by the irradiation up to 40 kGy. In addition, X-ray diffraction and melting patterns of the irradiated starches were similar to those of the native starch, indicating that crystalline regions in the starch granules were not changed by irradiation. However, the pattern of gel permeation column chromatography showed a significant increase in partial hydrolysis of gamma irradiated starch samples. The degree of polymerization and the paste viscosity of irradiated starch samples dose-dependently decreased significantly with irradiation, and increased solubility and clarity were observed in the irradiated starch solution. In addition, the degree of retrogradation decreased as irradiation dose increased. Irradiation of corn starch has advantages over the ordinary acid or the enzyme hydrolysis modification methods. It does not affect the granular shape and crystalline phase of starch during hydrolysis, and the process can be carried out in dry state.

Key words: gamma irradiation, corn starch, modified starch

INTRODUCTION

Starch contributes greatly to the textural properties of many foods and has many industrial applications as a thickener, colloidal stabilizer, gelling agent, bulking agent, water retention agent, adhesive, etc. Interest in new value-added commercial products has resulted in many studies being carried out on the morphological, rheological, thermal and textural properties of corn and potato starches (1-4). Although native starches have many uses in food products, modified starches have almost unlimited food and nonfood applications (5,6). As a useful method for the production of modified starch, gamma irradiation produces free radicals on starch molecules that can alter their size and structure (7-10). Gamma irradiation activates physicochemical transformations in starch granules. The breakdown of glycosidic bonds and decomposition of macromolecules accompanied by the creation of new macromolecules with shorter chains is the preferential reaction occurring under the influence of irradiation (11). Decreases in both the crystalline phase content and the ordered distributions of amylose and amylopectin macromolecules in starch granules were previously reported (12).

Several studies on the effects of ionizing radiation on wheat starch (13,14) and barley endosperm (15) have been conducted. Gamma irradiation is capable of hydrolyzing chemical bonds, thereby cleaving large molecules of starch into smaller fragments of dextrin that may be either electrically charged or uncharged as free radicals. These changes may affect the physical and rheological properties of irradiated foods, resulting in increased solubility of starch (16), decreased swelling property (17), and decreased relative viscosity (18) of starch paste. Other effects of irradiation include structural changes of starch molecules resulting in changes in sensitivity to enzymes, lowering of the melting point, changes of the absorption spectrum and cleavage of the α-1,4 chain of the starch molecule (18).

This study investigated changes in physicochemical properties of gamma-irradiated (0~40 kGy) corn starch as an economic and more environmentally friendly technique of modified starch production.

MATERIALS AND METHODS

Sample preparation

Corn starch containing 12% moisture, 0.1% ash, 0.38%

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protein and 37 ppm of SO_2 was obtained from Samyang Genex Co., Korea.

Gamma irradiation

Corn starch was packed in PVC bags (ϕ 5×H 8 cm) to reduce dose variations among samples. The gamma irradiation flux from the 100,000 Ci gamma irradiator (^{60}Co, Korea Atomic Energy Research Institute) to the sample was 1 kGy per hour at ambient temperature (18±4°C) and the total dose applied was 0~40 kGy, which was confirmed by ceric cerous dosimeter (USA) and Harwell Amber Perspex dosimeter (3042 Batch H). All the materials were kept at room temperature under constant atmosphere and humidity.

Electron microscopy

Electron microscopy studies were performed on the native and gamma-irradiated corn starch samples with an S-2380N Scanning Electron Microscope (Hitachi, Ltd., Tokyo, Japan). The samples were covered with a ca. 20 nm thick gold layer in a vacuum evaporator. Amplification of 2000 was applied.

Molecular weight distribution of starch

The starch solution was prepared by solubilizing a 0.5 g (dry basis) of starch with 50 mL of 90% dimethyl sulfoxide (DMSO) solution in a boiling water bath for 1 hr with constant stirring for 24 hr at room temperature. Starch was precipitated from an aliquot of DMSO solution with excess absolute ethyl alcohol and centrifuged at 3000×g for 20 min. The precipitated amorphous starch pellet was resolubilized in deionized water (15 mL, 95°C) and stirred with a magnetic stirrer in a boiling water bath for 30 min.

Each starch solution was filtered through a 1.0 μm syringe filter (Whatman, UK), and then the filtrate (5 mL) was injected into a GPC column (26 mM×100 cm, Sepharose CL-2B). The mobile phase was 0.02% NaCl containing 0.02% NaN_3 at a flow rate of 0.5 mL/min. The acid-alcohol modified starch solution was prepared by solubilizing 10 mg (dry basis) of starch in 15 mL deionized water and stirring with a magnetic stirrer in a boiling water bath for 1 hr. The total sugar content of the fractionated solution was measured according to phenol-sulfate method (19).

Solubility

Starch (3.0 g, dry weight) was dissolved in 100 mL of water at room temperature for 5 min, and was filtered by using a glass filter (GF/C, Whatman, UK). The solubility was the ratio in weight of the dried supernatant to the initial weight of the dry starch.

Hydrolysis residue

Starch (3.0 g, dry weight) was dissolved in 100 mL of distilled water at room temperature for 5 min, and was filtered by using a glass filter (GF/C, Whatman, UK).

The total sugar content of the filtrate was measured according to the phenol-sulfate method (19) The hydrolysis ratio was denoted as follows;

Hydrolysis ratio = amount of total solubilized sugar/ weight of original starch (dry basis).

In addition, the hydrolysis residue was also denoted as follows;

Hydrolysis residue (%) = (1 − Hydrolysis ratio) × 100.

Number-average degree of polymerization (DP_n)

The number-average degree of polymerization (DP_n) of native and gamma-irradiated corn starch samples was measured according to the phenol-sulfate method (19) and modified Park-Johnson method (20). Starch samples (15 mg) were dispersed in 1 mL of concentrated mobile phase (1.0 M KOH) for 8 hr under gentle magnetic stirring and then diluted to 10 mL with distilled water. After filtration (0.45 μm; regenerated cellulose syringe filter), fractionation was performed with a Sepharose CL-2B column (74×1.6 cm^2, Amersham Pharmacia Biotech, Sweden) at a flow rate of 30 mL/hr using degassed 0.1 M potassium hydroxide containing 0.02% (w/v) sodium azide. Fractions (5 mL) were collected and their iodine binding (620 nm) was examined.

Starch viscosity determination

The viscosity of the starch paste was determined using a Rapid Visco-Analyzer (New Port Scientific Pty Ltd Narrabean, Australia). The starch sample was added to an aluminum canister containing distilled water at room temperature. The canister was placed in an RVA heating block. Thermocline software controlled the heating and cooling cycles: The 7% suspension of corn starch (dry weight basis) was heated with gentle mixing from 25°C to 95°C at a speed of 3.5°C/min, maintained for 10 min at 95°C and then cooled to 50°C at a speed of 4.5°C/min, and then held at this temperature for 5 min. The temperature of initial gelatinization was measured when the curve reached 20 B.U.

Transmittance of the starch solution

The transmittance of native and gamma-irradiated corn starch samples was analyzed using a spectrophotometer (UVIKON 930, Swiss) at 650 nm. Ten mg of isolated corn starch was dispersed in 10 mL (0.1%, w/v) of distilled water and heated to 100°C on a hot plate with gentle stirring. Transmittance of the solution was measured at room temperature.

X-ray diffraction measurement

Monochromatic Cu-Ka radiation (wavelength=1.542

Å) was produced by an X-ray diffractometer (Rigaku Geigerflex G/max II-A, Japan). The starch powders were packed tightly in a rectangular aluminium cell (20×20 mm, thickness 0.15 cm). The samples (density about 1.10 ×10 g/cm^3) were exposed to the X-ray beam with the X-ray generator running at 30 kV and 30 mA. The scanning regions of the diffraction angle 2θ were 10°~40°, which covers all the significant diffraction peaks of starch crystallites (21). The degree of crystallinity of samples was quantitatively estimated following the method of Nara & Komiya (22). A smooth curve which connected peak baselines was computer-plotted on the diffractograms. The area above the smooth curve was taken to correspond to the crystalline portion (A_c), and the lower area (A_a) between the smooth curve and a linear baseline which connected the two points of intensity at 2θ of 40° and 10°. The upper diffraction peak area and total diffraction area over the diffraction angle 10°~40° 2θ were integrated. The ratio of upper area to total diffraction area, $A_c/(A_c+A_a)$, was taken as the degree of crystallinity.

IR

The IR spectra of dried powdered corn starch samples were run in the form of KBr pellets on a Bio-Rad FTIR 60 spectrophotometer in the frequency range of 4000 to 400 cm^{-1}.

Differential scanning calorimetry (DSC)

Native and gamma-irradiated corn starch samples were thermally analyzed using a differential scanning calorimeter (Seiko Instrument Inc., DSC 6100, Chiba, Japan) calibrated with indium. The sample pan was prepared with starch and distilled water (1:4, w/w) and analyzed from 20 to 180°C at a scanning rate of 10°C/min under nitrogen. An empty pan was used as a reference. Samples for retrogradation experiments were heated in an oven for 20 min at 105°C and then stored at 4°C for 28 days, before DSC analysis. DSC scans were performed for gelatinization studies. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures together with gelatinization enthalpy (ΔH) were quantified. All results are the means of at least three measurements from each of three tubers. Degree of retrogradation was denoted as follows;

Degree of retrogradation=(ΔH for melting of retrograded starch / original ΔH for melting).

Statistical analysis

Analysis of variance (ANOVA), correlation analyses (SAS Institute, Cary, NC, USA), and the t-test were used to compare means of data and to determine least significant difference (LSD) at α=0.05. Significant values were accepted at $p<0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Morphology of starch granules

The dry, native and gamma-irradiated (5~40 kGy) corn starch samples (12% of moisture) were examined by using SEM, revealing that not only native corn starch granules, but all gamma-irradiated (5~40 kGy) corn starch granules showed polygonal surface structure, which is the typical shape of corn starch (23). As shown in Fig. 1, the granule structure of corn starch was not changed even after the treatment with 40 kGy of gamma irradiation. However, Kwon et al. (24), observed a significant increase in roughness of the granule surface of gamma-irradiated acorn (39% of moisture) with the intensity of 10 kGy, but no significant configuration changes were observed at 1 kGy, meaning that gamma irradiation may affect the granule structure, according to the type and water content of starch.

Hydrolysis and number-average degree of polymerization (DP_n)

As shown in Fig. 2, the hydrolysis of gamma-irradiated

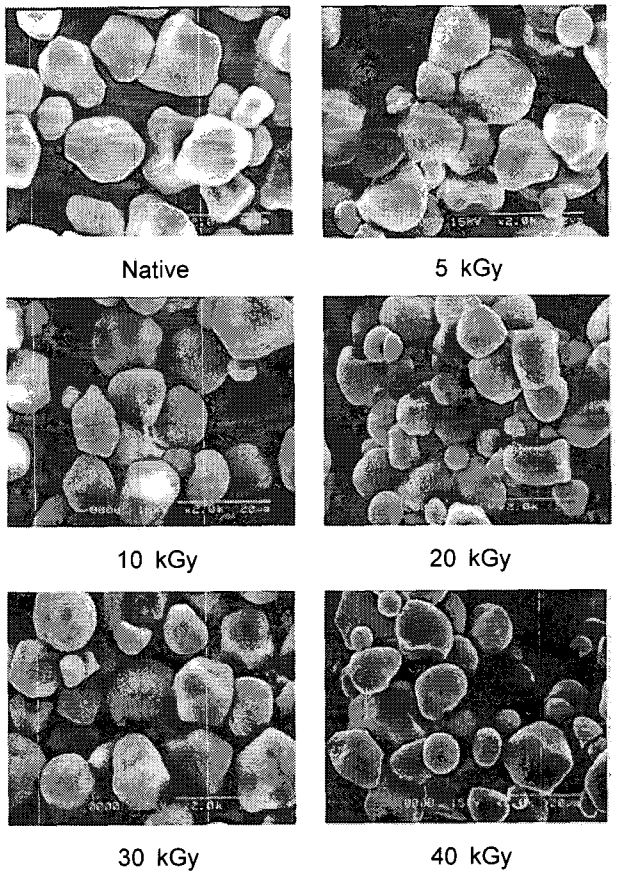

Fig. 1. Scanning electron microphotographs of native and gamma-irradiated corn starches (×2000).

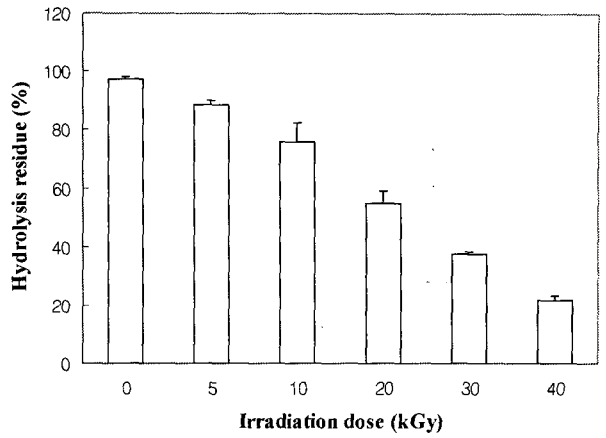

Fig. 2. Effect of gamma irradiation on change in hydrolysis residue of corn starch.

corn starches decreased with increased irradiation dose; native corn starch showed 97.2% of hydrolysis residue, and gamma-irradiated starch samples showed 88.9% ~ 21.8%.

Fig. 3 shows the changes in the number-average degree of polymerization (DP_n) of native and gamma-irradiated corn starches as a function of hydrolysis time. DP_n of corn starch samples obviously decreased with the increase in gamma irradiation intensity. The results indicated the decrease of DP_n mostly occurred at the level of 5 kGy. Native corn starch showed an 1150 DP_n, but DP_n of gamma irradiated starch at 5 kGy sharply decreased to 620. There was a gradual decrease in DP_n at 10 kGy, and more gently sloping decrease at the higher intensity of 20 kGy and 30 kGy, and reaching 170 of DP_n at 40 kGy. These results showed the close relationship between the hydrolysis and the number-average degree of polymerization of starch samples, and were similar to the result of Cho et al. (25). Besides, according to Kang and Byun (26), the solubility of corn starch increased with the intensity of gamma irradiation

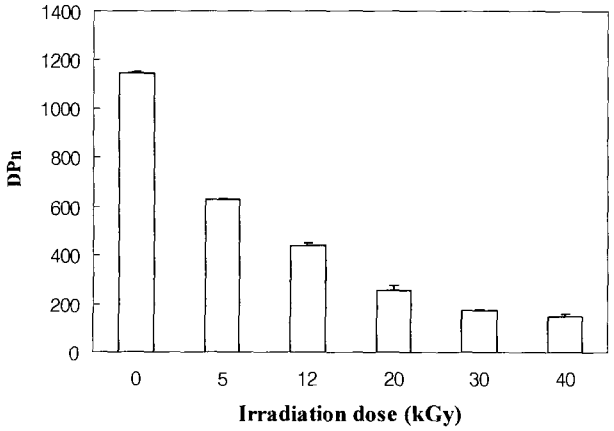

Fig. 3. Effect of gamma irradiation on change in number average degrees of polymerization (DP_n) of corn starch.

up to 50 kGy, because of the increase in the number of terminal aldehyde groups occurring from the partial damage in amylose and amylopectin chains. In addition, according to Radley (27), corn starch irradiated at the high dose of 1000 kGy, was dissolved and formed opaque solution even in cold water.

Molecular weight distribution of starches

Solubilized native and gamma-irradiated corn starches in DMSO were fractionated into two main components by Sepharose CL-2B gel (Fig. 4), as the high molecular component of the starch eluted first as a void volume fraction (the first fraction) and that of smaller molecular weight molecules (the second fraction) eluted subsequently. The first fractions in the profiles corresponded to amylopectin, and the second fractions to the low molecular weight molecules (28). For the gamma-irradiated starches, the areas of the first fractions decreased with increased irradiation dose, while the areas of the second fractions increased. This indicated that the degradation of amylopectin to low molecular weight molecules was due to the partial hydrolysis of corn starch by gamma irradiation. Especially, corn starch irradiated at 40 kGy was almost all composed of degraded amylopectin fractions, therefore, gamma irradiation of corn starch seemed to cause the degradation of amylopectin, leading to increases in low molecular weight molecules.

Pasting properties of starches

Pasting properties of starch samples determined by a Rapid Viscoanalyzer (RVA) are shown in Fig. 5 and Table 1. Native corn starch exhibited a larger peak

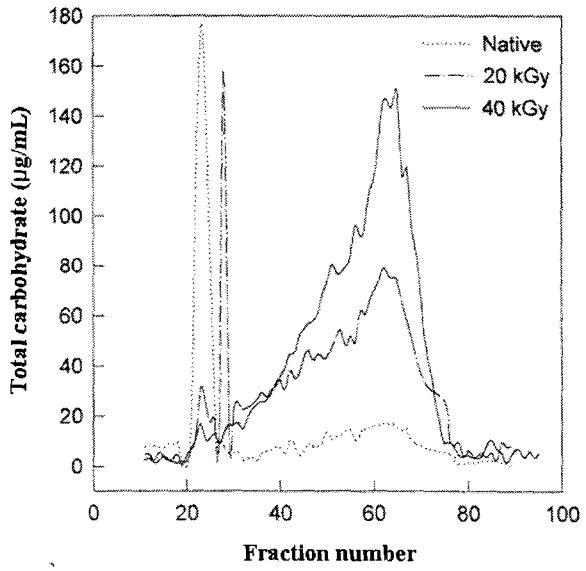

Fig. 4. Effect of gamma irradiation on change in molecular weight distribution of corn starch by Sepharose CL-2B gel fractionation.

Table 1. Effect of gamma irradiation on change in viscosity of 7% pasting solution of corn starch

Condition	Pasting temperature (°C)	Peak viscosity (cP)	Break down (cP)	Holding (cP)	Final viscosity (cP)	Set back (cP)
Native	60.6	1242	439	803	1357	554
5 kGy	59.2	1183	817	366	700	334
10 kGy	57.5	974	756	218	395	177
20 kGy	55.6	502	408	94	158	64
30 kGy	54.7	187	137	50	82	32
40 kGy	51.2	139	98	41	71	30

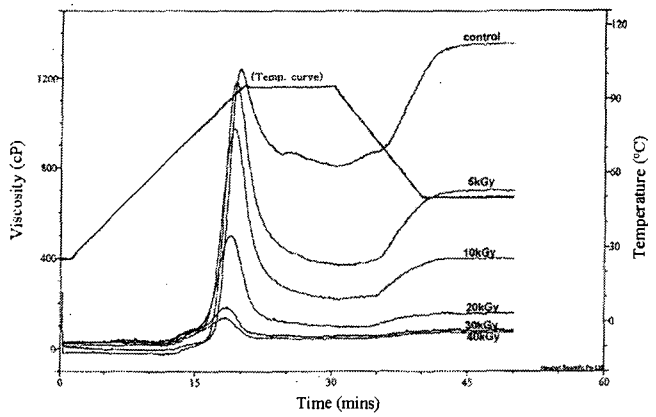

Fig. 5. Effect of gamma irradiation on change in pasting viscosity of corn starch (7%, w/v).

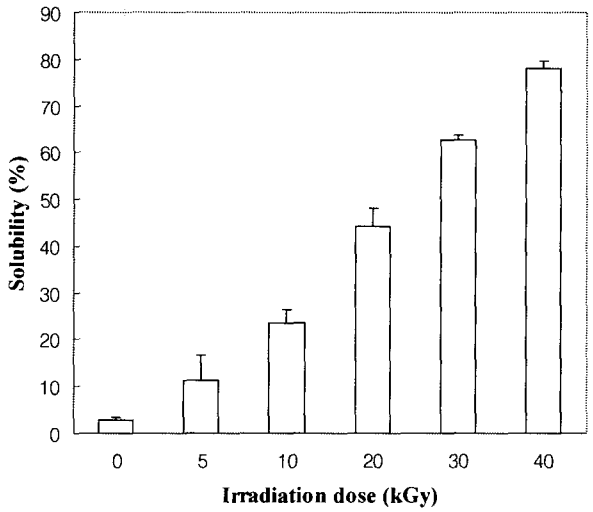

Fig. 6. Effect of gamma irradiation on changes in solubility of corn starch.

viscosity than gamma-irradiated corn starch samples; peak viscosity decreased with the increase in gamma irradiation intensity. The peak viscosities of each of the gamma-irradiated corn starches, ranging from 974 cP to 139 cP, were greater than that of the native starch (1242 cP). It decreased about 90% at 40 kGy, compared with native corn starch. In addition, initial pasting temperature decreased with the increase in gamma irradiation intensity. Results of pasting properties of starches measured by the RVA confirmed the high solubility of gamma-irradiated starches. As shown in Fig. 6, the solubility of native corn starch was less than 3%, but it obviously increased with the increase in gamma irradiation intensity; starch sample irradiated at 40 kGy showed 78% of solubility. The degradation of amylopectin could cause the disruption of the granular structure. There seemed to be a high negative correlation between solubility and pasting viscosity, and also a high positive correlation between solubility and hydrolysis of gamma-irradiated starches.

Pasting temperatures were 60.6°C and 51.2°C for native corn starch and gamma-irradiated corn starch at 40 kGy, respectively. Gelatinization was formed when the crystalline structure of the granules collapsed due to excessive swelling and the consequent loss of granular structure (29,30).

The decrease in peak viscosity of gamma-irradiated starches with the increase in irradiation dose could be due to its weaker water binding capacity and granular rigidity (31), and the higher setback (554 cP) value of the native corn starch was associated with a greater tendency of the starch to retrograde (32). The decrease in peak viscosity of the modified starches was attributed to the formation of low molecular weight molecules due to the degradation of amylopectin. However, because the pasting peak could still be observed even in the gamma-irradiated samples, low molecular weight molecules seemed to retain the crystalline structure internally.

According to the profile of final viscosity, which was measured after samples cooled to 50°C at a speed of 4.5°C and were then held at this temperature for 5 min, native sample showed an increase in final viscosity to 1357 cP (higher than its peak viscosity, 1242 cP), but all gamma-irradiated starch samples at different intensities had decreased final viscosities (lower than their peak viscosity). Degradation of starch has been considered to be responsible for the viscosity changes caused by gamma irradiation (33-35). A similar decrease in amylogram units of irradiated-wheat starch has been reported (36). Deschreider (37) has attributed these changes to shortening of polysaccharide chains, depending on the

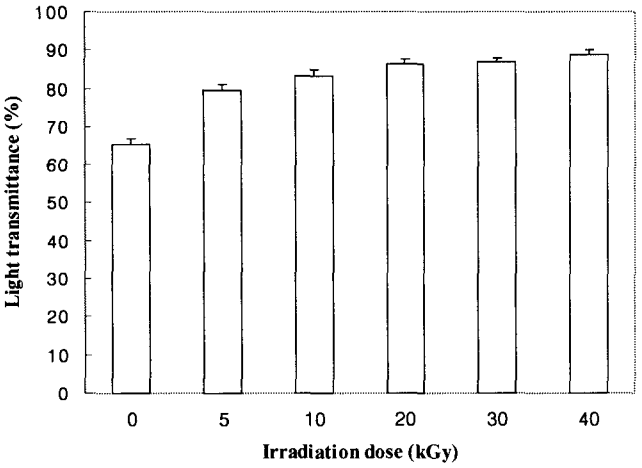

Fig. 7. Effect of gamma irradiation on change in light transmittance of corn starch.

irradiation dose. The shift in the iodine complex toward shorter wave lengths with irradiated-amylose also supports this view (38).

Optical transmittance pattern

When the transmittance of 0.1% starch solution was measured, native corn starch showed 63.7%, but gamma-irradiated corn starches at 5 kGy increased sharply to 80.0%, and then gradually from 10 kGy (Fig. 7). This indicated that the hydrolysis of corn starch induced by gamma irradiation increased the transmittance of starch solution, making it more transparent. Clarity, turbidity and whiteness of starch solutions are very important for a variety of industrial applications. In addition, content and structure of amylose, amylopectin, lipid, sugar and salt affect the optical transmittance pattern of starch (39, 40).

IR analysis

As shown in Fig. 8-1, native and gamma-irradiated corn starch samples showed a similar IR patterns to each other. Both showed the characteristic peak of hydroxyl group at the frequency, 3000~4000 cm^{-1}, and the characteristic peak of carbonyl groups at 1650~1850 cm^{-1} (41). However, when Fig. 8-2 showed the more detailed region at 1600~1700 cm^{-1} for better comparison, some variations were observed; starch sample treated at 40 kGy showed a decrease in absorbance, compared to native starch, indicating a loss of carbonyl groups. In other words, gamma irradiation could affect the basic structure of anhydrous glucose through the IR analysis.

Degree of crystallinity

The X-ray diffractograms of native and gamma-irradiated corn starches are presented in Fig. 9. There was no significant difference in X-ray diffractograms between native and gamma-irradiated corn starches at all intensities, i.e., both native and all gamma-irradiated corn starches showed typical A-type diffraction pattern with

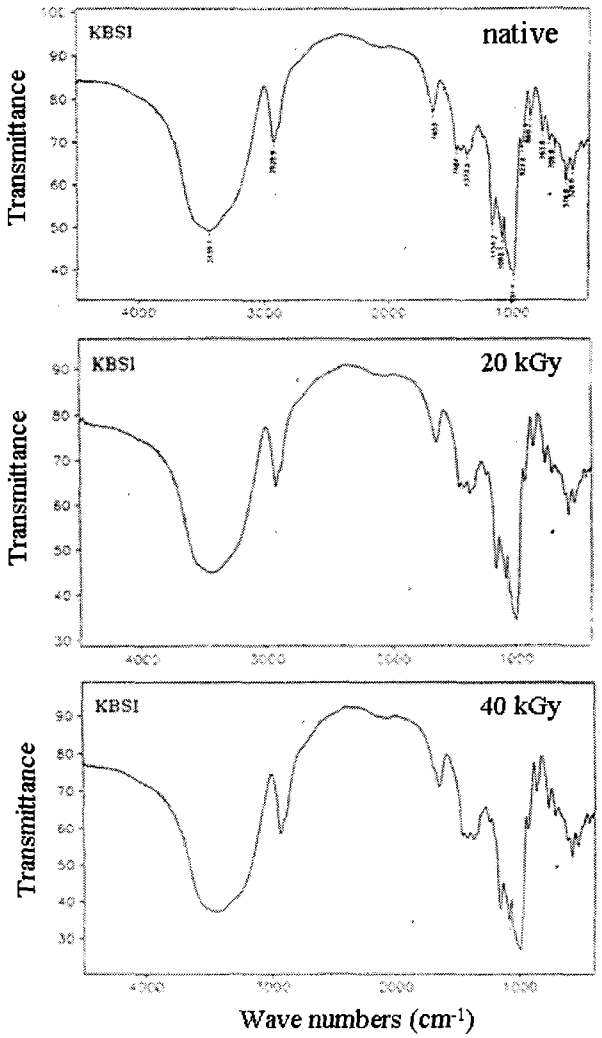

Fig. 8-1. Effect of gamma irradiation on change in IR spectrum of corn starch.

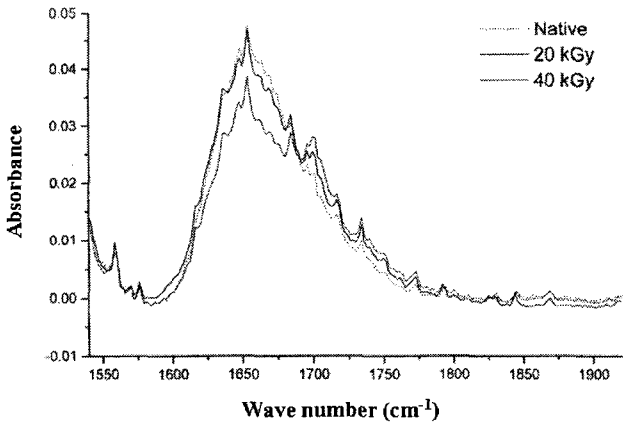

Fig. 8-2. Effect of gamma irradiation on change in IR spectrum of corn starch at the frequency of 1600~1700 cm^{-1}.

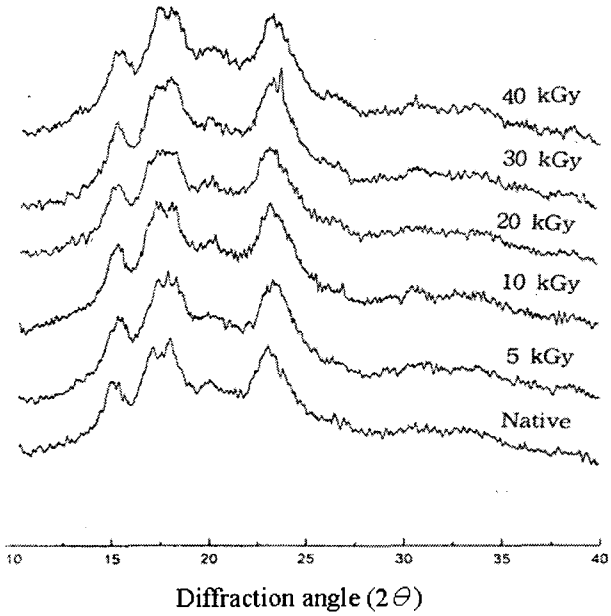

Fig. 9. Effect of gamma irradiation on change in X-ray diffractogram of corn starch.

strong reflection at 15°, 17.5° and 23° (42). The similar X-ray diffraction pattern indicates that the organization of crystalline structure of starch was not affected by gamma irradiation. There was no peak at higher than 30°, which is considered an area for amorphous structure. Consequently, gamma irradiation of corn starch did not significantly affect its crystalline structure, but severely affected amorphous structure, which seemed to contain weak interactions, compared with crystalline structure. The result was in agreement with previous finding, which evaluated modified acorn starch after gamma irradiation at 10 kGy (43). Even though the hydrolysis of corn starch with gamma irradiation occurred, there was no change in relative degree of crystallinity, as well as in crystallinity. It seemed that gamma irradiation of dry corn starch did not provide any molecular movement of starch, so that it may not be any interactions between starch molecules, leading no change in crystallinity.

Thermal properties of starches

Thermal properties of starch samples during heating determined by DSC are shown in Table 2. Onset and peak temperatures of starch gelatinization were the highest for native starch. Onset and peak temperatures decreased slightly as gamma irradiation dose increased. The T_o (gelatinization onset temperature) of gamma-irradiated starches at 5 kGy~40 kGy decreased from 64.7°C for native starch to 64.2°C~62.5°C. Defective crystalline structure of corn starches irradiated at higher

Table 2. Effect of gamma irradiation on change in melting temperature and enthalpy of corn starch

	Native	5 kGy	10 kGy	20 kGy	30 kGy	40 kGy
T_o (°C)	64.7	64.2	63.7	63.5	62.6	62.5
T_p (°C)	69.3	68.8	68.3	68.2	67.3	67.2
T_c (°C)	74.2	73.6	73.4	73.6	73.1	71.7
ΔH (J/g)	14.63	14.56	15.64	14.60	13.83	12.67

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature.

intensity may account for its lower onset gelatinization temperature. Transition enthalpy (ΔH) of gamma-irradiated starches at 5 kGy decreased from 14.63 J/g to 14.56 J/g for native starch, but increased at 10 kGy, and then decreased again with the further irradiation intensity. Inouchi et al. (44) reported that increasing amylose content decreased the enthalpy change. However, T_p (peak temperature) for native corn starch was 69.3°C, and that of gamma-irradiated starches decreased insignificantly. Wootton and Bamunuarachchi (45) have observed the distinctive increase in peak temperature and the decrease in enthalpy of dextrin obtained from wheat starch, compared to the native wheat starch. Suh and Kim (46) have reported the decrease in enthalpy during heating of corn starch at temperatures above 190°C.

In addition, retrogradation properties were studied by analysing the melting endotherm of recrystallized amylopectin by DSC; thermograms are shown in Table 3. A significant difference in retrogradation between native and gamma-irradiated corn starches was observed from the investigation on the degree of recrystallization after storing the gelatinized corn starch samples for 28 days at 4°C. Native corn starch did not show any change in ΔH, but gamma-irradiated starches showed some changes: samples treated at 5 kGy showed increases in ΔH from 14.56 mJ/mg at 0 day to 18.42 mJ/mg at 28 day; sample at 10 kGy showed an increase from 15.64 mJ/mg to 23.54 mJ/mg during the period, and; sample at 40 kGy showed the increase from 12.67 mJ/mg to 18.72 mJ/mg. In other words, ΔH in 28 day increased with the increased gamma irradiation intensity up to 30 kGy, but decreased at 40 kGy. The results implied that starch hydrolysis by gamma irradiation could lead to the retrogradation due to the reunion between the hydrolyzed starches to some degree, but excessive hydrolysis of starch might bring the degradation of starch to very small molecules for which recrystallization would not be possible.

Table 3. Effect of gamma irradiation on change in melting temperature and enthalpy of corn starch before and after retrogradation

Samples		T_O (°C)	T_P (°C)	T_C (°C)	ΔH (mJ/mg)	Degree of retrogradation
0 day	0 kGy	64.73±0.09[1)a2)]	69.47±0.09[a]	74.43±0.12[a]	14.65±0.01[b]	-
	5 kGy	64.37±0.12[a]	68.73±0.12[b]	73.73±0.09[b]	14.58±0.01[c]	-
	10 kGy	63.97±0.22[b]	68.27±0.09[c]	73.37±0.09[cd]	15.63±0.01[a]	-
	20 kGy	63.53±0.09[c]	68.07±0.09[c]	73.60±0.06[bc]	14.62±0.01[b]	-
	30 kGy	62.73±0.09[d]	67.50±0.12[d]	73.27±0.09[d]	13.83±0.01[d]	-
	40 kGy	62.50±0.12[d]	68.73±0.12[b]	71.67±0.09[e]	12.67±0.01[e]	-
28 days	0 kGy	45.57±0.09[a]	53.03±0.09[a]	60.33±0.09[d]	14.52±0.01[f]	0.99±0.01[f]
	5 kGy	38.33±0.12[d]	48.27±0.09[d]	60.23±0.09[d]	18.42±0.01[e]	1.25±0.01[e]
	10 kGy	39.03±0.09[b]	49.37±0.09[c]	63.33±0.12[a]	23.53±0.01[c]	1.53±0.01[c]
	20 kGy	37.73±0.09[c]	49.23±0.09[c]	61.60±0.06[c]	24.06±0.01[b]	1.67±0.01[b]
	30 kGy	38.70±0.12[e]	50.10±0.12[b]	63.03±0.09[b]	24.25±0.01[a]	1.78±0.02[a]
	40 kGy	39.27±0.09[b]	49.83±0.09[b]	55.90±0.06[e]	18.74±0.01[d]	1.47±0.01[d]

T_O: onset temperature, T_P: peak temperature, T_C: conclusion temperature.
[1)]Mean±SEM (standard error of mean).
[2)]Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test ($p<0.001$).

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