Gamma Radiation of Wheat. II. Effects of Low-Dosage Radiations on Starch Properties[1]

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ABSTRACT

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The effect of gamma-irradiation treatments (50, 100, 200, and 300 Krad) on the starch properties of three hard red spring wheat cultivars was investigated. Starch pasting properties of the isolated starches from the treated flour samples showed a decrease in peak height, 15-min hold height, and height at 50° C, whereas starch water-binding capacity and damaged starch values increased. Swelling power decreased, whereas solubility of the starches increased with radiation. Intrinsic viscosity of the various starches and the starch fractions derived from them decreased with increasing radiation, whereas certain X-ray diffraction patterns showed an increase in percent crystallinity. No appreciable differences were observed in the granules themselves by scanning electron microscopy.

The majority of published studies about radiation effects on starch have employed large dosages of radiation, dosages often in the megarad range. Included in these studies are those by Raffi et al (1980a, 1980b) and by Michel et al (1980a, 1980b). With high levels of irradiation, many reactions occur that involve progressive changes in the smaller molecules that form when the macromolecules are destroyed. Changes in carbohydrates are reported to be brought about either through a release of ionizing energy directly upon the target molecule or through indirect action of secondary intermediates such as free radicals. As a continuation of our previous studies on wheat, using low dosages of gamma irradiation (50–300 Krad) (MacArthur and D'Appolonia 1983), we examined the effects on starch, using the same low levels.

MATERIALS AND METHODS

Samples

A description of the three hard red spring (HRS) wheat flour samples and radiation treatments used in this study were previously reported (MacArthur and D'Appolonia 1983).

Moisture, ash, and protein (14% mb) were determined on the milled flour according to AACC methods 08-01, 46-01, and 44-15A (AACC 1961). Flour protein was 13.2, 13.4, and 11.7 for the control samples of Waldron, Olaf, and the experimental variety, respectively. Although starch content was not determined, it would be reasonable to expect starch content to be higher in the lower protein flour.

Starch Isolation

Starch was isolated from the control and irradiated flour samples according to the dough-kneading procedure described by Walden and McConnell (1955).

Starch Pasting Properties

Pasting properties of the different starches were investigated by means of the Brabender Visco/Amylograph, with and without the incorporation of carboxymethyl cellulose (CMC). The technique of D'Appolonia and MacArthur (1975) was followed for those starches without the addition of CMC, and the procedure described by Sandstedt and Abbott (1964) was followed when using CMC.

Starch Fractionation

Amylose and amylopectin were obtained from the starch through use of the aqueous leaching procedure described by Montgomery and Senti (1958).

Intrinsic Viscosity

Starches and starch fractions were dissolved in 1*N* sodium hydroxide as described by Lansky et al (1949). Intrinsic viscosity was determined at 25° C as described by Leach (1963), using a Ubbelohde (Cannon Fenske) viscometer, capillary size 75, equipped with a Wescan automatic viscosity timer (Wescan Instruments Inc., Santa Clara, CA).

Swelling Power and Solubility

Swelling power and solubility determinations were performed for the temperature range 60–100° C by the procedure of Leach et al (1959).

Solubility in Dimethylsulfoxide (DMSO)

A modification of the method of Leach and Schoch (1962) was used to determine the solubility of starch in DMSO. Starch samples (100 mg, db) were dispersed in 20 ml of anhydrous DMSO in 50-ml Erlenmeyer flasks. The flasks were stoppered and shaken gently at 25 ± 0.01° C so as to maintain the starch in suspension. After a specified period of time (10–40 hr), a flask was removed and the starch suspension centrifuged for 15 min at 1,700 × *g*. An aliquot of the clear supernatant was removed, properly diluted with distilled water, and the total carbohydrate measured by the phenol sulfuric acid method of Dubois et al (1956).

Scanning Electron Microscopy of Starch Granules

Scanning electron microscopy (SEM) was performed by adhering the starch sample onto a stub using double-stick tape, then sputter coating the stub with gold-palladium. The samples were photographed with a JEOL JSM-35 scanning electron microscope.

X-Ray Diffraction Studies

X-ray patterns of the starch (equilibrated to 96% rh) were taken with CuKα radiation on a Philips X-ray diffractometer. Operation was at 35 kV and 18 mA. Relative crystallinity of the starch was determined according to Herman's method as described by Nara et al (1978), in which the area of the crystalline fraction (a_c) is divided by the diffraction area for a 100% crystalline substance (A_c). The area of the crystalline fraction in the X-ray diffraction pattern of the control starch was used as the value of A_c.

Starch Damage

The method proposed by Williams and Fegol (1969) was used to determine starch damage colorimetrically, with results expressed in Farrand Equivalent Units (FEU).

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TABLE I
Yield of Starch and Gluten from Irradiated Flours[a]

Variety	Radiation (Krad)	Starch (%)	Gluten (%)	Gluten Protein (%)
Waldron	0[b]	61.9	14.4	75.6
	50	61.5	15.1	72.0
	100	60.9	15.0	71.6
	200	60.2	15.3	71.2
	300	59.1	16.2	69.6
Olaf	0[b]	60.6	13.7	78.0
	50	60.4	14.1	76.0
	100	59.0	14.9	74.0
	200	61.4	14.8	72.8
	300	60.4	14.6	72.8
Experimental	0[b]	59.6	13.3	70.3
	50	61.8	12.1	73.2
	100	60.4	13.6	72.0
	200	60.6	13.0	72.0
	300	60.9	15.0	68.8

[a] Recovery on a dry flour weight basis.
[b] Control.

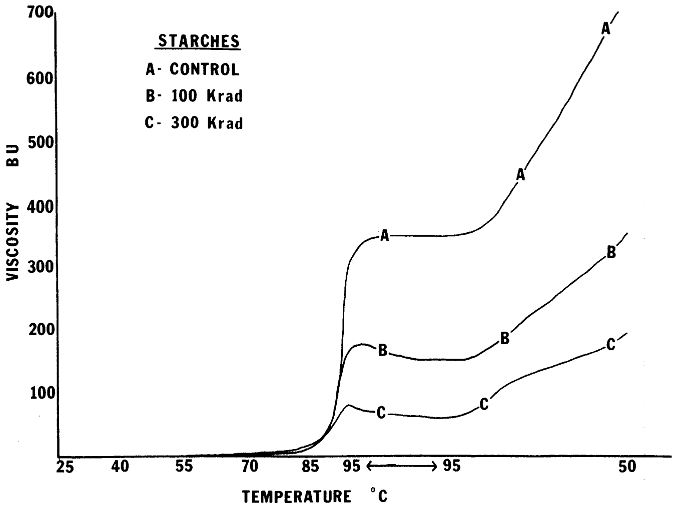

Fig. 1. Amylograph starch pasting curves for Waldron at two levels of radiation.

TABLE II
Pasting Properties of Starch Isolated from Controls and Irradiated Flour Samples

Variety	Radiation (Krads)	Pasting Temperature (°C)	Peak Height (BU)[a]	Peak Time at 95°C (min)	15 min Height at 95°C (BU)[a]	Height at 50°C (BU)[a]	Rate of Retrogradation (BU/min)[a]
Waldron	0[b]	84.0	345	...[c]	350	730	12.7
	50	84.0	230	95 + 3.50	225	435	7.0
	100	84.0	170	95 + 1.50	150	355	6.8
	200	83.0	110	95 + 0.50	95	255	5.3
	300	83.0	75	95 + 0.00	55	190	4.5
Olaf	0[b]	83.0	380	...[c]	385	730	11.5
	50	82.0	275	95 + 2.00	260	525	8.8
	100	82.0	195	95 + 0.75	170	375	6.8
	200	82.0	115	95 + 0.00	90	255	5.5
	300	83.5	75	95 + 0.00	50	180	4.3
Experimental	0[b]	82.5	300	95 + 2.75	300	650	11.7
	50	81.5	210	95 + 1.00	195	420	7.5
	100	84.0	150	95 + 0.50	130	310	6.0
	200	82.0	95	94.5	70	215	4.8
	300	83.5	55	93.5	40	160	4.0

[a] BU = Brabender unit.
[b] Control.
[c] No definite peak time established.

TABLE III
Pasting Properties of Starch Isolated from Control and Irradiated Flour Samples Using Carboxymethyl Cellulose

Variety	Radiation (Krad)	Temperature of Initial Pasting (°C)	Peak Height (BU)[a]	50°C Height (BU)[a]	Rate of Retrogradation (BU/min)[a]
Waldron	0[b]	58	195	325	4.7
	50	58	155	245	3.5
	100	58	155	225	3.2
	200	58	110	155	2.5
	300	58	100	125	2.3
Olaf	0[b]	55	230	330	3.7
	50	61	190	260	3.0
	100	61	165	225	3.0
	200	61	165	200	2.8
	300	61	110	130	2.2
Experimental	0[b]	58	225	330	3.8
	50	58	185	260	3.2
	100	58	175	255	3.2
	200	58	145	175	2.3
	300	58	110	125	2.0

[a] BU = Brabender unit.
[b] Control.

Water-binding Capacity

The procedure for water-binding capacity initially described by Yamazaki (1953) and later modified by Medcalf and Gilles (1965) was used.

Granule Density

Absolute density of the different starch samples was determined by the xylene-displacement method described by Schoch and Leach (1964). All determinations were done on defatted starch, in triplicate, and an average of the results recorded.

RESULTS AND DISCUSSION

Starch Isolation

Table I shows the yield of starch and gluten obtained from the various flour samples. The results show an increase in gluten recovery with flour irradiation and a decrease in starch yield for the variety Waldron. Also, gluten protein generally decreased as radiation increased, whereas starch protein (not shown) remained constant (0.2%) for all samples. The percentage of protein in the gluten based on gluten recovery was very similar for the different irradiation treatments within a variety. The results therefore indicate that with irradiation the isolated gluten contained more nonproteinaceous material.

TABLE IV
Intrinsic Viscosity of Isolated Starches and Starch Fractions

Variety	Radiation (Krad)	Starch (η)	Amylose (η)	Amylopectin (η)
Waldron	0[a]	1.70	3.00	2.23
	50	1.68	···	···
	100	1.50	···	···
	200	1.45	···	···
	300	1.32	1.62	1.82
Olaf	0[a]	1.52	2.64	2.05
	50	1.48	···	···
	100	1.45	···	···
	200	1.45	···	···
	300	1.35	1.49	1.85
Experimental	0[a]	1.80	2.63	2.11
	50	1.36	···	···
	100	1.33	···	···
	200	1.30	···	···
	300	1.29	1.65	1.50

[a] Control.

TABLE V
Starch Damage, Water-binding Capacity and Absolute Density Measurements

Variety	Radiation (Krad)	Starch Damage (flour, FEU)[a]	Water-binding Capacity (%)	Absolute Density
Waldron	0[b]	26.7	71.1	1.52
	50	30.8	73.0	1.51
	100	33.8	72.4	1.51
	200	39.1	84.0	1.51
	300	44.0	77.4	1.51
Olaf	0[b]	34.0	75.5	1.51
	50	36.4	81.0	1.51
	100	39.2	76.1	1.53
	200	44.5	80.0	1.52
	300	48.2	80.0	1.52
Experimental	0[b]	29.9	70.5	1.52
	50	35.5	73.0	1.52
	100	38.3	71.3	1.51
	200	44.4	74.0	1.52
	300	51.6	75.0	1.53

[a] FEU = Farrand Equivalent Units.
[b] Control.

TABLE VI
Percent Swelling Power of Control and Irradiated Starches[a]

Variety	Radiation (Krad)	Pasting Temperatures (°C) 60	70	80	90	100
Waldron	0[b]	5.5	7.1	7.8	9.7	14.1
	100	5.1	6.8	7.7	9.3	13.4
	300	4.6	6.4	7.5	8.8	13.1
Olaf	0[b]	4.9	7.1	8.5	9.5	16.9
	100	4.8	6.9	8.0	9.2	14.5
	300	4.4	6.7	7.6	8.9	13.2
Experimental	0[b]	4.8	7.4	8.3	9.9	13.9
	100	4.7	6.9	7.5	9.2	13.8
	300	4.5	6.6	7.4	8.6	13.2

[a] Average of three determinations.
[b] Control.

TABLE VII
Percent Solubility of Control and Irradiated Starches

Variety	Radiation (Krad)	Pasting Temperatures (°C) 60	70	80	90	100
Waldron	0[a]	2.1	3.3	4.9	5.5	16.1
	100	2.5	3.3	6.1	9.0	18.6
	300	3.2	6.9	10.6	12.3	30.0
Olaf	0[a]	2.0	3.5	4.7	7.6	18.0
	100	2.5	4.5	6.7	10.4	22.3
	300	4.2	8.6	10.5	14.7	31.5
Experimental	0[a]	2.4	4.1	5.4	6.8	18.0
	100	3.3	5.8	6.6	10.1	21.4
	300	4.9	8.6	12.9	18.3	25.5

[a] Control.

TABLE VIII
Percent Solubility of Control and Irradiated Starches in Dimethylsulfoxide[a]

Variety	Radiation (Krad)	Hours 10	20	30	40
Waldron	0[b]	7.4	8.8	18.0	38.0
	100	5.0	9.2	15.9	35.5
	300	4.9	8.7	15.0	19.5
Olaf	0[b]	7.0	16.0	28.1	33.5
	100	5.7	9.9	16.4	31.8
	300	5.0	8.7	15.4	25.6
Experimental	0[b]	8.9	18.6	23.8	47.2
	100	5.8	9.5	17.8	34.2
	300	5.7	9.9	20.8	27.3

[a] Average of two determinations.
[b] Control.

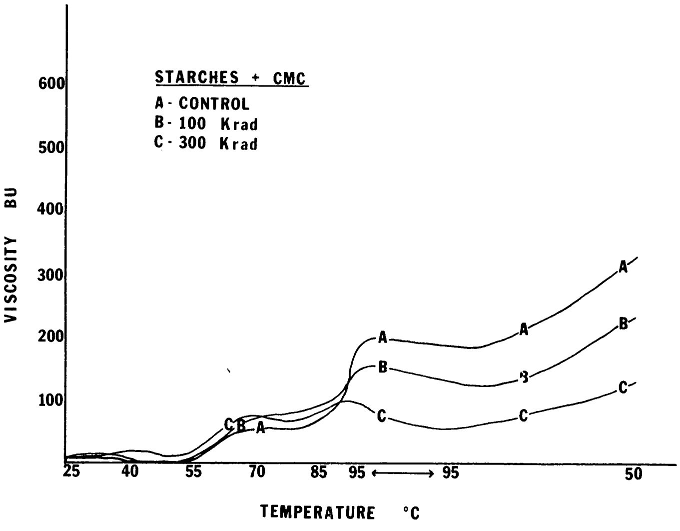

Fig. 2. Amylograph starch pasting curves for Waldron at two levels of radiation with the addition of carboxymethyl cellulose.

Starch Pasting Properties

The pasting property data of the starches isolated from the control and from the irradiated flour samples are shown in Table II. A noticeable decrease in peak viscosity, 15 min hold height at 95° C, and peak height at 50° C was observed as the level of radiation increased. A similar decrease was noted in the amylograph peak viscosity and falling number values of the corresponding flours (MacArthur and D'Appolonia 1983). Pasting temperature was similar for all three varieties and at all levels of irradiation; however, viscosity characteristics of the starch were altered. The effects are shown in Fig. 1 for the variety Waldron. With increasing radiation, the peak viscosity was reached sooner, and breakdown of the gel occurred more readily. Peak height values at 50° C showed a decrease as radiation levels increased, which might suggest a decrease in the "rate" of retrogradation. Table II gives values designated as rate of retrogradation. These values were calculated by subtracting the 15-min height at 95° C from the peak height at 50° C and dividing by 30 (which represents the 30-min cooling cycle). The values obtained showed a decrease as radiation levels increased. The decrease is in proportion to the decrease in peak height. Similar results can be obtained with the amylograph by changing the concentration of the starch slurry. The amylograph data indicate that gamma radiation does alter the viscosity characteristics of a starch paste but may not affect the rate of retrogradation since the slope of the amylograph curve during the cooling phase is dependent upon the peak viscosity.

The starch pasting curves with the incorporation of CMC for the variety Waldron are illustrated in Fig. 2; pertinent data for all three varieties are shown in Table III. Carboxymethyl cellulose has been used in starch studies to show the two-step gelatinization pattern characteristic of wheat starch and to magnify the viscosity effect of small changes in granule size during heating. The curves in Fig. 2 show an increase in viscosity in the first step of gelatinization for the irradiated samples. The increased peak viscosity in the initial stage of gelatinization with irradiation was not noted with the second peak. Results similar to those obtained without incorporation of CMC were noted after initial pasting. The initial increase in viscosity observed may be caused by increased competition between the starch and the CMC for water as a result of starch granule disruption caused by the radiation treatments.

Intrinsic Viscosity

Table IV shows the intrinsic viscosity values obtained for the starches and amylose and amylopectin fractions derived from the control and high-level irradiated starches. The viscosity data support the starch pasting property results and suggest that radiation exposure could possibly modify the shape of the starch molecule and its configuration and perhaps its molecular weight, resulting in reduced intrinsic viscosity. The results of this study are in agreement with Roushdi et al (1983), who found a decrease in viscosity values for gamma-irradiated corn starch while the starch chain length decreased.

Starch Damage, Water-binding Capacity, and Granule Density

Starch damage values, expressed as FEU, increased as radiation exposure increased (Table V). The increase in starch damage values in the irradiated samples cannot be attributed to mechanical damage as occurs during the wheat-milling operation. The increase

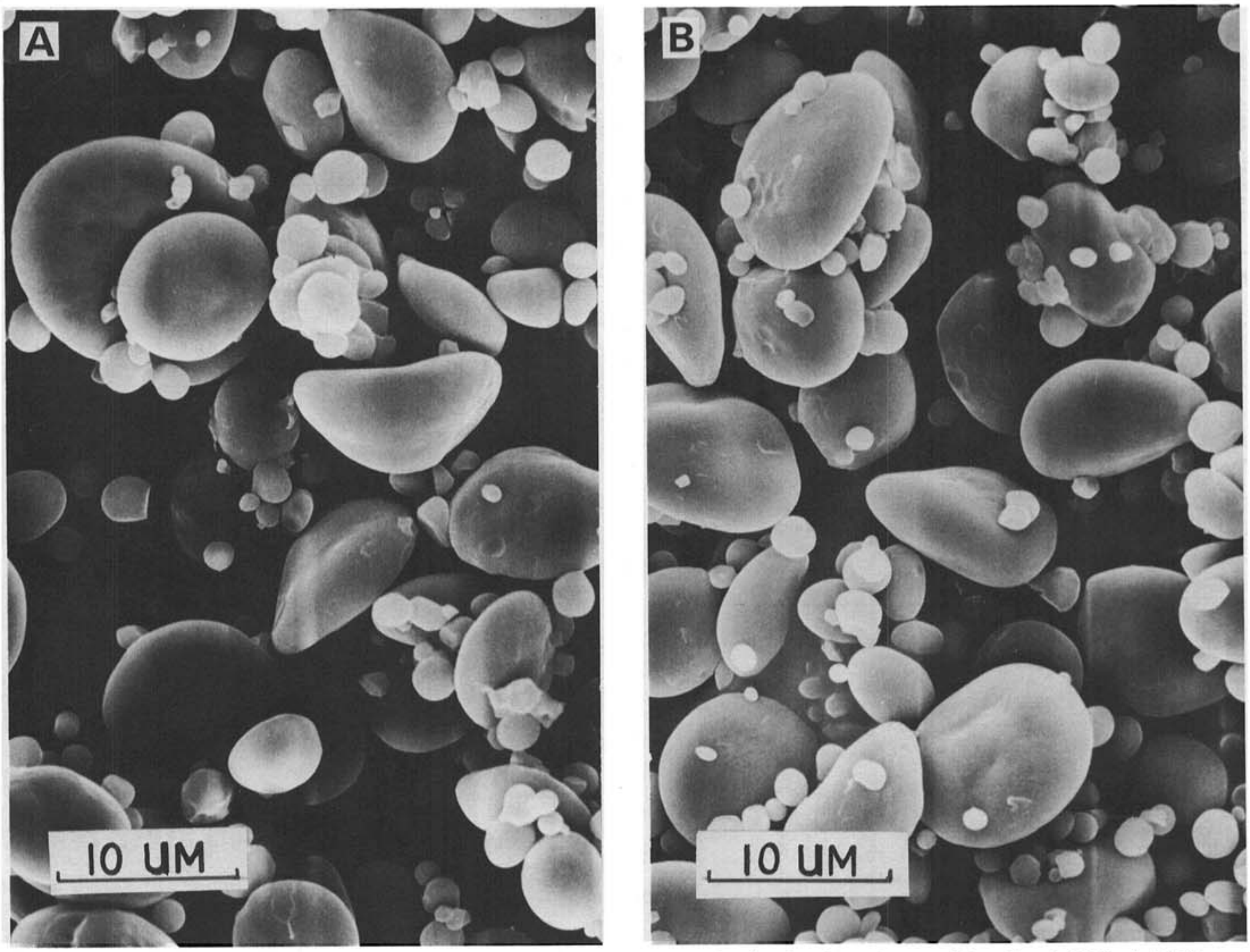

Fig. 3. Scanning electron micrographs of starch granules derived from Waldron hard red spring wheat. **A,** control; **B,** radiated, 300 Krad. (×860)

is most likely due to the measurement of increased soluble starch as a result of some type of alteration in the starch with radiation. Water-binding capacity values increased (Table V), particularly at the higher levels of gamma radiation. The increase in water-binding capacity may be due to either the destruction of some intermolecular bonds by low doses of gamma radiation or to competition for available water by the production of soluble, low-molecular weight compounds as a result of irradiation. Absolute density of the starches (Table V) remained essentially the same at all levels of radiation and for all three varieties.

Swelling Power and Solubility of Starches

Table VI gives the swelling power values of the starches derived from the control and irradiated flour samples. Swelling power increased with temperature, as expected, but decreased with increasing radiation treatment. The decrease in swelling power can possibly be attributed to depolymerization of the starch due to irradiation treatment. The lower-molecular-weight fractions formed are not able to bind water for swelling and gel formation as a result of being more soluble in water. These results agree with studies reported by Deschreider (1960), Korotchenko and Semionov (1966), and Reuschl and Guilbot (1966). Starch solubility increases as the temperature is increased, because of the dissociation of hydrogen bonds in the amylose and amylopectin fractions. With a continuous rise in temperature, additional hydrogen bonds are ruptured with a subsequent formation of soluble matter. The solubilities of the starch samples (Table VII) increased considerably with increased radiation exposure, most likely because of losses of molecular-weight components formed by the degradation of the starch. The solubility of the control starches increased only slightly between 60 and 90° C. This small increase in solubility, followed by a greater increase after 90° C, suggests the presence of homogeneous and strong binding forces maintaining the granular matrix of these starches in comparison to the irradiated samples. Greenwood and MacKenzie (1963) suggested that the increase in solubility, with radiation, might be caused by degradation or modification of the amylose and amylopectin structure, or by both. Robin et al (1978) showed, with the enzymatic sequential method, that high radiation doses (10, 30, 50 Mrad) on corn and on waxy corn starches had a "hydrolytic" effect on both amylose and amylopectin, and DP = 15 and DP = 25 material were preferentially liberated by irradiation. Abd Allah et al (1974), however, reported that increased solubility may be a result of a secondary cross-linkage reaction occurring because of formation of free radicals during irradiation. However, in both of these studies, large dosages of radiation were used.

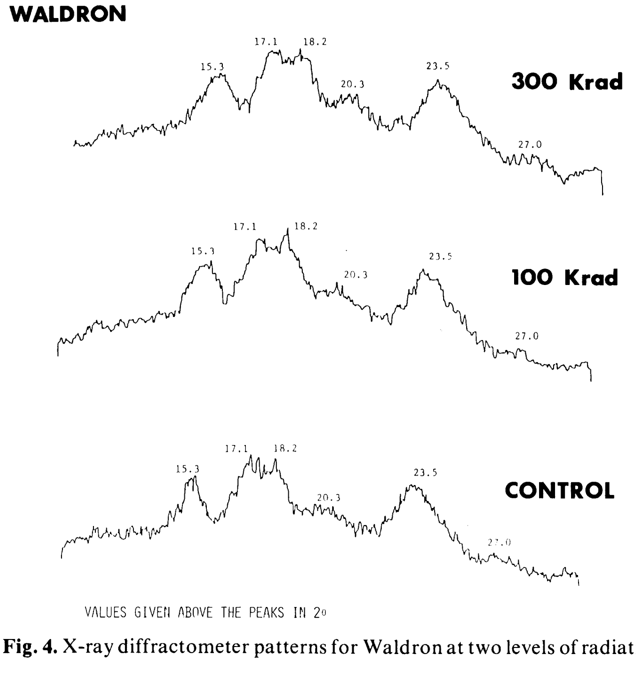

Fig. 4. X-ray diffractometer patterns for Waldron at two levels of radiation.

Solubility in DMSO

The percent solubility for the control and irradiated starches in DMSO are shown in Table VIII. Dimethylsulfoxide has been used in starch studies because it slowly dissolves granular starches without appreciable swelling and without molecular degradation of the starch substance. The control starches solubilized at a faster rate than the irradiated starches, with the 300 Krad starches being the least soluble during the 40-hr digestion period. The reduced solubility of the irradiated starches in DMSO may be due to poor solvent penetration because of modifications occurring in the starch chain, or certain chemical changes within the starch matrix as a result of radiation. At megarad dosages, Jencie and Samec (1960) found that irradiation of starch produced a form of soluble starch they called "nuclear starch." Traubenberg et al (1965) stated that high doses of radiation increased water-soluble substances but did not form mono- or disaccharides; however, solubility and total acidity both increased with increasing radiation dosage. Putilova et al (1966) reported that irradiation of starch led to the formation of free radicals, organic acids, and dihydroxy acetone. More recently, Ananthaswamy et al (1970) characterized glucose and maltose as well as a series of oligosaccharides with low R_f values as degradation products of starch irradiated at megarad levels. They also found a series of oligosaccharides obtainable at low dosage levels (20–1,000 Krad), with glucose appearing only above 200 Krad.

SEM

Starch granules isolated from Waldron variety and examined by SEM are shown in Fig. 3A and B. Irradiation exposure did not appear to cause large differences in the granules. This same observation also was made for the other two varieties.

X-Ray Diffraction Pattern

Figure 4 shows the X-ray diffractometer patterns for the variety Waldron at 0, 100, and 300 Krad levels of gamma radiation. The values for percent crystallinity in the starches, subjected to 100 and 300 Krads, were 104.5 and 112.4%, respectively. The small increase in crystallinity as determined by X-ray analysis might indicate that starch fragments contribute to this increase.

CONCLUSION

This study, involving low dosage levels of gamma radiation, showed effects on starch properties similar to the ones reported in the literature for very high dosages. The damage to the starch from gamma-radiation treatments was reflected by several factors; the decrease in peak viscosity of the starch pastes, the decreased intrinsic viscosity values, and the increased water-binding capacities. The decrease in swelling power and reduced solubility values of the irradiated starch samples might suggest molecular degradation of the starch granule.

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