The Effects of Low-Dose Gamma Irradiation and Storage Time on Carotenoids, Antioxidant Activity, and Phenolics in the Potato Cultivar Atlantic

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ABSTRACT

Potatoes are stored to ensure a continuous supply; however, losses due to shrinkage and sprouting can be large. It is believed that low-dose ionizing irradiation will become more prominent for sprout inhibition due to the increasingly higher operating costs of low-temperature storage and the possible phase-out of chemical sprout inhibitors. The effects of storage and gamma irradiation on carotenoid content, antioxidant activity (AOA), and phenolic content were analyzed for the potato cultivar Atlantic. Tubers were subjected to 0, 75, and 200 Gy gamma irradiation doses, stored at 20 C, and analyzed after 0, 10, 20, 75, and 110 days in storage. Total carotenoid content determined via spectrophotometry decreased, while lutein content increased with storage. AOA appeared to first decrease and then increase, possibly due to dehydration, concentration, and/or induced stress. Phenolic content increased more with storage than with gamma irradiation. However, levels of some phenolic compounds, such as quercetin dehydrate, decreased with storage. Irradiation dose exerted a limited influence on AOA and phenolic and carotenoid contents. Interaction between storage time and irradiation dose was significant for AOA and phenolic content, but not for carotenoid content. Overall, storage exerted a much greater influence on carotenoid content, AOA, and phenolic content than did low-dose gamma irradiation.

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RESUMEN

Las papas son almacenadas para asegurar el abastecimiento continuado, pero las pérdidas por reducción y por brotamiento pueden ser grandes. Se cree que una dosis baja de irradiación ionizante sería más prominente en la inhibición del brotamiento debido a los cada vez mayores costos de operación para mantener baja la temperatura de almacenaje y la posible eliminación de inhibidores químicos. Se analizaron los efectos del almacenaje y la irradiación gamma del contenido carotenoide, actividad antioxidante (AOA) y del contenido fenólico en el cultivar Atlantic. Los tubérculos fueron sometidos a dosis de 0, 75 y 200 Gy de irradiación gamma, almacenados a 20 C y analizados a los 0, 10, 20, 75 y 110 días. Con el almacenaje, el contenido total de carotenoides, determinado por medio de espectrofotometría, disminuyó, mientras que aumentó el contenido de luteína. AOA parece disminuir inicialmente y luego incrementarse, debido posiblemente a la deshidratación, concentración y/o estrés inducido. El contenido fenólico aumentó más con el almacenaje que con la irradiación gamma, sin embargo, disminuyeron los niveles de algunos compuestos como el dehidrato de quersitina. La dosis de irradiación ejerció una influencia limitada sobre AOA y contenidos fenólicos y carotenoides. La interacción entre tiempo de almacenaje y dosis de irradiación fue significativa para AOA y contenido fenólico, pero no para contenido carotenoide. El almacenaje ejerció mayor influencia sobre el contenido carotenoide, AOA y contenido fenólico que las dosis bajas de irradiación gamma.

INTRODUCTION

Numerous epidemiological studies have correlated diets rich in fruits and vegetables with low levels of certain diseases (Ames et al. 1993). Other studies have attempted to explain this phenomenon by studying compounds in fruits and vegetables and correlating their effect on preventing and/or controlling chronic diseases (Dillard and German 2000). Currently, much research is focused on identifying and quantifying the levels of these phytochemicals found in fruits and vegetables; however, only limited studies have been conducted on the effect of processing on these potentially important compounds. Significant levels of these phytochemicals, such as antioxidants, phenolics and carotenoids, have been identified in cultivars and advanced breeding lines from the Texas Potato Variety Development Program, and results show that their content is genotype-dependent (Al-Saikhan 1994, 2000; Hale 2003). It is unknown how these levels are affected by storage, low-dose gamma irradiation, and the interaction between the two. The objectives of this investigation were to determine the effects of storage time and low-dose gamma irradiation on carotenoid content, antioxidant activity (AOA), and phenolic content in 'Atlantic', a popular processing cultivar.

MATERIALS AND METHODS

Plant Material

The early-market chipping cultivar Atlantic was used in this study. Atlantic is a standard for potato chip quality in the United States and is also a good cultivar for other cooking methods such as boiling, baking, and frying. Potatoes used for the investigation were planted near McCook, TX, close to the Mexican border in the Lower Rio Grande Valley, 30 mi northwest of McAllen in west central Hidalgo County. Tubers were harvested in bulk from the field and samples taken at random from the bulked harvest. Three to five potato tubers were subjected to each treatment combination, i.e. storage and irradiation. Single tubers from each set were prepared separately by dicing the tuber into 64 mm cubes with a manual vegetable dicer (The Redco Insta Cut 3.5, Lincoln Foodservice, Fort Wayne, IN), and taking three samples of 5 g each. Samples were then frozen at -20 C until extraction.

Gamma Irradiation Treatment

Potato tubers were subjected to gamma irradiation via a Cesium-137 source at the USDA/APHIS Moore Air Field Base near Mission, TX. The gamma irradiation dosage was determined on the basis of dose per time rates (40 Gy per minute) that are calculated periodically based on the degradation of the irradiation source and confirmed by dosimeters. Tubers were surrounded with alanine dosimeter pellets (Bruker, Billerica, MA) to verify irradiation exposure using a PC-interfaced benchtop electron paramagnetic resonance (EPR) spectrometer (Bruker, Billerica, MA). The EPR spectrometer used in this study was not calibrated to read such low doses, so the pellets were exposed to a previously larger dose before exposure to low dosages with the tubers, and dosage was then determined by measuring the dose difference. The spectrometer measurements were analyzed over 24 hours after the samples were exposed to the irradiation, so the measurements are considered as estimates. The average dosage measured for the pellets exposed to the 75 Gy treatment was 90 Gy, while the average dosage measured for the pellets exposed to the 200 Gy treatment was 200 Gy.

Storage Treatments

All tubers, both irradiated and non-irradiated, were stored at 20 C for 0, 10, 20, 75, and 110 days. After every storage period, samples were analyzed for carotenoid content, antioxidant activity, and phenolic content. Samples stored for 0 days were analyzed fresh or within 24 h after irradiation.

Extraction of Carotenoids

The carotenoids (only xanthophylls) were extracted with methanol (plus 1 g/l of BHT for stabilization). Tuber samples (5g) with extraction solvent were homogenized and centrifuged at 14,500 rpm for 15 min. The supernatant was collected and analyzed for carotenoid content following the method of Scott (2001). A standard curve of lutein ($y = 3028.6x + 8.1063$, $R^2 = 0.99$) was prepared and used to equate spectrophotometric absorbance readings of the methanol extract at 445 nm to lutein equivalents, where x was the absorbance at 445 nm and y was the µg lutein equivalents per hundred g fresh weight (µg LE/100gfw).

HPLC Analysis for Individual Carotenoid Compounds

Six ml of extracted sample were concentrated under nitrogen gas and filtered through a 0.45 µm syringe filter (Hale 2003). A PC-operated Waters high performance liquid chromatograph (HPLC) system was used to analyze individual

carotenoid compounds through spectra and retention time. The system was comprised of two binary pumps (Waters 515), an autoinjector (Waters 717 Plus), a photodiode detector (Waters 996), and a column heater (SpectraPhysics SP8792) maintained at 35 C. A 4.6 x 250 mm, 5 µm, YMC Carotenoid Column (C-30 reverse phase) (Waters, Milford, MA) was used to separate the carotenoid compounds. The compounds analyzed included: violaxanthin, neoxanthin, antheraxanthin, β-cryptoxanthin, canthaxanthin, zeaxanthin, and lutein. Standard compounds were obtained from CaroteNature (Lupsingen, Switzerland) and Hoffman La Roche (Basel, Switzerland). The two solvents used for carotenoid identification were Solvent A: methanol, water, and triethylamine (90:10:0.1), and Solvent B: methanol, MTBE, and triethylamine (6:90:0.1). The following was the gradient used for the analysis: (min / %A) 0/99, 8/99, 45/0, 50/0, and 53/99 with a flow rate of 1 ml/min (Breithaupt and Barmedi 2002; Hale 2003).

Extraction of Phenolics and Total AOA

For the evaluation of total phenolic content, individual phenolic compounds, and total AOA a single extraction was performed. Fifteen ml of methanol were added to a 5 g sample of diced potato. Samples were homogenized and centrifuged at 17,000 rpm for 20 min. The supernatant was collected for analysis.

DPPH Assay for Total AOA

Total AOA was measured using DPPH (2,2 Diphenyl-1 picrylhydrazyl), a colorimetric assay first described by Brand-Williams et al. (1995). The reduction of the DPPH solution is positively correlated with a change in absorbance, allowing for a simple assessment of AOA. The sample extracts were allowed to react with DPPH until stabilization at 24 h. After this time, the level of reduction was determined by absorbance at 515 nm. A standard curve using a known antioxidant, trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid), was prepared, and a regression curve was calculated to convert the change in absorbance into AOA. AOA was reported as trolox equivalents and determined using the following equation: $y = 891.69x$, where x is the change in absorbance (calculated by subtracting the sample absorbance from the blank of methanol and DPPH at 515 nm), and y was the µg trolox equivalents per g fresh weight (µgTE/gfw) (R^2 = 0.997).

Total Phenolic Content

The Folin-Ciocalteu phenol method to determine phenolic content was first described by Swain and Hillis (1959) and modified by Singleton and Rossi (1965). Absorption was determined at 725 nm in plastic UV-spectrophotometeric cuvettes. Phenolic content was determined by preparing a regression curve, $y = 0.5775x - 0.0279$, where x was the absorbance at 727 nm and y was the µg chlorogenic acid equivalents per g fresh weight (µgCE/gfw).

HPLC Analysis for Individual Phenolic Compounds

Six ml of the extracted sample were concentrated under nitrogen gas to completion, resuspended in 0.5 ml of water and 0.5 ml ethanol, and filtered through a 0.45 µm syringe filter (Hale 2003). A PC-operated Waters HPLC system was used to analyze individual phenolic compounds in the samples by comparing their spectra and retention times to those of standards. The system used to separate phenolic compounds was comprised of two Waters 515 binary pumps (Waters 515), an autoinjector (Waters 717 Plus), a photodiode detector (Waters 996), along with a column heater (SpectraPhysics SP8792) maintained at 40 C. A 4.6 x 150 mm, 5 µm, Atlantis C-18 reverse-phase column (Milford, MA) was used. Pure phenolic compounds — 5,7-trihydroxyflavanone, sinapic acid, keampherol, (-)epicatechin, catechin, quercetin dehydrate, rutin hydrate, protocatechuic acid, salicylic acid, myricetin, syringic acid, vanillic acid, t-cinnamic acid, p-coumaric acid, ferulic acid, caffeic acid, and chlorogenic acid, obtained from Agros Organics (Pittsburgh, PA) — were used as standards. Two filtered and de-gassed solution solvents were used for HPLC analysis: Solvent A 100% acetonitrile, and Solvent B nanopure water adjusted to pH 2.3 with HCL. The following gradient was used: (min/%A) 0:85, 5:85, 30:0, and 35:0 with a flow rate of 1 ml/min (Hale 2003).

Experimental Design

The experiment was a 3 x 5 factorial design with three doses of gamma irradiation (0, 75, or 200 Gy) and five storage periods (0, 10, 20, 75 and 110 days after irradiation treatment). Three to five potato tubers were subjected to each treatment combination and data was collected on individual tubers of each set, resulting in three to five replicates of each treatment combination. Analysis of variance (ANOVA) was performed

using the general linear model (GLM). Given the nature of the treatment factors (quantitative) regression analysis or model fitting was done to ascertain the relationships between treatment variables and the response variables by estimating the regression coefficient (β value) corresponding to each treatment variable. All statistical analyses were performed with SPSS software version 11.5 (SPSS 2002). Second degree models (polynomial) were fitted with SAS/GRAPH software (SAS 2002) to approximate the shape of the response surface.

RESULTS

Phenolic compounds identified and quantified with HPLC were chlorogenic acid, rutin hydrate, protocatechuic acid, quercetin dehydrate, catechin, p-coumaric acid, caffeic acid, vanillic acid, (-) epicatechin, myricetin, sinapic acid, and t-cinnamic acid, while carotenoid compounds detected and quantified with HPLC analysis were lutein, zeaxanthin, and canthaxanthin (Table 1). ANOVA results revealed significant storage effects for AOA, carotenoid content and phenolic content. In addition, lutein, zeaxanthin, and canthaxanthin exhibited significant storage effects, and among the phenolic compounds quantified, significant storage effects were displayed by chlorogenic acid, caffeic acid, t-cinnamic acid, epicatechin, quercetin dehydrate, catechin, and vanillic acid. Gamma irradiation had significant effects on AOA, carotenoid content, t-cinnamic acid, and quercetin dehydrate. Interaction between storage time and gamma irradiation dose significantly affected AOA, phenolic content, lutein, t-cinnamic acid, quercetin dehydrate, and vanilllic acid (Table 1).

When multiple factors in a study are compared, some factors have a greater influence than others, regardless of significance levels. The extent of influence or the magnitude of strength of a specific factor is the amount of variability created due to that factor. Levine and Hullett (2002) suggested using eta squared values to compare the magnitude of effects. These values attempt to explain how strongly two or more variables are related, with a percent of the total variability attributed to each factor in an experiment, including the error term, totaling 100 %. We estimated the magnitude of strength of each variance component by computing the proportion of the total variance (total mean squares) attributed to their effects. This was done only for those dependent variables (parameters) that exhibited significant effects for any of the variance components (Table 2). The most influential factor for carotenoid content, AOA, and phenolic content was storage time. It accounted for 90, 15, and 17 % of the total variability in carotenoid content, AOA, and phenolic content, respectively. HPLC results of individual carotenoid and phenolic compounds indicated that storage was the most influential factor. Proportion of total variance attributed to interaction effects was greater than that attributed to irradiation dose, except for

TABLE 1—*ANOVA of antioxidant activity, carotenoid content, phenolic content, and individual carotenoid and phenolic compounds.*

Parameter	Variance components‡ Storage [4]	Irradiation [2]	S x I [8]
AOA	132417.09**	8691.93**	43162.17**
Carotenoid content	226364.98**	4353.57**	721.99
Lutein	1830.07**	218.57	488.65**
Zeaxanthin	368.11**	4.31	7.33
Canthaxanthin	193.74**	33.32	25.16
Phenolic content	289659.55**	5334.35	71907.22*
Chlorogenic acid	10095.24**	291.69	1578.59
Caffeic acid	1.25**	0.16	0.39
T-cinnamic acid	0.13**	0.21**	0.04*
Rutin hydrate	308.15	682.47	979.04
Sinapic acid	178.34	45.07	45.05
Epicatechin	0.61*	0.09	0.18
Quercetin	48.06**	70.96**	17.44**
Protocatechuic acid	3674.07	228.94	1473.69
Myricetin	15.39	2.21	7.01
P-coumaric acid	20.37	7.95	20.47
Catechin	5.13*	0.16	0.92
Vanillic acid	2.52**	0.85	0.91*

‡ Values in brackets are degrees of freedom for each component.
* Indicates significance at $P < 0.05$.
** Indicates significance at $P < 0.01$.

TABLE 2—*Percentage of total variation attributed to storage time, gamma irradiation dose, and their interaction.*

Parameter	Variance components Storage	Irradiation	S x I
AOA	15.0	1.0	10.0
Carotenoid content	89.9	0.9	0.6
Lutein	36.9	2.2	19.7
Zeaxanthin	44.1	0.3	1.8
Canthaxanthin	32.8	2.8	8.5
Phenolic content	17.0	0.2	8.5
Chlorogenic acid	27.2	0.4	8.5
Caffeic acid	23.9	1.5	14.8
T-cinnamic acid	28.6	21.9	16.5
Epicatechin	17.2	1.4	10.3
Quercetin	28.8	21.2	20.9
Catechin	20.5	0.3	7.4
Vanillic acid	28.3	4.8	20.5

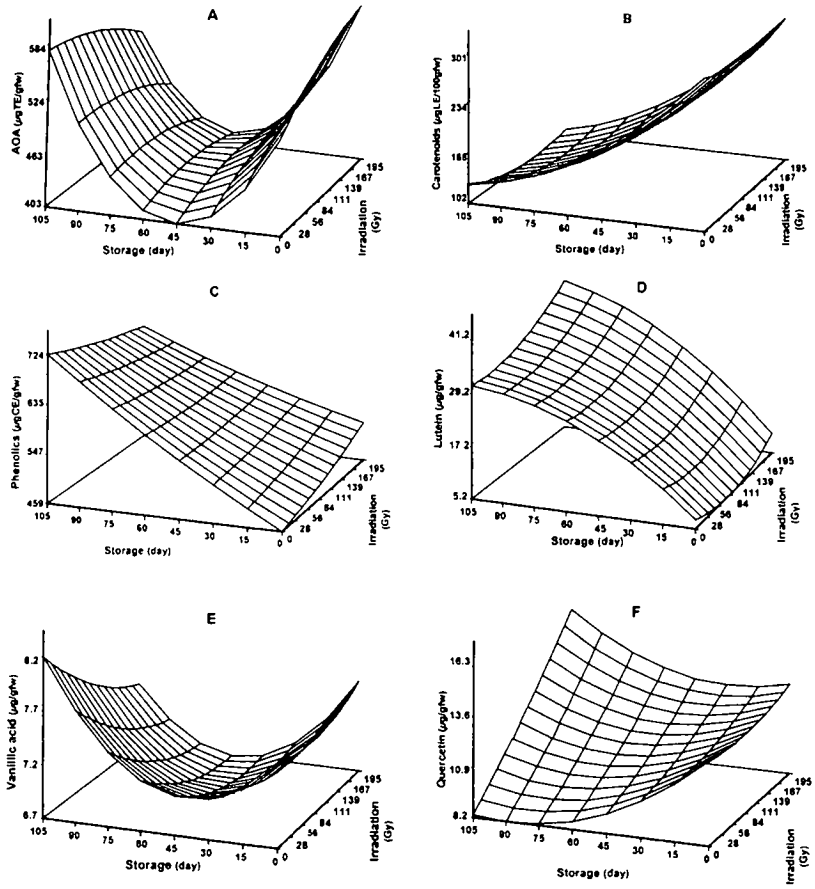

FIGURE 1.
Response surface plots of the effect of storage time and gamma irradiation on antioxidant activity, phenolic content, and carotenoid content in potato cultivar Atlantic. (A) antioxidant activity (AOA); (B) carotenoid content; (C) phenolic content; (D) lutein; (E) vanillic acid; (F) quercetin.

TABLE 3—*Estimated regression coefficients of the treatment variables for each response variable.*

Dependent variable	Estimated regression coefficients (β values) Intercept	Storage	Irradiation
AOA	484.18	0.21	0.17
Phenolic content	471.56	1.98	0.16
Carotenoid content	285.29	-1.70	-0.01
Lutein	6.44	0.26	0.03
T-cinnamic acid	7.45	0.00	0.00
Quercetin dehydrate	10.87	-0.01	0.02
Vanillic acid	7.36	0.00	-0.00

t-cinnamic acid and quercetin dehydrate. Also, the proportion of total variance in AOA and phenolic content attributed to interaction effects was greater than for carotenoid content. There was a large amount of variability in the dependent variables not explained by the three variance components, with the exception of carotenoid content, for which more than 90 % of the variability was accounted for by the treatment factors (Table 2).

To further quantify the effects of storage time and gamma irradiation, linear models were fitted to the data of the dependent variables that exhibited significant effects for storage and gamma irradiation and/or significant effects for one of the treatment factors and the interaction effect (Table 3). Storage exerted more influence on AOA, phenolic content, and carotenoid content than did gamma irradiation. The coefficient estimates suggest that there was a 0.21 units (µgTE/gfw) increase in AOA for each unit (day) increase in storage, and 0.17 units increase in AOA with a 1 unit (Gy) increase in gamma irradiation dosage. Phenolic content also increased with storage by 1.98 units each day and 0.16 units for 1 Gy increase in irradiation. However, carotenoid content decreased with storage by 1.7 units per day (Table 3). Gamma irradiation had a slight increasing effect on lutein and quercetin dehydrate, and a decreasing effect on carotenoid content and vanillic acid.

Second-degree models (polynomial) were fitted to approximate the shape of the response surface. Polynomials are popular due to their versatility in fitting a wide variety of different surface shapes (Cornell 1995). Response surfaces provide a visual image of how the dependent variables were affected by the treatments during the course of the experiment (Figure 1). AOA was highest at the early and late stages of storage, while between d 10 and d 75 it was low (Figure 1A). AOA may have steadily decreased with storage; however, due to dehydration and subsequent concentration of solids, AOA was again high at the end of the storage treatment.

Phenolic content also increased more with storage time than with gamma irradiation dose (Figure 1C). Quercetin dehydrate increased with storage, while vanillic acid first decreased, then increased at later stages of storage (Figure 1E and F). Zafrilla et al. (2001) also noted that certain phenolic

compounds increased during storage. The gain in phenolic content may be due to dehydration, leading to concentration of solids at the end of the storage period. Additionally, stimulation of synthesis of both antioxidants and polyphenols is known to occur with stress, which may have increased at the end of the storage period due to dehydration (Friedman 1997; Ghanekar et al. 1984; Kang and Saltveit 2002). For example, the activity of PAL (phenylalanine ammonia lyase), which is a precursor to phenolic compounds, has been reported to increase under stressful conditions, and this is associated with the accumulation and synthesis of phenolic compounds (Blankenship and Unrath 1988; Kang and Saltveit 2002). Carotenoid content decreased with subsequent storage (Figure 1B), probably due to low stability of carotenoid compounds. However, lutein increased with storage and slightly increased with gamma irradiation (Figure 1D).

During later stages of storage, tubers not subjected to 0 Gy of irradiation sprouted, while those exposed to 75 and 200 Gy remained dormant. This may have caused the 0 Gy tubers to dehydrate and concentrate their solids, resulting in higher AOA at later stages of storage. Also, stress due to sprouting may have induced antioxidants in the 0 Gy tubers. Bergers (1981), Patil et al. (1999), and Penner and Fromm (1972) reported a time-dependent change with irradiation and storage in the antioxidant-rich phenolics, chlorogenic acid, scopoletin, and quercetin. Pendharker and Nair (1975) also reported an increase in PAL activity with irradiation. Storage time and, to a limited extent, irradiation dose affected certain compounds; however, the variability of these compounds did not fluctuate much, and the potato retained a large proportion of these health-promoting compounds.

DISCUSSION

Storage had the greatest influence on all variables analyzed, with an increase in phenolic content and a decrease in carotenoid content. Low-dose gamma irradiation had less influence on AOA, phenolics, and carotenoids, as indicated by their magnitude of strength and regression coefficients. A trend with AOA was identified where tuber samples had higher AOA values immediately after exposure, lower after 30-60 days of storage, and then higher values with continued storage. This is believed to be due to some induction mechanism; however, physical changes such as sprouting and subsequent dehydration are also believed to be responsible for this observed phenomenon. There was some variability in the levels of specific carotenoid and phenolic compounds due to storage and irradiation.

Sprout-controlling mechanisms such as irradiation will gain importance as more regulations are imposed on sprout-inhibiting chemicals. Consumer acceptance has been the largest hurdle for the implementation of irradiation treatment. However, acceptance levels may be increasing since irradiation has proven effective in inactivating spoilage and pathogenic microorganisms. Market tests have demonstrated that acceptance increases when consumers are provided with information about irradiated foods, such as potential increases in phytonutrients. Consumers now view irradiated meat and poultry positively, with most willing to purchase such products. Each food processing method has technical and economic advantages and disadvantages; therefore, food irradiation for sprout control, quarantine treatment, or food safety and nutritional quality should be considered as a viable treatment, especially if consumer education is provided. Continued research into the physical, chemical, and nutritional changes induced by storage and irradiation is warranted.

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