Postharvest Storage Quality of Gamma-irradiated 'Climax' Rabbit-eye Blueberries

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Abstract Postharvest quality of 'Climax' rabbiteye blueberries (*Vaccinium ashei* Read) was evaluated after exposure to dosages of 0, 0.75, 1.5, 2.25, or 3.0 kGy gamma irradiation (0.118 kGy·min⁻¹) and after subsequent storage. Irradiation did not affect weight loss, but irradiated berries were softer than nontreated berries. There was also a trend toward increased decay as dose increased. Irradiation had no effect on powdery bloom or surface color; total soluble solids concentration, acidity, and pH were affected slightly. Flavor preference was highest for nonirradiated berries and generally declined as dosage increased. Irradiation at 2.25 and 3.0 kGy resulted in increased levels of xylosyl residues in cell walls, and xylosyl residues were the most abundant cell-wall neutral sugar detected in blueberries. There was no evidence of cell wall pectin loss in irradiated berries. Irradiation at 21.5 kGy lowered the quality of fresh-market 'Climax' blueberries.

Blueberries are grown commercially in many parts of the United States and shipped fresh to domestic and export markets. Some of these markets require that the berries be certified free of insects such as the apple maggot [*Rhagoletis pomonella* (Walsh)], blueberry maggot (*R. mendax curran*), and plum curculio [*Conotrachelus nenuphar* (Herbst)]. The approved quarantine treatment is methyl bromide fumigation; however, continued approval of this chemical is uncertain.

Little information is available on how irradiation affects physiological and physical changes or on how effective irradiation is for either decay control or as a quarantine treatment for blueberries. Significant cultivar variation, however, was found in the physiological response of highbush blueberries to gamma irradiation ranging from 1.0 to 5.0 kGy (Eaton et al., 1970).

Tissue softening is an undesirable effect of irradiation treatment for small fruits, such as strawberry (*Fragaria × ananassa* Duch.)

(Maxie and Abdel-Kader, 1966; Maxie and Sommer, 1968). Tissue softening occurs during normal ripening, and changes in tissue firmness are associated with compositional changes of cell wall neutral sugars in a variety of fruits and vegetables, including blueberry (Gross and Sams, 1984). Proctor and Peng (1989) documented changes in total cell wall pectin and compositional changes of fractionated pectic substances during fruit development and ripening of 'Bluetta' blueberry.

Blueberries lose considerable market value because of postharvest rots (Ceponis and Cappellini, 1979). Even though blueberries can be stored at 1°C (Hardenburg et al., 1986), a temperature that slows the growth of many decay organisms, postharvest decays such as anthracnose (*Gloeosporium* spp.), gray mold rot (*Botrytis cinerea* Pers. ex Fr.), and alternaria (*Alternaria* spp.) result in spoilage. Market losses from decay vary depending on factors such as harvest method (Mainland et al., 1975), ripeness stage at harvest (Ballinger and Kushman, 1970; Galletta et al., 1971), and cultivar (Miller et al., 1988). Controlled atmospheres (Ceponis and Cappellini, 1983, 1985; Smittle and Miller, 1988), modified atmospheres (Day et al., 1990), fungicides (Ceponis and Cappellini, 1978), and temperature (Ballinger et al., 1978) vary in their decay-control efficacy. High CO₂ for either 24 or 48 h, was not detrimental to the quality of lowbush blueberries, but this treatment was not effective for blueberry maggot control (Prange and Lidster, 1992).

Gamma irradiation may be used as a single treatment for quarantine purposes or in combination with other treatments such as high- or

low-temperature storage to achieve the desired efficacy. Irradiation partially controlled decay in mangos (*Mangifera indica* Linn) (Spalding and Reeder, 1986). Johnson et al. (1990) also reported partial decay control in mangos with irradiation or fungicides alone, but hot benomyl followed by irradiation provided more effective control of anthracnose (*Colletotrichum gloeosporioides* Penz.) and stem-end rot (*Dothiorella dominicana* Petrak Cif.). Carambola (*Averrhoa carambola* L.) peels were not injured at 0.05 kGy (Gould and von Windeguth, 1991). Irradiation of food-stuffs is limited to 1.0 kGy by the U.S. Food and Drug Administration (USFDA, 1986).

The purpose of our study was to determine the maximum gamma irradiation dosage that 'Climax' blueberries could tolerate without physiological or physical degradation and to investigate a possible relationship between biochemical changes in cell wall neutral sugars and physical changes in berry softening following irradiation.

Materials and Methods

'Climax' blueberry fruit were harvested on three occasions at 1-week intervals (20 May–4 June 1991) from a plantation in Alachua County, Fla. Immediately after harvest, berries were manually packaged in 0.24-liter pulp baskets with a cellophane cap held in place with a rubber band. The baskets were placed, 12 each, into commercial master shipping trays. The trays of fruit were put into large plastic foam coolers, separated from crushed ice by plastic film, and transported by automobile to the U.S. Horticultural Research Laboratory in Orlando, Fla. On arrival, each basket of berries was weighed and placed randomly into six treatment (TRT) groups of 18 baskets each. TRT 1 berries were immediately placed in air at 1°C; all other fruit (TRT 2–6) were held overnight at 15°C. The following morning, berries of TRT 2–6 were placed in a portable cooler with ice and transported to the Subtropical Horticultural Research Laboratory, Miami. Berries of TRT 3–6 were irradiated in a Gammacell 220 irradiator (Atomic Energy of Canada, Ottawa, Ont.) containing 26,060 Ci (1 Ci = 37 GBq) of Co⁶⁰ (Jan. 1983). The dose rate at the center of the chamber was 117.5 ± 0.5 Gy·min⁻¹. About 1250 g of berries in a 2-liter glass beaker was irradiated on each occasion. The six TRTs were as follows: 1) control A—held continuously at 1°C and not irradiated, 2) control B—held in the same environment as irradiated fruit but not irradiated, 3) 0.75 kGy (6 min 19 sec), 4) 1.5 kGy (12 min 38 sec), 5) 2.25 kGy (18 min 56 sec), and 6) 3.0 kGy (25 min 15 sec). After treatment, the fruit were repackaged as previously described. All berries were weighed before and after treatment, and returned the same day to the Orlando laboratory for storage and inspections.

Three baskets of berries from each of the six treatments were inspected after six storage regimes: 1) overnight at 1°C, 2) 3 days at 1°C, 3) 7 days at 1°C, 4) 7 days at 1°C + 2 days at 15°C, 5) 14 days at 1°C, and 6) 14 days at 1°C + 2 days

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at 15C. At each inspection, all berries were evaluated subjectively and individually for juice leakage, decay, and cullage (immature berries) (Miller and Smittle, 1987). Berry firmness was determined subjectively and objectively. Subjective firmness was determined by rotating berries between the thumb and index finger and applying moderate pressure. Berries yielding to light pressure were rated soft; those not yielding were classified as firm. Objective firmness of 10 berries per basket was measured on an Instron Food Testing System (Instron, Canton, Mass.). It was calibrated to read 98 N full scale, and elongation was set at 3 mm at a speed of 50 mm·min⁻¹. Fruit were also evaluated for skin discoloration, shrivel, powdery bloom, and moisture on the fruit surface. After each inspection, 10 berries from each basket were evaluated for powdery bloom (1 = full bloom, 2 = half bloom, and 3 = no bloom) and then wiped free of moisture and powdery bloom before peel color (L*, a*, b*) was measured with a chroma meter (model CR 200; Minolta, Osaka, Japan) in the CIE(1976) mode. After each inspection, 50 g of berries was taken from each basket of each treatment-storage combination, frozen, and later assayed for soluble solids concentration (SSC), titratable acidity (TA), and PH. An additional 50-g sample was taken for cell wall analysis. Undamaged fruit from each inspection lot were evaluated by an informal nine-member panel for flavor anti mastication texture (MT) on a modified hedonic-type scale with a quantifying index ranging from 0 to 100 (unacceptable 10 excellent, respectively).

The 50 g of berries used for cell wall analysis was frozen in liquid nitrogen and stored at -80C for subsequent extraction. These berries were homogenized in 80% ethanol using a Polytron homogenizer (Brinkman Instruments, Westbury, N.J.) and filtered through Miracloth (Cal biochem, Novabiochem, LaJolla, Calif.). The residue was thoroughly rinsed with three volumes of 20 mM Hepes-NaOH (pH 6.9), stirred in two volumes of 2 phenol : 1 acetic acid : 1 H₂O (by volume) for 20 min to inactivate endogenous wall-associated enzymes (Jarvis, 1982), and filtered through Miracloth. The residue was stirred in two volumes 1 chloroform : 1 methanol (v/v) for 20 min, transferred 10 a sintered-glass filter, and washed with two additional volumes of chloroform : methanol followed by three volumes of acetone. Cell wall material was dried ≥3 days over P₂O₅ *in vacuo* at 37C.

For cell-wall neutral sugar analysis, cell walls (5 mg) were treated in scaled tubes with 2 N trifluoroacetic acid for 1 h at 121C to hydrolyze noncellulosic neutral sugars. The hydrolyzed sugars were dried under N₂, and alditol acetate derivatives were prepared according to a modification of the procedure used by Blakeney et al. (1983). Alditol acetates were separated and quantified using a gas chromatography (model 5880; Hewlett Packard, Palo Alto, Cal if.) as described by Mitcham and McDonald(1992). Alloose served as the internal standard.

Quality attributes and taste-panel data were averaged over three replications of harvest

dates and subjected to analysis of variance (ANOVA) or a regression analysis to test for differences among treatments after each storage duration. Data for neutral sugars were combined over inspection days for ANOVA and regression analysis of treatment differences.

Results and Discussion

Berry firmness (subjective). The percentage of firm fruit over the three harvests (replications) averaged 63% initially and declined to 34% after 14 days at 1C; conversely, the amount of soft fruit increased from 26.5% to 47.5% during the same storage time at 1 C. In general, irradiation resulted in significantly reduced fruit firmness after 1 C storage (Fig. 1, Table 1). However, the percentage of firm berries increased in all treatments during the two additional days at 15C after storage (Fig. 1) or 14 days at 1C (data not shown). The reason for the increased firmness of the blueberries is unexplained; there was no decrease in weight that could be associated with berry

shriveling or drying and subsequently firming. A higher percentage of berries were rated soft after 7 days at 1 C than after 2 additional days at 15C regardless of treatment (Fig. 1), and differences among treatments were not significant (Table 1). After the final inspection (14 days at 1C + 2 days at 15 C), visual observations of the pulp tissue indicated increased internal damage in fruit given the higher irradiation doses (2.25 and 3.0 kGy) compared to control fruit or fruit irradiated at 0.75 or 1.5 kGy (data not shown). The tissue at the cut surfaces of injured berries was translucent, watery, and mottled compared with the consistent white-blue tissue of normal or noninjured pulp.

Decay and culls. The incidence of decay averaged 7%, 10%, and 14% over all treatments after 3, 7, and 14 days of storage at 1 C, respectively, and ≈ 16% after 14 days at 1C + 2 additional days at 15C.

Regardless of dosage, irradiated fruit had more decay than nonirradiated berries after all storage regimes, although differences by dose were not significant (*P* < 0.05). For example,

Fig. 1. Percentage of firm, soft, decay, and cull 'Climax' blueberries after irradiation and storage for 7 days at 1C or 7 days at 1C + 2 days at 15C.

Table 1. Mean squares for quality characteristics of irradiated blueberries.

Variable	df	Mean squares			
		Firm ²	Soft	Decay ²	Culls
7 Days 1C					
Treatment	5	440.3*	172.7 ^{NS}	111.0 ^{NS}	2.1 ^{NS}
Error		97.3	73.1	61.1	2.1
7 Days 1C + 2 days 15C					
Treatment	5	262.8 ^{NS}	23.6 ^{NS}	147.3 ^{NS}	3.0 ^{NS}
Error		101.8	24.9	92.5	2.1

²Firm berries, those with no yield to moderately applied finger pressure.

³Including mushy and leaky fruit.

^{NS} Nonsignificant or significant at *P* ≤ 0.05, respectively.

after 7 days at 1C, berries exposed to 0.75 kGy had more decay than control fruit but less decay than fruit receiving a higher irradiation dose (Fig. 1).

The amount of cull fruit [consisting mostly of immature fruit, mummified berries, and plant material other than berries (stem and leaves)] was relatively constant, ranging from 2% to 5% among the various experimental treatments during storage.

Flavor and texture. Flavor of fruit from the first of the three harvests was generally preferred over that of later-harvested berries (data not shown). Averaged over all inspections, flavor declined as irradiation dose increased, ranging from a score of 80 for nonirradiated fruit to 68, 61, 57, and 53 for 0.75, 1.5, 2.25, and 3.0 kGy, respectively. The negative effect of dosage on flavor and MT was detected in fruit held overnight at 1C and continued throughout storage at 1C for 7 or 14 days. There was as light, but noticeable, improvement in flavor and MT when berries were held at 15C, regardless of prior storage duration at 1C (Table 2). The cause for this change in flavor and MT is unknown because there was no environmental change in atmosphere and only $\approx 1\%$ change in fresh weight during 2 days at 15C. Although the same panelists conducted all flavor and MT evaluations at each inspection, they were comparing relative differences of berries among treatments within a particular inspection lot only. Scores < 50 for flavor or MT indicated consumer rejection (Table 2). At doses 0.75 kGy, MT was generally unacceptable because of berry softening. Flavor was generally rated unacceptable at doses > 1.5 kGy, especially when storage at 1C exceeded 7 days. There was a trend for flavor and MT to decline as storage duration at 1C increased. Panelists found MT of irradiated fruit to be soft or mushy when chewed compared to nonirradiated fruit. Mean objective texture measurements indicated that berries were not significantly softened as dosage increased; however, a general tendency for softening is indicated (Table 2). Objective measurements show berries were more resistant to compression after two additional days at 15C storage than during prior 1C storage. At each inspection, berries were cut in half, perpendicular to the stem/blossom-end axis, and irradiated fruit pulp was visibly more gelatinous than nonirradiated pulp.

Cell wall analysis. There was little change in cell-wall neutral sugar composition within the same treatment from 1 day after treatment to 14 days after treatment (data not shown). Cell walls from blueberries irradiated at 2.25 and 3.0 kGy had more noncellulosic neutral sugars than the control B fruit (Table 3). The amount of xylosyl residues in cell wall from fruit irradiated at 2.25 and 3.0 kGy was also significantly higher than for control B fruit.

There was no evidence for a loss of insoluble cell wall pectin with irradiation, as there was no decrease in rhamnosyl, galactosyl, or arabinosyl residues (Table 3). However, this finding does not preclude a change in pectin molecular weight, which can occur without changes in neutral sugar composition. In

irradiated pickle mesocarp tissue softening was primarily associated with changes in the solubility characteristics of cell wall pectic substances, and a decrease in galactose, which increased with increased dosage (Howard and Buescher, 1989). A decrease in pectin molecular weight after irradiation has been demonstrated in citrus (Skinner and Kertesz, 1960) and apple (*Malus domestica* Borkh.) pectin (Ayyad et al., 1990) through a reduction in viscosity, and this may be the cause for blueberry softening in our study. Further analysis of pectin molecular weight of irradiated blueberries is required to confirm this theory.

The increase in cell wall xylosyl residues may indicate an increase in xylosyl-containing polymers such as hemicellulosic polymers. An increase in hemicellulose with increasing dosage of irradiation has been demonstrated in tomato (*Lycopersicon esculentum*

Mill) and peach (*Prunus persica* L. Batsch) fruit; however, a smaller increase in hemicellulose also occurred in nonirradiated, ripening tomatoes and peaches (Foa et al., 1980), indicating irradiation may have stimulated ripening. Alternatively, hemicellulose-degrading enzymes may be more sensitive to irradiation damage than synthetic enzymes, resulting in a net increase in hemicellulose.

Weight loss. Regardless of irradiation dosage, weight loss was similar in all storage regimes. Average weight loss increased from 1.3% after storage for 1 day at 1C to 5.9% after 14 days at 1C + 2 days at 15C. Slight shriveling occurred at the stem end on some fruit when weight loss reached $\approx 4\%$. When weight loss increased to $\approx 6.0\%$, most berries were shriveled, usually at the stem end.

Bloom and surface color. The initial average bloom index was 1.6 and the indices

Table 2. Means for flavor and texture (sensory or objective) of 'Climax' blueberries after gamma irradiation treatment (TRT) and storage for 14 days at 1C or 14 days at 1C + 2 days at 15C.

Dose (kGy)	Source	df	14 days 1C			14 days 1C + 2 days 15C		
			Sensory		Objective texture	Sensory		Objective texture
			Flavor ^a	Texture ^b	(N) ^c	Flavor	Texture	(N) ^c
0.0 (control A) ^w			62	63	2	82	77	3
0.0 (control B) ^y			71	69	2	78	75	2
0.75			57	46	2	72	65	2
1.5			59	44	2	69	57	2
2.25			54	46	2	56	53	2
3.0			46	34	2	49	42	2
TRT		5	204.1 [*]	511.4 [*]	0.1 ^{NS}	475.8 [*]	553.1 [*]	0.05 ^{NS}
Error		10	33.3	52.4	0.08	86.9	55.6	0.07
Regression ^u		1	833.2 [*]	1461.6 [*]	0.02 ^{NS}	1576.9 [*]	1880.2 [*]	0.12 ^{NS}
Error		10	41.5	86.9	0.09	90.0	53.8	0.05

^aFlavor was measured on a hedonic scale (0–100); < 50 = unacceptable; 100 = excellent.

^bTexture was measured on a hedonic scale (0–100); < 50 = unacceptable; 100 = excellent.

^cN = Newton measurement of force to compress berry 3 mm.

^wControl A = fruit held continuously at 1C.

^yControl B = nonirradiated fruit given same environmental conditions as irradiated fruit (see text for details).

^uLinear regression on treatments excluding control A only.

NS, *Nonsignificant or significant at $P \leq 0.05$, respectively, by analysis of variance.

Table 3. Noncellulosic neutral sugar content of cell walls from control and irradiated blueberry fruit.

Dose (kGy)	Cell wall (%)	Total noncellulosic neutral sugars ^a					
		RHM	ARA	GAL	XYL	MAN	GLC
		µg/mg cell wall					
0.0 (control A) ^y	36 ^a ab ^w	28 a	106 a	92 a	1,498 ab	28 ab	59 a
0.0 (control B) ^y	33 a	26 a	112 a	114 a	1,287 a	32 b	62 a
0.75	36 ab	27 a	94 a	84 a	1,490 ab	26 a	55 a
1.5	36 ab	26 a	97 a	109 a	1,482 ab	28 ab	60 a
2.25	42 bc	31 a	112 a	102 a	1,800 bc	31 ab	69 a
3.0	46 c	32 a	116 a	103 a	1,943 c	30 ab	66 a
Source	df	Mean squares					
Treatment ^v	5	97.8 [*]	24.4 ^{NS}	308.2 ^{NS}	485.3 ^{NS}	227,733 [*]	24.4 ^{NS}
Error	18	26.1	14.2	266.9	198.2	64,846	15.2
Regression ^u	1	435.6 ^{**}	84.1 [*]	220.9 ^{NS}	7.2 ^{NS}	15.1 ^{**}	46.4 ^{NS}
Error	15	27.2	11.9	283.6	233.4	68,757	16.9

^aRHM = rhamnosyl, ARA = arabinosyl, GAL = galactosyl, XYL = xylosyl, MAN = mannosyl, GLC = glucosyl.

^yControl A = blueberries held continuously at 1 C; control B = nonirradiated blueberries held at same environmental conditions as irradiated berries (see text for details).

^wMean of four samples from each treatment (harvest 1, 1 and 14 days after treatment; harvest 2, 1 and 7 days after treatment).

^vMean separation of control A vs. means of all other treatments only within columns at $P \leq 0.05$.

^uAnalysis of variance for all treatments.

^uRegression analysis excluding control A treatment.

^{NS}, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

increased (bloom decreased) to 2.7 after the final storage duration with no difference among treatments. The presence of powdery bloom decreased as storage duration increased.

The average CIE b^* (blue-yellow) value was -0.37 (range -0.31 to -0.57) over all storage durations, with no difference > 0.5 units in peel color in the CIE b^* value among fruit of the three tests. L^* (darkness-lightness) and a^* (green-red) values averaged 29.37 (range 29.32-29.47) and 0.41 (range 0.33-0.47), respectively, during all storage/temperature durations (detailed data not shown). The surface color of berries was not affected by treatment and did not change during storage.

SSC, TA, and pH. Initially, SSC, TA, and pH averaged 13.9%, 0.52%, and 3.14, respectively, and remained relatively constant during storage (data not shown); SSC and TA were not affected by irradiation treatment. In general, pH was not affected by irradiation dosage except after 7 and 14 days of storage at 1C, when pH averaged 3.21 in fruit treated at 2.25 and 3.0 kGy and 3.16 for all other treatments ($P \leq 0.05$).

In general, berry quality was seriously reduced at > 1.5 kGy. Loss of quality was demonstrated through increased berry softening, increased decay, loss of internal tissue integrity, and reduced flavor acceptability. Because the composition of major neutral sugars was not significantly affected, softening did not appear to be due to a loss of cell wall pectin, but it could be related to changes in polymer molecular weight or loss of uronic acids. Changes in xylosyl residue content indicated a change in cell wall metabolism at higher doses. We did not observe increased peel darkening or notable increases in SSC as reported by Eaton et al. (1970), but we confirmed the observation of increased fruit softening due to irradiation.

Additional experiments should be conducted to more precisely determine the threshold tolerance of 'Climax' berries to irradiation. In addition, the effect of irradiation on other major blueberry cultivars should be evaluated because previous research has indicated that cultivar response to irradiation is inconsistent (Eaton et al., 1970). Previous research has shown that treatment of grapefruit (*Citrus paradisi* Macf.) at 0.6 kGy (von Windeguth, 1982) and carambola at 0.05 kGy provided Probit 9 mortality (Gould and von Windeguth, 1991) against Caribbean fruit fly [*Anastrepha stuspensa* (Loew)] eggs and larvae. If Probit 9 mortality can be achieved ≤ 0.75 kGy, an irradiation protocol might be developed as a feasible nonchemical quarantine treatment against blueberry pests. However, irradiation alone likely will not be a viable treatment for decay

control because dosages from 2 to 3 kGy are reportedly required for efficacy against post-harvest rots such as gray mold or alternaria in some other fruits (Maxie et al., 1971).

In summary, our findings indicate that 'Climax' blueberries tolerate ≈ 0.75 kGy of irradiation, and we expect that low-dose irradiation may be developed as an alternative to the methyl-bromide 'quarantine treatment.

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