Chemical and Molecular Properties of Irradiated Starch Extrudates[1]

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ABSTRACT

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Corn starch samples containing 0, 25, 50, and 70% amylose were γ-irradiated at 0 (native), 5, 10, 20, and 30 kGy. All starch samples were extrusion cooked at 140°C barrel temperature, 140 rpm screw speed, and 18% moisture content (db) using a C. W. Brabender single-screw extruder. Starches irradiated at a 20-kGy dosage were extrusion cooked with and without hydrogen peroxide, potassium persulfate, or ceric ammonium nitrate. The quantity of free radicals produced on the starch increased with increasing irradiation dosages (0–30 kGy). Stability of the free radicals was greater for high-amylose starches than for those with low amylose. Extrusion-cooked starches had traces of free radical activity. Acidity of the irradiated starches increased (pH decreased) with increasing irradiation dosages. Gel permeation chromatographic separation of variously treated starches gave three fractions. Fraction I, mostly amylopectin, eluted at the void volume, whereas fraction II, mostly amylose, eluted at the latter part of the gel. Fraction 0, degraded products of amylopectin and amylose, mostly eluted closer to the total volume of the gel. Fraction I quantities of irradiated starches decreased with increasing irradiation dosages, whereas fraction II and III quantities correspondingly increased. Native starches with 0% amylose exhibited more than a fourfold decrease in fraction I content, whereas 70% amylose native starches showed less than a twofold decrease due to increasing irradiation dosages from 0 to 30 kGy. Extrusion cooking accelerated the degradation of fraction I for 0% amylose starches more than for 70% amylose starches. Both 2.5 and 5% concentrations of chemical additives caused excessive degradation of fraction I of starches irradiated at 20 kGy, consequently increasing reducing powers. Ceric ammonium nitrate caused the highest decrease in the iodine binding capacity of the starches. Fraction I clearly suffered more degradation due to irradiation, extrusion, addition of chemical agents, or a combination of these.

Starch, one of the most abundant ingredients in many food systems, provides structure, texture, consistency, and appeal (Rogols 1986). Two major starch fractions, amylose and amylopectin (Banks and Greenwood 1975, Takeda et al 1990), are present in different proportions in starches. These fractions are responsible for different functional properties in the food systems (Jane and Chen 1992). A better understanding of the structural and functional properties exhibited by food systems with different proportions of amylose and amylopectin (Mercier 1973; Takeda et al 1988, 1989) has inspired many food scientists to design new products or modify traditional products.

Although native starches have many uses in food systems, modified starches have limitless food and nonfood applications (Mercier and Feillet 1975, Rogols 1986). One type of modification, γ-irradiation, produces free radicals on starch molecules that can alter their size and structure (Raffi et al 1980, Ciesla et al 1991, Grant and D'Appolonia 1991, Sabularse et al 1991). Food products subjected to γ-irradiation are widely recognized as safe for consumption (FAO/IAEA/WHO 1976). A γ-irradiation dosage of 10–50 kGy could improve nutritional quality of foods by destroying microorganisms, destroying enzymes, or sterilizing starchy foods (Radley 1960, Ehlermann 1983). Adam (1983) reported that γ-irradiation could convert an aqueous D-glucose to D-glucuronic acid or formaldehyde and convert crystalline D-glucose to arabinose or ribose. Although the radiolytic products produced by starch irradiation were similar, some configurational differences were reported (Raffi et al 1981). Applications of irradiation include the destruction of pathogens and other microorganisms causing food spoilage in meat, poultry products, vegetables, and fruits, and the modification of physiological stages, such as ripening and sprouting, of fruits and vegetables (Maehler 1985). Free radicals produced during irradiation are high-energy unpaired electrons. They can either subside over time, combining with other molecules or quenchers, or they can fragment the starch molecules (Phillips 1980, Sonntag 1980).

Extrusion cooking generates heat and shear, accelerating the rate of cooking, which brings about molecular degradation, changes in functional properties of the starch extrudates, or a combination of these (Harper 1981, Wen et al 1990, Kokini et al 1992). Della Valle et al (1991) diversified the use of extrusion cooking by producing chemical reactions inside the extruder barrel. Chinnaswamy and Hanna (1991) successfully used reactive extrusion processing to graft polymerized styrene onto starch. γ-Irradiated vinyl monomers and starches have been polymerized in the presence of a ceric (Ce^{+4}) ion (Jones and Elmquist 1973). Twin-screw extruders can continually produce starch-vinyl graft copolymers within brief residence times of 3–7 min (Carr et al 1992).

The objective of this research was to determine the effects of extrusion cooking on γ-irradiated starches that could possibly impart different functional properties to help design new food and nonfood systems.

MATERIALS AND METHODS

Samples

Four corn starch samples (0, 25, 50, and 70% amylose contents) were donated by American Maize Products Co., Hammond, IN. Ceric ammonium nitrate (C-3654, lot 129F3471), potassium persulfate (P-9392, lot 28F0841), and hydrogen peroxide (H-1009, lot 30H3542) used to process starches during extrusion cooking were purchased from Sigma Chemical Co., St. Louis, MO. Powdered starch was agglomerated by steadily dribbling distilled water onto starch vibrating in a rotary pan. The agglomerated starch was removed continuously, dried at room temperature for 48 hr to 9–12% moisture, and stored in polyethylene bags for future use.

Irradiation

All starch samples (400 g, "as is" basis) were irradiated at room temperature for 0, 9.1, 18.2, 36.5, and 54.7 hr at γ-irradiation dosages of 0 (native), 5, 10, 20, 23 or 30 kGy using a ^{60}Co irradiator (Nuclear Materials and Equipment, Apollo, PA). The dosage rate was standardized by Landuer Inc. (Glenwood, IL) at a level of 0.54879 kGy/hr.

Chemical Treatments

A portion of starches treated with 20-kGy radiation dosage was mixed with 2.5% potassium persulfate (PPS), ceric ammonium nitrate (CAN), or hydrogen peroxide (HP), on a dry weight basis. Control samples were run with no added chemical additives.

Extrusion

Starch samples were cooked in a single-screw extruder (C. W. Brabender, South Hackensack, NJ) with a 1.90-cm barrel diam-

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eter, a 20:1 ratio of barrel length to diameter, and a 3-mm cylindrical die. The extruder screw had a compression ratio of 3:1. Extrusion conditions (140°C barrel temperature, 140 rpm screw speed, and 18% sample moisture content) were kept constant throughout the study unless stated otherwise. Extrudates used for physical and chemical analyses were ground to pass through a 0.5-mm sieve in a Tecator Powdertec 3090 mill (Tecator, Inc., Germany).

Physicochemical Properties

The intensities of free radicals on the irradiated (0–30 kGy) starch samples were determined by electron paramagnetic resonance (EPR) using a Bruker ECS-106 electron spin resonance spectrometer (Bruker Instruments, Millerica, MA). The native (0 kGy) starch samples were kept as control. The intensities of EPR signal recorded as peak-to-peak distance (cm) were adjusted to 0.1 g of starch (db).

Free acidity was determined by titrating a starch suspension against 0.023*N* standardized NaOH solution. The starch suspension was prepared by suspending 1 g of starch sample in 10 ml of degassed, boiled distilled water in a beaker and continuously shaking for 5–10 min. The results were expressed as total neutralized acidity. The pH of each starch suspension was determined using a digital pH/millivolt meter (model 611, Orion Research, Cambridge, MA) and a pH electrode (combination electrode). Starch samples for pH measurements were prepared by suspending 1 g of starch in 25 ml of water at 25°C and agitating for 5–10 min.

The iodimetric method of amylose determination (Schoch 1964) was used to determine iodine binding capacity (IBC). The amount of free iodine in the titrant was charted by changes in electromotive force readings. Starch (100 mg, db) was solubilized in 5 ml of 1*N* KOH solution and brought up to 100.9 g with 10 ml of 0.5*N* KI solution. The IBC was calculated as:

$$\% \text{ IBC} = \frac{\text{bound iodine (mg) at zero intercept} \times 100}{\text{mg of sample weight (db)}}$$

Molecular Properties

Native and irradiated samples of 0, 25, 50, and 70% amylose starch, before and after extrusion cooking, were dispersed (~0.1%) in 95% dimethyl sulfoxide. About 5 ml of the 5-mg starch solution was fractionated by ascending gel permeation chromatography (GPC) (Chinnaswamy and Bhattacharya 1986) using a Sepharose CL 2B column (Pharmacia Fine Chemicals, Sweden) operating at a flow rate of 30 ml/hr. Distilled water containing 0.01% sodium azide was used as an eluent. The gel column was standardized for void volume (K_{av} 0) using blue dextran and total volume (K_{av} 1) using potassium chloride.

Fractions (4 ml) were collected; 2-ml aliquots from these fractions were used to determine carbohydrate content using phenol-sulfuric acid (Dubois et al 1956). These aliquots were measured at 490 nm against a glucose standard using a spectrophotometer and expressed on a starch basis for total volume fraction. The remaining 2-ml aliquots were mixed with 0.2 ml of 0.2% iodine solution and 3 ml of distilled water; absorbencies were read at 630 and 520 nm using a scanning spectrophotometer (Beckman DU-64). Amylose contents of these fractions were determined using a standard of corn amylose (essentially free of amylopectin [Sigma]) and expressed on a total volume fraction basis. The ratios of amylose or amyloselike contents of each fraction was calculated and reported as the 630/520 nm ratio. The λ_{max} of iodine-polysaccharide complexes of native, irradiated or PPS-, HP-, and CAN-treated samples were also determined.

Chain Length

The average chain lengths of the 0, 25, 50 and 70% amylose native and irradiated (20 kGy) starch samples, before and after extrusion, were determined using a pullulanase enzyme method (Biliaderis et al 1981). The pullulanase enzyme (0.7 ml, 32 IU, Sigma) was added to a solution of 0.1*M* acetate buffer containing 40-mg starch samples, pH 4.8. The solution was incubated for 48 hr at 37°C and boiled for 20 min to inactivate the enzymes. Insoluble materials were removed by centrifugation at 5,000 × *g* for 30 min. The supernatant was analyzed for starch and reducing sugar content using standard methods of Dubois et al (1956) and Dygert et al (1965), respectively. Chain length was determined using the following formula (Marshall 1974):

$$\text{Chain length} = \frac{\text{total carbohydrates (as glucose)}}{\text{increased reducing capacity after debranching (as glucose)}}$$

Statistical Analysis

All experimental values were a mean from two or more replicates. A statistical package (generalized linear model, SAS version 6, SAS Institute, Cary, NC) was used to analyze the data, to fit curves, and for the statistical validation of trends and patterns.

RESULTS AND DISCUSSION

Physicochemical Changes

The stability of free radicals on starches during extrusion

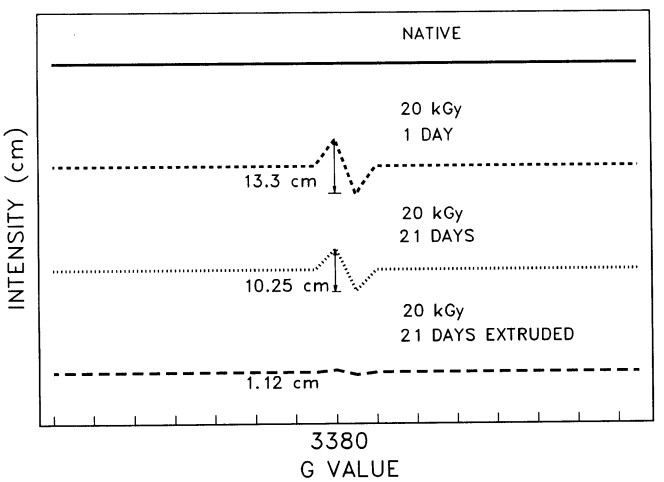

Fig. 1. The intensity of each treatment and the effect of storage time and extrusion cooking on the intensity of free radicals on 70% amylose starch irradiated at 20 kGy. Native = starches before extrusion and irradiation; 1 day and 21 day = starches irradiated at 20 kGy but not extruded.

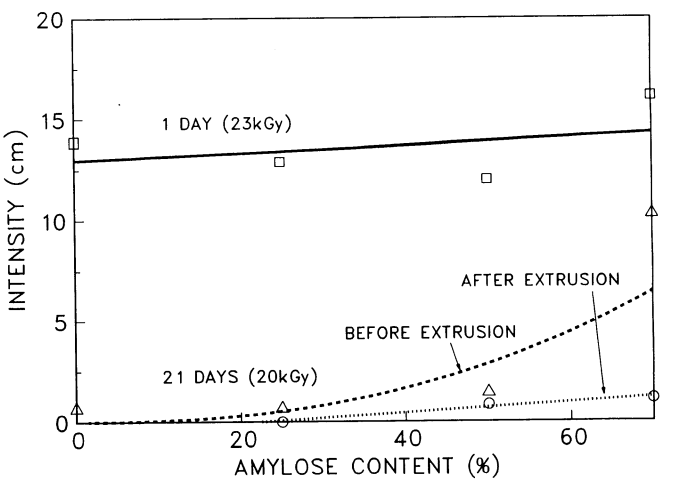

Fig. 2. Relationship between amylose content and the free radicals intensity index (cm/0.1 g of starch) of various starches irradiated at 20 and 23 kGy.

TABLE I
Effect of Irradiation Dosages on Starch Acidity (meq/g), Before and After Extrusion Cooking

Irradiation Dosage (kGy)	Amylose Content (%)							
	0		25		50		70	
	Before	After	Before	After	Before	After	Before	After
0 (Native)	0.52	0.37	0.66	0.69	0.76	0.66	0.81	0.75
5	0.58	0.43	0.77	0.76	0.94	0.73	0.95	0.81
10	0.70	0.59	0.94	0.92	1.00	0.93	1.09	0.88
20	1.21	1.07	1.25	1.18	1.28	1.16	1.32	1.10
30	1.68	1.45	1.55	1.51	1.45	1.43	1.52	1.26

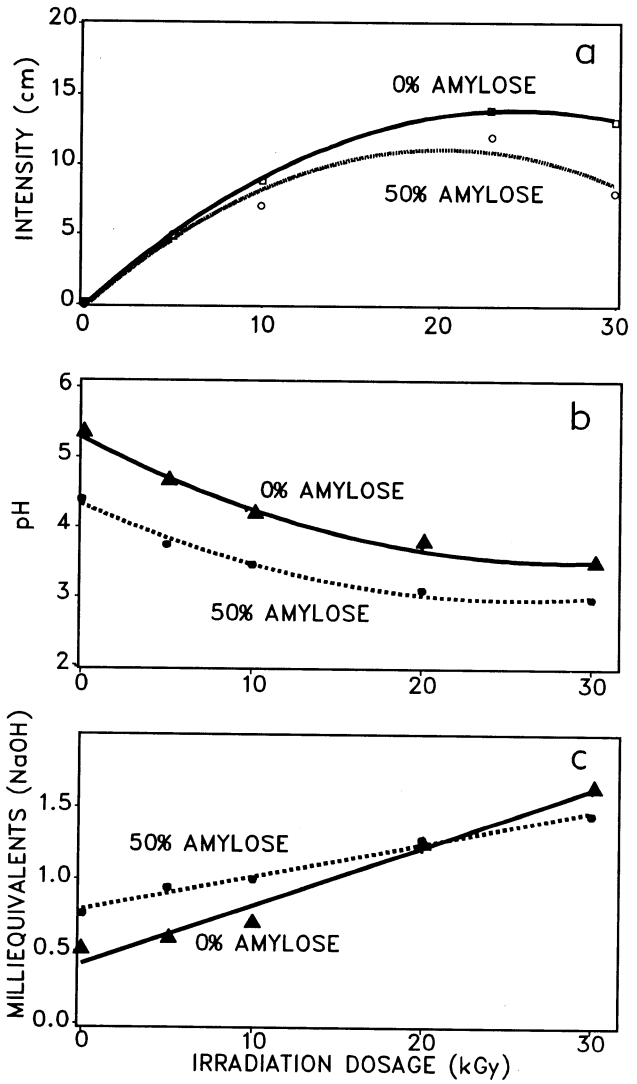

Fig. 3. Relationship between irradiation dosages and various properties of starch extrudates. **a**, electron paramagnetic resonance intensity. **b**, pH. **c**, acidity (meq of NaOH).

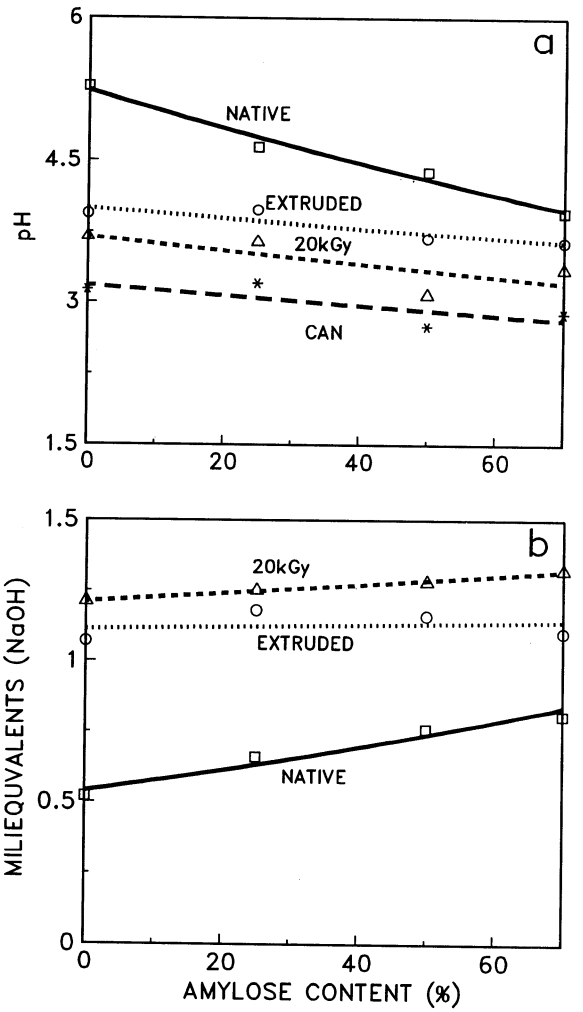

Fig. 4. Relationship between amylose content of starches and pH (**a**) and acidity (meq of NaOH) (**b**). Native = unextruded and unirradiated; Extruded = unirradiated, extruded; 20 kGy = irradiated at 20 kGy and extrusion cooked; PPS = treated with 2.5% potassium persulfate and extruded; HP = treated with 2.5% hydrogen peroxide and extruded; CAN = treated with 2.5% ceric ammonium nitrate and extruded.

cooking is an important factor in monitoring the irradiation-induced chemical reactions of starches. Therefore, to find the stability of the free radicals, 70% amylose starch was irradiated at 20 kGy and the intensity of free radicals was measured using EPR at one and 21 days after irradiation and extrusion. The intensity of free radicals on 70% amylose starch decreased from 13.3 to 10.25 cm after 21 days of storage (Fig. 1). The free radicals on extrusion-cooked irradiated starch (20 kGy) decreased in intensity to 1.12 cm after 21 days. The intensity of free radicals generally decreased with time, which is in agreement with the findings of Rayas-Solis (1987) and Stevenson and Gray (1989).

The EPR intensity of 0, 25, 50, and 70% amylose starches irradiated at 23 and 20 kGy were measured one and 21 days after irradiation, respectively, to investigate the effect of increasing amylose contents on the free radicals. The range of EPR intensities of 0–70% amylose starches after one day of irradiation was 12.0–16.1 cm, which decreased to 0.66–10.25 cm after 21 days of storage (Fig. 2). Extrusion cooking apparently terminated the free radicals on 0 and 25% amylose starches and decreased the intensity of free radicals on 50 and 70% amylose starches to 0.84 and 1.12 cm, respectively. These results indicated that the stability of the free radicals was higher in starches with higher amylose content. No previous work reported the effects of starch molecule structure, such as amylose and amylopectin, on the stability of

free radicals. This aspect needs further investigation.

Irradiation produces COOH groups in starch, so it was of interest to study the interrelationships between irradiation dosages and the levels of acidity and pH values. Increasing irradiation dosages from 0 to 30 kGy increased the acidity of native 0, 25, 50, and 70% amylose starches (0.52 to 1.68, 0.66 to 1.55, 0.76 to 1.45, and 0.81 to 1.52 meq/g, respectively, Table I). However, after extrusion cooking, the acidity values for all dosages (0–30 kGy) of irradiation decreased slightly. Increased acidity of irradiated starches could also be due to the breakdown of starch molecules, perhaps inducing COOH formation. Radley (1960) and El Saadany et al (1974) reported similar results, indicating that the breakdown of glycosidic linkages by the action of free radicals may cause an increase in the starch acidity. A slight decrease in acidity values after extrusion cooking could be due to the formation of salt forms of phosphates present naturally in starch.

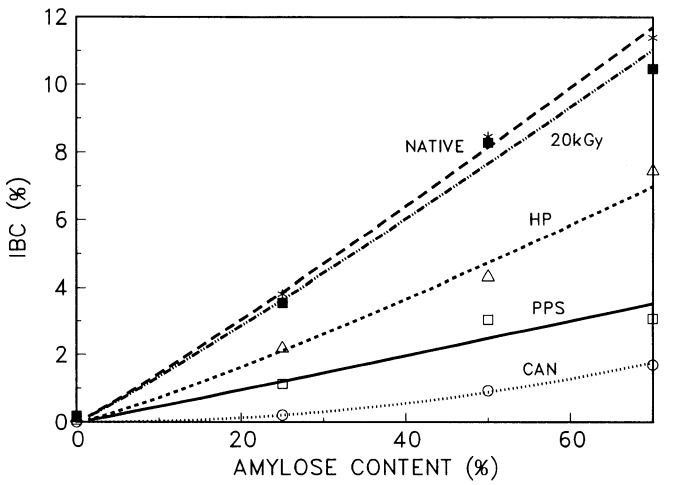

Fig. 5. Relationship between amylose content and iodine binding capacity (IBC) of native and PPS-, HP- and CAN-treated starches. Native = unextruded and unirradiated; 20 kGy = irradiated at 20 kGy and extrusion cooked; PPS = treated with 2.5% potassium persulfate and extruded; HP = treated with 2.5% hydrogen peroxide and extruded; CAN = treated with 2.5% ceric ammonium nitrate and extruded.

Intensity of free radicals and pH of starches exhibited quadratic relationships, and acidity exhibited linear relationship with the irradiation dosages. The results of free radical intensities, pH, and acidities of 0 and 50% amylose starches over irradiation dosage ranges of 0–30 kGy are shown in Figure 3. With increasing irradiation dosages of 0–30 kGy, intensities of free radicals of 0 and 50% amylose starches increased from 0 to 13.2 and from 0 to 8.0 cm, respectively. Maximum values were at 23 kGy (Fig. 3a). The pH of 0 and 50% amylose native starches decreased from 5.28 to 3.48 and from 4.38 to 2.96, respectively (Fig. 3b). The acidity for 0 and 50% amylose starches increased from 0.52 to 1.68 and 0.76 to 1.45 meq/g, respectively (Fig. 3c). The increases observed in EPR intensities and acidities are consistent with earlier reports of Radley (1960) and El Saadany et al (1974).

Increasing amylose contents (0–70%) of native starches decreased the pH from 5.28 to 3.95 and increased the acidity from 0.52 to 0.81 meq/g (Table I, Fig. 4a and b). A 20-kGy irradiation dosage drastically reduced the pH of 0% amylose native starch from 5.28 to 3.69 but marginally decreased the pH of 70% amylose native starch from 3.95 to 3.35 (Fig. 4a). Similarly, the acidity of 0 and 70% amylose native starches increased from 0.52 to 1.21 and 0.81 to 1.32 meq/g, respectively. Decreases in pH values and increases in acidity values for 0% amylose native starch were larger than those for 70% amylose native starch (Fig. 4a and b). Extrusion-cooked 0–70% amylose starches, irradiated at 20 kGy, shifted the pH values marginally upwards and the acidity values marginally downwards (Fig. 4a and b). Adding 2.5% CAN to starches irradiated at 20 kGy decreased pH to a 2.89–3.20 range (Fig. 4a), indicating that reactive chemicals, such as CAN, further accelerate the breakdown of irradiated starches.

IBC reflects the linear chains in starch. It is well established that longer linear chains or a higher number of linear chains show higher IBC. The IBC for native, irradiated, and HP-, PPS-,

TABLE II
Characteristics of Gel Permeation Chromatography Fractions of Irradiation-Modified Starches

Irradiation Dosage (kGy)	Amylose (%)	Fraction I[a] K_{av}	Fraction I Content (mg)	Fraction I Molecular Weight ($\times 10^7$)	Fraction II K_{av}	Fraction II Content (mg)	Fraction II Molecular Weight ($\times 10^6$)	Fraction III[b] Content (mg)	Fraction III[b] Molecular Weight ($\times 10^5$)
0 (Native)	0	0.2	4.69	4.5	0.3–1.2	0.31	3.7	...	...
	25	0.2	3.56	4.5	0.3–0.7	0.92	1.7	0.43	1.4
	50	0.2	2.95	4.5	0.4–1.2	2.05	0.4	...	...
	70	0.2	2.24	3.5	0.4–1.2	2.76	0.1	...	...
5	0	0.2	3.25	4.5	0.2–1.2	1.75	2.2	...	...
	25	0.2	2.40	3.5	0.2–1.2	1.63	4.7	0.97	1.4
	50	0.4	2.52	3.5	0.4–1.2	2.48	0.2	...	...
	70	0.4	2.15	2.7	0.4–1.2	2.85	0.1	...	...
10	0	0.2	2.91	3.5	0.2–1.2	2.09	3.7	...	...
	25	0.2	1.84	3.5	0.2–1.2	1.96	2.9	1.20	3.0
	50	0.4	2.46	3.5	0.4–1.2	2.54	0.4	...	...
	70	0.4	1.68	3.5	0.4–1.2	3.32	0.2	...	...
20	0	0.2	1.45	3.5	0.2–1.2	3.55	1.4	...	...
	25	0.2	1.40	3.5	0.2–1.2	2.18	3.7	1.42	2.3
	50	0.4	2.10	2.1	0.4–1.2	2.90	0.3	...	...
	70	0.4	1.51	2.7	0.4–1.2	3.49	0.2	...	...
30	0	0.2	1.06	3.5	0.2–1.2	3.94	0.8	...	...
	25	0.2	0.97	3.5	0.2–1.2	2.29	3.7	1.74	1.1
	50	0.4	1.68	0.6	0.4–1.2	3.32	0.4	...	...
	70	0.4	1.16	0.8	0.4–1.2	3.84	0.1	...	...

[a] K_{av} ranges begin at −0.1.
[b] K_{av} ranges fall between 0.7 and 1.2.

and CAN-treated starches are given in Figure 5. All three chemical agents (HP, PPS, and CAN) induce free radicals on the starch (Fanta and Doane 1989) that are enhanced by 20 kGy γ-irradiation. The IBC values of 0–70% amylose native starches decreased from 0.22–11.39% to 0.18–10.47% with 20-kGy irradiation. CAN decreased the IBC values 0–1.7% for 0–70% amylose starches.

Molecular Changes

The 0, 25, 50, and 70% amylose starch samples irradiated at 0–30 kGy, before and after extrusion cooking, were fractionated using GPC. The quantities of fractions I, II, and III of native, irradiated, and extrusion-cooked starch samples are given in Tables II and III. The molecular weights of the peak fractions given in Tables II and III were determined using the molecular weight standard curve given in Figure 6.

The 0% amylose native starch sample contained 4.69 mg of starch in fraction I, which decreased to 3.25, 2.91, 1.45, and 1.06 mg, respectively, for irradiation at 5, 10, 20, and 30 kGy (Table II). Similarly, fraction I quantity in 25, 50, and 70% amylose native starch samples decreased from 3.56 to 0.97, 2.95 to 1.68, and 2.94 to 1.16 mg, respectively, for 30-kGy irradiation. Molecular weights of fraction I in the 0 and 25% amylose native starch samples decreased from 4.5×10^7 to 3.5×10^7 for 30-kGy irradiation. Molecular weight of fraction I of 50 and 70% amylose native starch samples, however, decreased from 3.5 to 4.5×10^7 and 0.6 to 0.8×10^7, respectively, for increased irradiation. Fraction I corresponded mostly to amylopectin and fraction II corresponded mostly to amylose, which is in agreement with the findings of Chinnaswamy and Bhattacharya (1986), Chinnaswamy and Hanna (1990), and Jane and Chen (1992).

The 0 and 25% amylose native starch samples, irradiated at 30 kGy, showed almost a fourfold decrease in fraction I quantities, whereas the 50% amylose starch sample showed less than a twofold decrease. Decreases in the fraction I quantities of all starch samples were followed by corresponding increases in fraction II and III quantities (Table II). It appears that γ-irradiation causes breakdown of starch structure, resulting in a release of low molecular weight molecules. Ciesla et al (1991) found that irradiation destroys the order in which crystalline and amorphous regions of the molecule within a starch granule are arranged; this may explain the decreases observed in fraction I quantities of irradiated starch samples.

Starch samples with 0–70% amylose content and irradiated at 0–30 kGy, or those treated with HP, PPS, and CAN, were extrusion cooked and fractionated using GPC. The fraction I quantities of the native 0, 25, 50, and 70% amylose starch samples decreased to 2.33, 2.82, 1.53, and 1.39 mg after extrusion, (decreases of 50, 48, 48, and 38%, respectively) (Tables III and IV). Similarly, extrusion cooking of starch samples irradiated at 5, 10, 20, and 30 kGy decreased fraction I quantities by 33–76% (Tables III and IV). CAN treatment of the irradiated 0–50%

TABLE III
Characteristics of Gel Permeation Chromatography Fractions of Irradiation-Modified Starches after Extrusion Cooking

Irradiation Dosage (kGy)	Amylose (%)	Chemical Additive[a]	Fraction I[b] K_{av}	Fraction I Content (mg)	Fraction I Molecular Weight ($\times 10^7$)	Fraction II K_{av}	Fraction II Content (mg)	Fraction II Molecular Weight ($\times 10^6$)	Fraction III[c] Content (mg)	Fraction III Molecular Weight ($\times 10^5$)
0	0	...	0.2	2.33	4.5	0.2–1.2	2.67	2.9	...	...
	25	...	0.3	2.82	3.5	0.3–0.6	1.23	3.7	0.95	3.0
	50	...	0.2	1.53	3.5	0.2–0.6	1.75	4.7	1.73	2.3
	70	...	0.2	1.39	3.5	0.2–0.6	1.21	4.7	2.40	1.1
5	0	...	0.2	1.63	4.5	0.2–1.2	3.37	1.1	...	...
	25	...	0.3	1.62	3.5	0.3–0.6	1.86	6.1	1.53	6.4
	50	...	0.2	1.12	2.7	0.2–0.6	1.53	6.1	2.35	2.3
	70	...	0.2	1.04	3.7	0.2–0.6	1.27	0.5	2.69	1.4
10	0	...	0.2	1.04	3.5	0.2–1.2	3.96	2.2	...	...
	25	...	0.2	1.08	2.1	0.3–0.6	1.87	3.7	2.06	3.9
	50	...	0.2	0.72	1.7	0.2–0.6	1.48	0.4	2.80	1.8
	70	...	0.2	0.75	2.1	0.2–0.6	1.13	...	3.12	1.1
20	0	...	0.2	0.39	...	0.2–1.2	4.61	0.8	...	...
		HP	...	0.02	2.1	...	0.47	...	4.51	1.4
		PPS	...	...	...	...	0.15	...	4.85	1.1
		CAN	...	...	...	...	...	...	5.00	2.3
	25	...	0.2	0.64	2.1	0.3–0.6	1.74	1.7	2.62	3.9
		HP	...	0.53	1.7	...	0.78	...	3.68	0.9
		PPS	...	0.36	2.7	...	0.53	6.1	4.11	0.9
		CAN	...	...	...	...	0.04	...	4.96	3.0
	50	...	0.2	0.64	2.1	0.2–0.6	1.61	3.7	2.75	1.4
		HP	...	1.19	3.5	...	1.02	...	2.80	3.0
		PPS	...	0.43	2.7	...	0.36	4.7	4.21	0.5
		CAN	...	...	...	...	0.06	...	4.94	0.7
	70	...	0.2	0.64	2.1	0.2–0.6	0.98	...	3.38	1.4
		HP	...	1.06	3.5	...	0.46	...	3.48	0.7
		PPS	...	0.45	2.7	...	0.59	...	3.96	0.3
		CAN	...	0.16	...	...	0.19	...	4.65	0.3
30	0	...	0.2	0.25	...	0.2–1.2	4.75	0.4	...	...
	25	...	0.2	0.47	...	0.3–0.6	1.46	...	3.07	3.0
	50	...	0.2	0.70	1.7	0.2–0.6	1.70	1.7	2.60	1.8
	70	...	0.2	0.43	...	0.2–0.6	1.20	4.7	3.37	0.5

[a] 2.5% chemical treatments; HP = hydrogen peroxide; PPS = potassium peroxide; CAN = ceric ammonium nitrate.
[b] K_{av} ranges begin at −0.1.
[c] K_{av} ranges between 0.6 and 1.2.

amylose starch samples shifted the entire fraction I to fraction II. PPS and HP were less effective than CAN was on 25–70% amylose starch samples because fraction I quantities of 0.36–1.19 mg remained unchanged (Table III). Extrusion cooking of irradiated 0–70% amylose starch samples not only decreased the fraction I quantities but also decreased molecular weights from $3.5–4.5 \times 10^7$ for native samples to 2.1×10^7 for samples irradiated at 20 kGy (Table III).

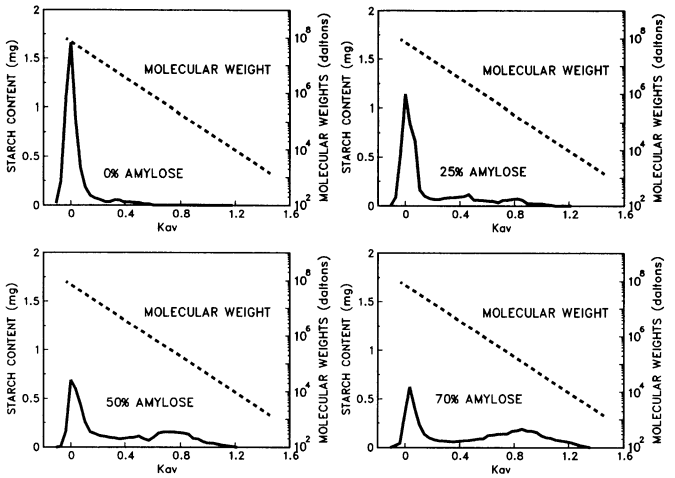

Fig. 6. Gel chromatographic fractionation profiles of 0–70% native amylose starches determined using a phenol-sulfuric acid method. The molecular weight standard curve is given on a log scale. The K_{av} ranges of fractions are given in Tables 2, 3, 5, and 7.

TABLE IV
Decrease (%) in Fraction I Contents of Irradiation-Modified Starches Due to Extrusion Cooking

Irradiation Dosage (kGy)	Amylose Content (%)			
	0	25	50	70
0 (Native)	50	48	48	38
5	50	33	56	52
10	64	41	70	55
20	73	54	70	58
30	76	52	58	63

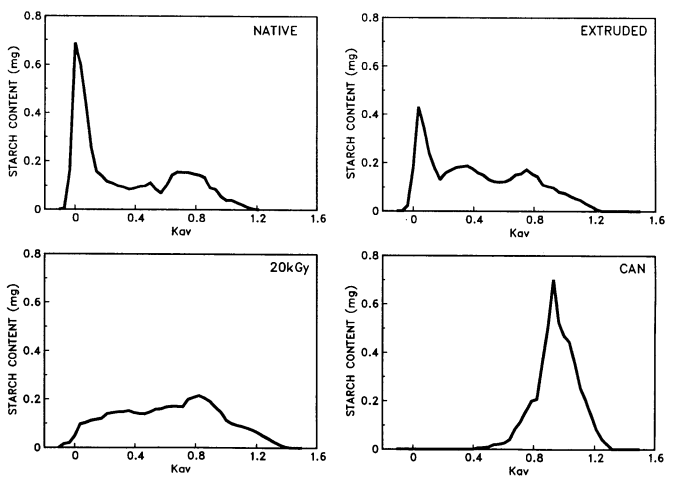

Fig. 7. Gel chromatographic fractionation profiles of 50% amylose starch. Native, extruded, irradiated (20 kGy), or treated with ceric ammonium nitrate (CAN). Determined by phenol-sulfuric acid method and expressed on starch basis.

The GPC patterns of native 0–70% amylose starch samples showed that 0% amylose starch had the largest fraction I eluted at the void volume of the gel (Fig. 6). As the amylose content increased from 0 to 70% amylose, the fraction I quantities consistently decreased, and the fraction II and III quantities correspondingly increased. This observation is consistent with the findings of Sokhey and Chinnaswamy (1992) and Chinnaswamy and Hanna (1990).

To study the changes in molecular weights of starches, 50% amylose starch samples were treated under various conditions and then subjected to GPC analyses using standard methods. The results are given in Figures 7–9 and Table V. Starch content of fraction I of 50% amylose native starch decreased from 2.95 to 1.53 mg after extrusion cooking and to 0.64 mg after both 20-kGy irradiation and extrusion cooking (Fig. 7). CAN reduced the fraction I quantity to zero. A similar pattern of starch degradation was found in the amylose blue values of various GPC fractions (Fig. 8).

The absorbance maxima of amylopectin, branched or short chain fractions, is ~520 nm; for amylose, linear or long-branched chain fractions, it is ~630 nm. The ratio of absorbencies of the amylose-iodine complex at 630/520 nm for 50% amylose native starch sample is given in Figure 9. Extrusion cooking of 50%

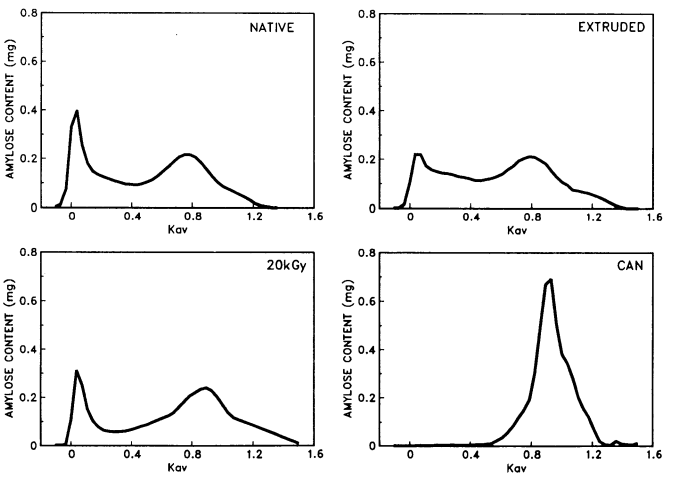

Fig. 8. Gel chromatographic fractionation profiles of of 50% amylose starch. Native, extruded, irradiated (20kGy), or treated with ceric ammonium nitrate (CAN). Determined from the measurements of iodine-amylose complex at 630 nm.

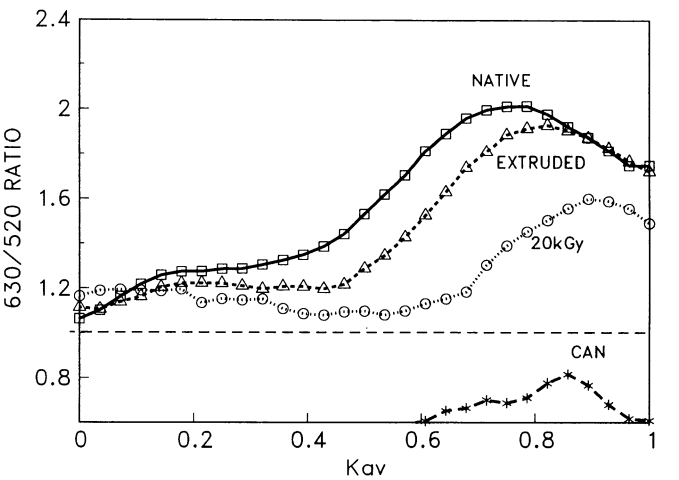

Fig. 9. Ratio of 630/520 nm of iodine-amylose complex fractionated by ascending gel permeation chromatography. Native, extruded, irradiated (20kGy), or treated with ceric ammonium nitrate (CAN).

TABLE V

λ_{max} (nm) of Absorbance of Amylose-Iodine Complex for Irradiated Starches Treated With or Without Chemical Additives

Chemical Additives	Amylose (%)	Fraction I						Fraction II[a]						Fraction III[a]			
		0%		2.5%[a]		5.0%[a]		0%[a]		2.50%[a]		5.0%[a]		2.5%[a]		5.0%[a]	
		K_{av}	λ_{max}	K_{av}	λ_{max}	K_{av}	λ_{max}	K_{av}	λ_{max}	K_{av}	λ_{max}	K_{av}	λ_{max}	K_{av}	λ_{max}	K_{av}	λ_{max}
None	0	0.04	530	...	...	...	...	0.39	553	...	...	...	...	...	...	...	...
	25	0.04	574	...	...	...	...	0.71	638	...	...	...	...	...	...	...	...
	50	0.04	575	...	...	...	...	0.79	624	...	...	...	...	...	...	...	...
	70	0.04	575	...	...	...	...	0.82	617	...	...	...	...	...	...	...	...
Hydrogen	0	...	...	0.11	579	...	...	0.57	525	0.86	518	0.89	514	1.29	500	...	...
Peroxide	25	0.21	586	0.11	584	0.11	584	0.82	608	0.93	581	0.96	570	...	...	...	...
	50	0.04	578	0.04	577	0.04	578	0.89	604	0.75	586	0.79	585	...	...	0.89	591
	70	0.11	585	0.04	581	0.04	580	0.93	608	0.93	600	0.96	595	...	...	...	...
Potassium	0	...	...	0.07	583	...	...	0.57	525	0.89	513	1.00	515	1.25	500	...	...
Pensulfate	25	0.21	586	0.07	575	0.36	581	0.82	608	1.07	569	0.93	561	...	...	...	...
	50	0.04	578	0.04	576	0.36	584	0.89	604	0.93	569	0.79	562	...	...	...	...
	70	0.11	585	0.07	579	...	...	0.93	608	0.96	573	1.07	559	...	...	1.39	546
Ceric Ammonium Nitrate	0	...	...	...	...	...	...	0.57	525	1.04	491	0.93	512	...	...	...	...
	25	0.21	586	...	...	...	...	0.82	608	0.93	530	1.04	525	...	...	...	...
	50	0.04	578	...	...	...	...	0.89	604	0.93	549	1.07	539	...	...	1.32	527
	70	0.11	585	0.11	575	...	...	0.93	608	1.04	558	1.11	546	...	...	...	...

[a] % concentration of the chemical additives.

TABLE VI

Reducing Power Values of Irradiation-Modified Starches With or Without Chemical Additives

Amylose (%)	Irradiated (20 kGy)	Hydrogen Peroxide		Potassium Persulfate		Ceric Ammonium Nitrate	
		2.5%[a]	5%[a]	2.5%	5%	2.5%	5%
0	1.08	3.16	5.43	2.34	7.42	5.53	5.89
25	1.04	2.68	3.81	4.12	9.19	7.36	8.97
50	0.96	2.17	1.49	5.09	6.88	6.10	9.28
70	0.99	1.85	2.42	4.32	7.45	5.59	12.01

[a] % concentration of additive.

TABLE VII

Effect of Extrusion Cooking on Fraction Quantities (mg) and Chain Lengths of Native and Pullulanase-Treated Starches

Amylose (%)	Starch	Fraction I[a]			Fraction II		
		K_{av}[b]	$\overline{CL}$	Content (mg)	K_{av}	$\overline{CL}$	Content (mg)
0	Native	(0.1)–0.1	24	9	0.2–1.2	6	991
	Irradiated	(0.2)–0.1	26	56	0.2–1.3	7	944
	Extrusion cooked only	(0.2)–0.2	23	72	0.2–1.2	7	928
	Extrusion cooked and irradiated, no chemicals	(0.2)–0.1	24	47	0.2–1.2	7	953
	Extrusion cooked, irradiated, 2.5% CAN added[c]	...	...	...	0–1.2	6	1,000
25	Native	...	...	...	0.1–1.2	7	1,000
	Irradiated	(0.2)–0.2	24	247	0.2–1.4	7	753
	Extrusion cooked only	(0.2)–0.2	24	251	0.2–1.2	7	749
	Extrusion cooked and irradiated, no chemicals	(0.2)–0.1	25	182	0.2–1.2	8	818
	Extrusion cooked, irradiated, 2.5% CAN added[c]	...	...	...	0.1–1.2	5	1,000
50	Native	(0.1)–0.2	23	300	0.2–1.2	7	700
	Irradiated	(0.2)–0.5	17	764	0.6–1.2	4	236
	Extrusion cooked only	(0.2)–0.4	24	688	0.5–1.2	6	312
	Extrusion cooked and irradiated, no chemicals	(0.2)–0.3	20	596	0.4–0.9	5	404
	Extrusion cooked, irradiated, 2.5% CAN added[c]	(0.1)–0.5	14	470	0.5–1.1	5	530
70	Native	(0.1)–0.5	18	737	0.6–1.2	4	263
	Irradiated	(0.2)–0.4	19	678	0.5–1.2	6	332
	Extrusion cooked only	(0.1)–0.5	18	737	0.6–1.2	4	190
	Extrusion cooked and irradiated, no chemicals	(0.2)–0.5	18	779	0.5–1.0	5	221
	Extrusion cooked, irradiated, 2.5% CAN added[c]	0.1–0.6	11	586	0.6–1.2	4	414

[a] Average chain length ($\overline{CL}$) weighted over the content of each 4-ml fraction collected.
[b] Figures in parentheses are negative numbers.
[c] CAN = ceric ammonium nitrate.

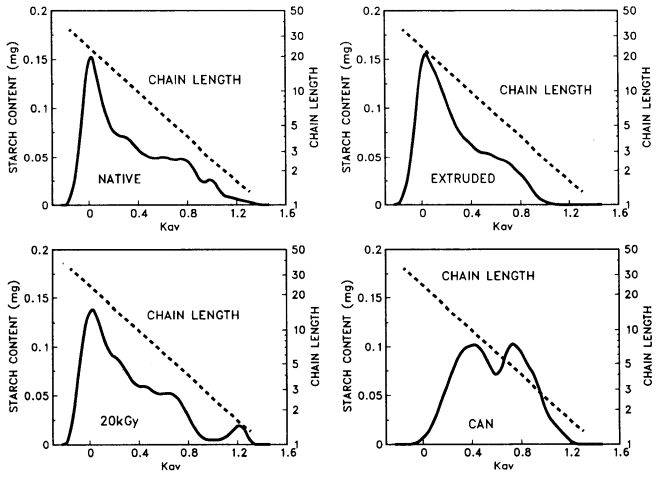

Fig. 10. Gel permeation chromatography profiles of pullulanase-treated starches. Left axis = native, extruded, irradiated (20kGy), or treated with ceric ammonium nitrate (CAN). Determined by phenol-sulfuric acid measurement. Right axis = chain length standard curve.

amylose starch generally decreased the 630/520-nm ratio of GPC fractions. However, the 630/520-nm ratio decreased drastically for 50% amylose starch irradiated at 20 kGy. Interestingly, the 630/520-nm ratio decreased below those of 50% amylose starch samples treated with 2.5% CAN (Fig. 9). These changes generally reflect the overall changes in the chain length population of starches.

The λ_{max} of native starches and starches treated with HP, PPS, and CAN was evaluated to assess the status of starch structure. The 0% amylose native starches contained a major fraction at void volume K_{av} 0.04 with λ_{max} at 530 nm. This fraction corresponded to fraction I (Table I, 4.69 mg) of amylopectin, branched or short chain fraction. Irradiation treatment at 20 kGy generally eliminated fraction I for 0% amylose native starch. The λ_{max} of fraction I of 25–70% amylose starches remained between 574 and 575 nm, suggesting that the linear chain lengths of these starches were relatively longer than those of the 0% amylose starches (Table V). This is consistent with the findings of Mercier (1973), who observed that amylopectin derived from high-amylose corn starches has longer outer chains resulting in a higher λ_{max} than that of 0% amylose starches. Extrusion with chemical treatments generally showed a slight reduction in the λ_{max} of fraction I for native starches. However, large decreases in λ_{max} of fraction II of 25, 50, and 70% amylose starches irradiated at 20 kGy with chemical treatments could be due to the severe degradation of linear chains, to the linking of starch molecules, or to a combination of these. This needs further investigation.

Reducing powers of extruded starches irradiated at 20 kGy and treated with 2.5 and 5% HP, PPS, and CAN were measured. Reducing powers (expressed as glucose) of extruded starches irradiated at 20 kGy without chemical treatments ranged from 0.96 to 1.08%, increasing to 1.85–7.36% for 2.5% chemical treatments, and further increasing to 1.49–12.01 for 5% chemical treatments (Table VI). Generally, 5% chemical treatment generated more reducing powers. Fragmentation of these radiation-disrupted starch granules was probably caused by a splicing action of highly reactive chemicals as explained by Ciesla et al (1991).

Chain Length

Native, extruded, irradiated, and CAN-treated starches were treated with pullulanase enzyme to hydrolyze the $\alpha(1\rightarrow6)$ bonds for chain length analyses. Biliaderis et al (1981) used pullulanase enzyme to debranch starches for chain length estimation. However, the effects of γ-irradiation and chemical treatment of starch on the pullulanase activity is not known. Pullulanase-treated starch samples were fractionated using GPC. Chain lengths of various fractions were determined. A representative GPC fractionation pattern for 50% amylose starch is shown in Figure 10. Elution volumes, chain lengths, and content of all starches are given in Table VII. Extrusion cooking appeared to have little effect on the chain lengths of 25, 50, and 70% amylose native starches, which, for the most part, remained at 24, 24, and 18, respectively. Generally, the average chain lengths of extrusion-cooked starches, with or without irradiation at 20 kGy, did not change appreciably. However, CAN-treated starches showed a sharp decrease in the average chain length from 18 to 11 (Fig. 10). This could be due to the extensive degradation of the chains. This pattern is reflected in reducing powers, IBC, and acidity values of all starches. This study could be useful in experiments involving starches obtained from other sources, such as potato, subjected to γ-irradiation

CONCLUSION

Free radicals produced on starches by γ-irradiation increased in intensity with increased irradiation at 0–30 kGy. Stability of free radicals on high-amylose starches irradiated at 20 kGy was greater than that of low-amylose starches. Extrusion cooking generally decreased free radicals. Some free radicals were, however, still found in 50 and 70% amylose starches. Acidity of irradiated starches increased and pH decreased with increasing irradiation dosages. Fraction I sizes of irradiated starches decreased with increasing irradiation dosages, while fraction II and III sizes increased correspondingly. Native starches of 0% amylose exhibited a more than fourfold decrease in fraction I content, whereas 70% amylose native starches showed less than a twofold decrease with increasing irradiation dosages. Extrusion cooking accelerated the degradation of fraction I of 0% amylose starches more than that of 70% amylose starch. Both 2.5 and 5% concentrations of chemical additives caused excessive degradation of fraction I of 20-kGy irradiated starches, consequently increasing reducing powers. CAN caused the highest decrease in the IBC of the starches. This trend is also reflected in chain length analysis. Fraction I, amylopectin, suffered more degradation due to irradiation, extrusion, and chemical additives.

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