Effect of γ Radiation on Polysaccharides and Calcium Distribution in Carrot Cell Walls

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Vegetable foods are drastically softened by high doses of γ radiation. Irradiated plant tissues also leach calcium which has been associated with this softening. To investigate the relation of this calcium to cell wall polysaccharide degradation, irradiated carrots were fractionated into their constituent polysaccharides and analyzed for calcium. Irradiation brought about a redistribution of cell wall polysaccharides which is generally consistent with solubilization. Calcium was associated with the most insoluble polysaccharide fraction. It was concluded that the observed release of calcium is either not associated with the cell wall or is associated only with the weakly bound calcium and probably not in itself responsible for the softening process.

Texture in vegetable foods is drastically affected by high doses of γ radiation (Glegg *et al.*, 1956; Roberts and Proctor, 1955). This softening is apparently the result of alterations in wall structure and cohesion (Massey and Faust, 1969; Romani, 1967; Sterling, 1963). There is considerable evidence as to the importance of calcium pectate on cell wall rigidity (Cormack, 1965; Laconti and Kertesz, 1940; Tagawa and Bonner, 1957; Taylor and Wain, 1966). Degradation has been detected in both pectin (Kertesz *et al.*, 1964; McArdle and Nehemias, 1956) and cellulose (Kertesz *et al.*, 1964) isolated from cell walls of irradiated fruits. Radiation degradation of isolated pectin has been associated with random fissure of the glycosidic linkage with no appreciable alteration in the degree of methylation (Skinner and Kertesz, 1960). Irradiated plant tissues have since been shown to leach inorganic calcium (Echandi and Massey, 1970) which may be related to loss in texture (Al Jasim and Markakis, 1965; Shah, 1966). In other studies, no redistribution of calcium was detected in the pectin fractions of tissue softened with the growth hormone indole acetic acid (Burling and Jackson, 1965; Cleland, 1960). This investigation was conducted to explore the relationship of calcium to cell wall polysaccharides in irradiated tissues.

MATERIALS AND METHODS

Preparation of Material. Twelve sound carrots of unspecified variety were washed, cut transversely into three sections of approximately the same length, and randomized in such a manner as to include four whole roots per treatment. The randomized portions were placed in cellophane bags which were closed in such a manner as to permit gas exchange with a minimum of moisture loss. Irradiation was accomplished with a 6000 Ci^{60} Co source at ambient temperature (24 ± 2° C) (Massey *et al.*, 1965). Doses of 100,000 rads (100 Krads) and 1000 Krads were obtained by placing the samples at varying distances from the source over a uniform period of 16 hr.

Two 200 g tissue samples were prepared from each treatment by slicing approximately equal amounts of tissue from each portion of each root. One of the samples was dried in an oven at 40° C, the other was extracted and fractionated.

Isolation of the Cell Wall. The method used in cell wall isolation was a modification of that by Kivilaan *et al.* (1959). Use of anhydrous glycerol (Bean and Ordin, 1961) enabled us to prepare a cell wall extract in which contamination by intact cells, cytoplasm, and cell organelles was minimal.

The samples were first comminuted in a Waring Blendor for 2 min in 800 ml of glycerol, and then homogenized in an Omni-Mixer for 10 min. The homogenate was filtered with suction through sharkskin filter paper, the residue resuspended in 800 ml of glycerol, and rehomogenized for an additional 5 min. The mass was then filtered with suction through sharkskin filter paper and the residue resuspended in 500 ml of glycerol and stirred vigorously by hand before refiltering. The remaining residue was dried by washing twice with 500 ml of absolute ethanol and once with an equal volume of acetone. Finally the residue was extracted in a Soxhlet apparatus for 24 hr with a mixture of absolute ethanol, benzene (1 to 2, v/v) after which it was dried under vacuum at 50° C. Four replications for each treatment were performed.

Fractionation of the Cell Wall. The cell wall material corresponding to each treatment was fractionated by a modification of the methods of Ito and Fujiwara (1967), Jermyn (1955), and King and Bayley (1963).

Pectin Fraction. The cell wall preparations were extracted twice with 0.5% ammonium oxalate-oxalic acid for 3 hr at 75° C. At the end of each extraction the residue was separated by filtering with suction through sharkskin filter paper. Each of the two filtrates were treated separately with 8 volumes of absolute ethanol and stirred. The resulting precipitates were collected by filtration, dried in vacuum at 50° C, and over P_2O_5. These fractions were designated pectin I and II, respectively.

Oxidized Lignin Fraction. The residue from above was suspended in 500 ml of deionized water to which 3 g of sodium chlorite and 3 ml of glacial acetic acid were added. This mixture was maintained at 75° C for 4 hr, adding 3 g of sodium chlorite and 3 ml of glacial acetic acid at hourly intervals. The mixture was then filtered through a fritted-glass funnel and the residue washed thoroughly with cold deionized water. Filtrate and washings were combined, and the volume reduced to approximately 200 ml under vacuum. Excess salt was removed by dialyzing the concentrate for 3 days against running tap water and for 1 day against deionized water. Finally the concentrate was freeze-dried.

Hemicellulose Fractions. The chlorite-treated residue (above) was treated first with 200 ml of 4% NaOH followed

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by 200 ml of 17.5% NaOH for 3 hr at room temperature, filtered, and the filtrates neutralized with acetic acid and treated with 10 volumes of 95% ethanol. The resulting precipitates were dried by washing with ethanol and acetone in vacuum at 50° C, and finally over P_2O_5. These fractions are designated hemicelluloses I and II, respectively.

α-Cellulose Fraction. The residue of the final alkali extraction (above) was washed thoroughly with deionized water and dried with absolute ethanol and acetone and in vacuum at 50° C.

Calcium Analysis. Calcium was analyzed on a Perkin-Elmer (Norwalk, Conn.) atomic absorption spectrophotometer, Model 303. The determination was conducted by dissolving the soluble polysaccharide fractions in glass distilled water and diluting with lanthanum stock solution (5% lanthanum as La_2O_3 in 25% concentrated HCl) to a final concentration of 1% lanthanum in 5% HCl. For insoluble polysaccharides, calcium determination was carried out by the method of Burling and Jackson (1965) in which the samples were ashed, the ash dissolved in concentrated HCl, and adjusted by the addition of lanthanum stock and water to 1% lanthanum in 5% HCl for assay.

RESULTS AND DISCUSSION

There are several factors which must be taken into consideration in interpreting the results of these experiments. The application of any isolation and fractionation scheme for cell wall and its carbohydrate components is based upon more or less arbitrary conditions which are known to produce fractions which are complex mixtures of various polysaccharides possessing not only the recognized range in weight-number and size-number molecular constituents of the fraction involved but also a certain degree of cross-contamination between fractions. Further, as radiation-induced fissure of polysaccharide molecules is known to occur (Massey and Faust, 1969), it is probable that some losses were experienced during extraction of the cell wall and its fractions from the irradiated tissues. Furthermore, it is also probable that the calcium in structural polysaccharides is in a highly dynamic state and that a high turnover rate exists between the free and the loosely-bonded form of the ion, which may be influenced by separation and fractionation procedures. We have analytical evidence, for example, that some contamination of the hemicellulose fraction I with pectin did occur, and that some calcium was lost during the aqueous alkali extraction. Although in each of these cases efforts were made to minimize the discrepancy, such problems are obviously unavoidable utilizing presently known techniques. In order to compensate for this, effort was made to standardize each step in the experimental protocol, particularly where it is known that relatively subtle differences may bring about significant variation in extraction conditions. Less significance should be attributed to the absolute quantities than to the relative quantities reported.

Table I. Weight (g) and Calcium Content (mg) of Oven-Dried γ-Irradiated Carrot Tissues and Extracted Cell Wall

		Oven dry tissues[a]	Extracted cell wall[a]	% of oven dried tissue
Control	weight	25.07 ± 1.98	6.44 ± 0.38	23.99
	Ca	83.03 ± 2.92	35.49 ± 2.45	42.74
100 Krads	weight	24.68 ± 1.81	6.46 ± 0.34	26.00
	Ca	79.02 ± 0.58	37.71 ± 3.92	47.72
1000 Krads	weight	24.76 ± 1.57	5.74 ± 0.22	23.36
	Ca	82.33 ± 2.31	38.14 ± 3.14	46.33

[a] Mean value and standard deviation.

Table II. Weight (g) and Calcium Content (mg) of γ-Irradiated Carrot Cell Wall Fractions

		Control			100 Krad			1000 Krad		
Fraction		Per[a] Fraction	% Dry Whole Tissue	mg Ca per g	Per[a] Fraction	% Dry Whole Tissue	mg Ca per g	Per[a] Fraction	% Dry Whole Tissue	mg Ca per g
Whole Tissue	Wt	25.07 ± 1.98	...	...	24.68 ± 1.81	...	...	24.76 ± 1.57	...	...
	Ca	83.03 ± 2.92			79.02 ± 0.58			82.33 ± 2.31		
Cell Wall	Wt	6.44 ± 0.22	25.68	...	6.46 ± 0.19	26.17	...	5.74 ± 0.12	23.18	...
	Ca	35.48 ± 1.73	42.73	...	37.71 ± 2.19	47.72		38.13 ± 2.22	46.31	
Pectin I	Wt	1.34 ± 0.14	5.34	0.96	1.56 ± 0.01	6.32	0.86	1.56 ± 0.13	6.30	0.99
	Ca	1.29 ± 0.16	1.57		1.35 ± 0.02	1.71		1.55 ± 0.29	1.88	
Pectin II	Wt	0.78 ± 0.11	3.11	1.47	0.68 ± 0.05	2.76	1.14	0.34 ± 0.01	1.37	1.74
	Ca	1.14 ± 0.40	1.37		0.78 ± 0.06	1.00		0.59 ± 0.07	0.71	
Total Pectin	Wt	2.12 ± 0.25	8.45	1.15	2.24 ± 0.06	9.08	0.95	1.90 ± 0.14	7.67	1.13
	Ca	2.43 ± 0.56	2.94		2.13 ± 0.08	2.71		2.14 ± 0.36	2.59	
Hemicellulose I	Wt	0.71 ± 0.06	2.83	8.97	0.52 ± 0.08	2.11	7.46	0.55 ± 0.09	2.22	7.95
	Ca	6.41 ± 0.64	7.72		3.87 ± 0.49	4.90		4.38 ± 0.03	5.32	
Hemicellulose II	Wt	0.26 ± 0.08	1.04	2.81	0.36 ± 0.05	1.46	4.90	0.42 ± 0.02	1.70	4.4
	Ca	0.72 ± 0.44	0.87		1.79 ± 0.59	2.27		1.89 ± 0.84	2.30	
Total Hemicellulose	Wt	0.97 ± 0.14	3.87	7.35	0.88 ± 0.13	3.57	6.43	0.97 ± 0.11	3.92	6.4
	Ca	7.13 ± 1.08	8.59		5.66 ± 1.08	7.16		6.27 ± 0.87	7.62	
α-Cellulose	Wt	1.98 ± 0.15	7.90	2.50	1.86 ± 0.11	7.54	2.67	1.83 ± 0.03	7.39	3.74
	Ca	4.97 ± 0.85	5.99		4.99 ± 0.09	6.31		6.85 ± 1.49	8.32	
Oxidized Lignin	Wt	0.83 ± 0.08	3.31	3.91	0.80 ± 0.08	3.24	2.60	0.63 ± 0.06	2.54	3.3
	Ca	3.23 ± 0.99	3.89		2.09 ± 0.28	2.64		2.09 ± 0.41	2.54	
Total Fractions	Wt	5.90 ± 0.62	23.53		5.78 ± 0.38	23.43		5.33 ± 0.34	21.52	
	Ca	17.76 ± 3.49	21.41		14.87 ± 1.54	18.83		17.35 ± 3.13	21.07	

[a] Mean value and standard deviation.

The results indicate that exposure of the carrot tissues to doses of radiation up to 1000 Krads had little significant effect on the amount of total cell wall material recovered. Total calcium content of the cell wall amounted to approximately 45% of the total cellular calcium and this also was not significantly affected by radiation (Table I). It should be noted that the use of an anhydrous extraction medium such as glycerol minimized possible losses due to enzymatic degradation which are sometimes experienced.

Total extracted pectin constitutes approximately $^1/_3$ of the dry weight of the cell wall. Irradiation does not significantly alter the amount of total extractable pectin, but it does affect a significant increase in the amount extracted in fraction I and a corresponding decrease in the amount extracted in fraction II (Table II). The calcium associated with these pectin fractions shows a similar and parallel trend. There is no significant overall change in total calcium content of these fractions.

Radiation doses of 100 Krads and above are known to increase the solubility of both *in vitro* and *in vivo* irradiated pectin (Kertesz *et al.*, 1964; McArdle and Nehemias, 1955). The results of these experiments are quite consistent with these reports. It is notable that calcium content of these fractions is more or less parallel to fraction weight. As the action of oxalate in bringing about the solubilization of pectin is by unbinding at least the more loosely associated calcium from the native pectin, it is apparent that there is no large displacement from the total fraction which may be associated with the establishment of a significant irradiation-weakened calcium-pectate bond. Thus the high levels of pectic calcium seem to remain associated with the more insoluble forms.

Hemicelluloses amount to about 15% of the cell wall, and as with the pectins, irradiation does not significantly alter the total amount of this constituent or its calcium content. However, irradiation does alter the constitution of this component in the opposite manner of that observed with the pectins, bringing about a decrease in fraction I and a corresponding increase in fraction II (Table II). Hemicellulose contains a relatively large proportion of calcium, both on an absolute and on a per-unit-dry-weight basis. Although there is no significant alteration in total calcium content, irradiation does cause a significant displacement of calcium from fraction I to fraction II in a manner similar to that in dry weight. This effect of increasing radiation apparently decreasing the solubility of this structural polysaccharide is unexpected, and no explanation for this reversal of effect from that of pectin can be offered. Although little information is available in the literature concerning the *in vitro* or *in vivo* irradiation effects on this polysaccharide, a radiation-induced solubilization would be anticipated on the basis of known effects on similar materials (Massey and Faust, 1969). Analysis for the presence of galacturonic acid and nitrogen in the hemicellulose fractions indicate only slight contamination by carryover of pectin or proteins.

The α-cellulose constitutes approximately $^1/_3$ of the total cell wall. This fraction is but slightly reduced and its calcium content insignificantly increased by radiation. It is well known that cellulose is degraded by doses of radiation over about 100 Krads. The slight loss of weight of this fraction is not then unexpected.

Irradiation has no significant effect upon lignin content of carrot tissue. Calcium content of this fraction is small, amounting to only about 7% of the total calcium content of the cell wall. An insignificant decrease in calcium content of this fraction was observed.

It is evident that γ radiation has a marked effect on the cell wall polysaccharides and on the calcium distribution in that part of the cell. Although about 90% of the cell wall was accounted for in these determinations, from an overall standpoint there is no significant effect of irradiation upon the total composition in terms of weight. A redistribution of some fractions did occur, however, which can be explained in terms of radiation-induced solubilization of the polysaccharide. A reduction in solubility of hemicelluloses was experienced, however, for which there is no obvious explanation. Owing to the methods of extracting the polysaccharide fractions, only approximately 50% of the original calcium in the cell wall was accounted for, probably the most firmly bound calcium in each fraction. It has been shown indirectly (Echandi and Massey, 1970; Sterling, 1968) and directly (Linehan and Hughes, 1969) that the most easily leached, weakly bound calcium has little effect on tissue texture. Hence it is concluded that the observed release of calcium from irradiated tissues is either not associated with the cell wall or is associated only with the weakly bound calcium and probably not in itself responsible for loss in texture of irradiated carrots.

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