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Ionizing Radiation Induces Formation of Malondialdehyde, Formaldehyde, and Acetaldehyde from Carbohydrates and Organic Acid

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A study was conducted to investigate irradiation-induced formation of malondialdehyde (MDA), formaldehyde (FA), and acetaldehyde (ACT) from fructose, sucrose, glucose, and malic acid solutions. MDA and FA were generated from the carbohydrate solutions upon irradiation while little was formed from malic acid solution. On the other hand, a much higher amount of ACT was formed from malic acid than from the carbohydrate solutions. The *G* values (number of molecules formed per 100 eV radiation) for MDA were 0.042, 0.0066, and 0.0026 from 0.9 mg mL^{-1} fructose, sucrose, and glucose solutions at pH 3.5, respectively. The *G* values for FA formation were 0.134, 0.233, and 0.0081 from the fructose, sucrose, and glucose solutions, respectively. As concentration of sugars in solutions increased from 0 to 90 mg mL^{-1}, the formation of these compounds increased rapidly. A further increase in sugar concentration from 90 to 900 mg mL^{-1} resulted in a lower rate of increase in MDA and FA formation. pH had a profound effect on the irradiation-induced formation of these compounds from carbohydrates, especially on MDA formation. The minimum amount of MDA from fructose and glucose solutions was observed at pH 5 while formation of MDA from sucrose solution decreased as pH decreased from 7 to 2. The results can be used by the food industry to optimize food formulation in order to minimize formation of these compounds.

KEYWORDS: **Carbohydrate; formaldehyde; irradiation; malondialdehyde; malic acid**

INTRODUCTION

Several recent outbreaks of illness have been linked to the consumption of unpasteurized fruit juice due to contamination of foodborne human pathogens (*1*, *2*). Ionizing radiation is a nonthermal technique highly effective for inactivating human pathogens in juice (*3*, *4*). Irradiation, however, induces accumulation of formaldehyde (FA), malondialdehyde (MDA), and acetaldehyde (ACT) in apple juice (*5*). MDA, FA, and ACT are naturally occurring compounds commonly found in a variety of foods. However, MDA and FA, at high concentrations, can induce toxic effects in animal systems (*6*−*8*), including carcinogenicity (*9*), and ACT can influence juice flavor (*10*). However, sources of these compounds are not completely clear.

Fruit juices are rich in carbohydrates and organic acids. Major carbohydrates and organic acids in fruit juice include fructose, glucose, sucrose, malic acid, and citric acid. These compounds may be precursors for aldehyde formation. In fact, high doses (>10 kGy) of radiation induce formation of MDA from carbohydrates in either solid state or aqueous solution (*11*−*13*). When irradiated at high doses, glucose and fructose solutions are cytotoxic in vitro (*14*). Although MDA can arise from carbohydrates, analytical methods used in most of the previous studies utilized nonspecific colorimetric techniques with strongly acidic and high temperature conditions. MDA can be artificially generated under these conditions (*15*). In the present study, a gas chromatography−mass spectrometry (GC-MS) method (*16*) was used to characterize factors influencing low dose irradiation-induced formation of MDA, FA, and ACT from carbohydrates and malic acid.

MATERIALS AND METHODS

All chemicals were purchased from Sigma (St. Louis, MO). Fructose, glucose, sucrose, and soluble starch (amylopectin) were all ACS grades while DL-malic acid had a 99% purity.

Sample Preparation. Solutions (∼4 mL) containing 90 mg mL^{-1} sucrose, glucose, fructose, or starch or 20 mg mL^{-1} malic acid prepared using deionized water were placed into 4 mL glass vials. The concentrations of carbohydrates and malic acid were chosen to simulate apple juice. The vials were sealed with Teflon-lined septa and screw caps and stored at 5 °C overnight before irradiated.

Irradiation and Dosimetry. Irradiation was performed at 5 ± 2 °C using a self-contained, Lockheed Corporation ^{137}Cs γ-radiation source (Marietta, GA). The unit has 23 ^{137}Cs pencils placed in an annular array around a 63.5 cm high stainless steel cylindrical chamber with a 22.9 cm internal diameter. The source strength at the time of this study was ca. 102 000 Ci with a dose rate of 0.099 kGy min^{-1}. The dose rate was established using alanine transfer dosimeters from the National Institutes of Standards and Technology (Gaithersburg, MD). Actual doses were typically within 5% of targeted doses. The maximum/minimum ratio of absorbed doses was 1.08. The variations in radiation dose absorption were minimized by placing the samples within a

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uniform area of the radiation field, by irradiating them within a polypropylene container (4 mm wall) to absorb Compton electrons, and by using the same geometry for sample irradiation during the entire study. Routine dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rjeomstettem, Germany), and the free radical signals were measured using a Bruker EMS 104 EPR Analyzer (*17*). The dosimeters were placed into 1.2 mL cryogenic vials (Nalgene, Rochester, NY), and the cryogenic vials were taped onto the tubes containing samples prior to irradiation. The temperature was maintained by injecting the gas phase from a liquid nitrogen tank into the radiation chamber.

Measurement of MDA, FA, and ACT. MDA, FA, and ACT were measured (*16*) by adding pentafluorophenylhydrazine (20 μL of 5 mg mL^{-1}) to 250 μL of solution with the pH adjusted to 3.0 using HCl or NaOH solutions. After it was vortexed for 5 s, the mixture was incubated at room temperature for 30 min, and then, 250 μL of hexane was added. 1,2-Dibromobezene was added as an internal standard. After this mixture was vortexed for 30 s, a 2 μL aliquot of the hexane layer was injected into a Hewlett-Packard 5890/5971 GC-MSD (Agilent Technologies, Palo Alto, CA) equipped with a HP-5 trace analysis column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The GC oven temperature was held at 50 °C for 1 min, then increased at 20 °C min^{-1} to 280 °C, and then held for 1 min. The temperatures of the injector and transfer line were 250 and 280 °C, respectively. Helium was the carrier gas at a linear velocity of 20.7 cm s^{-1}. Amounts of MDA, FA, and ACT were calculated using response factors generated from standard curves. MDA was prepared by hydrolyzing 10 μmol of malonaldehyde bis(diethyl acetal) with 10 mL of 0.01 N HCl at 50 °C for 1.5 h, and then, the concentration of the MDA solution was determined spectrophotometrically by measuring absorbance at 245 nm (ϵ = 13 700).

Dose Response. Sucrose, fructose, and glucose (90 mg m^{-1}) solutions were prepared in 0.1 M Na-phosphate, and the pH was adjusted to 3.5 with dilute HCl solution. The solutions were then irradiated with radiation doses ranging from 0 to 6 kGy at 5 °C. *G* values (number of species per 100 eV absorbed) were calculated by the following eq (*18*): $G = k \times 10^8$, where k is the slope (mol L^{-1}/kGy) of the linear curve of yield vs dose.

Influence of pH. Sucrose, fructose, and glucose were dissolved in 0.1 M Na-phosphate, and the solution pH was adjusted to 2, 3, 4, 5, 6, or 7 using diluted HCl or NaOH, and the final concentration of carbohydrates in the solution was 90 mg mL^{-1}. The solutions were placed into 4 mL vials, equilibrated at 5 °C for 4 h, and then irradiated with a dose of 3.0 kGy at 5 °C.

Influence of Carbohydrate Concentration. A series of concentrations (0, 0.09, 0.9, 9, 90, and 180 mg mL^{-1}) of fructose, glucose, or sucrose were prepared in deionized water. The solutions were then placed into 4 mL glass vials. The vials were sealed, stored at 5 °C overnight, and then irradiated (3.0 kGy) at 5 °C.

Statistical Analysis. There were four replicates per treatment. Each vial was regarded as a replicate. Data were subjected to statistical analysis using SAS Version 7 (SAS Institute, Cary, NC). Treatment effects were analyzed by the least significant difference (LSD) analysis of the general linear model. In the figures, mean standard deviations are presented. Differences between means that exceed the standard deviations were always significant when analyzed using the LSD procedure at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Formation of Aldehydes from Carbohydrates and Malic Acid. MDA and FA were formed from fructose, glucose, and sucrose solutions following irradiation (**Figure 1**). The irradiation-induced formation of MDA and FA decreased in the following order: fructose > sucrose > glucose. Very little MDA or FA was formed from malic acid. A large amount of ACT was formed from malic acid by irradiation while very little was formed from the three carbohydrates. Very low amounts of MDA, ACT, or FA were formed from starch.

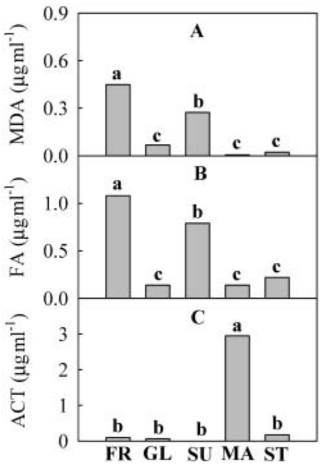

Figure 1. Irradiation-induced formation of MDA (**A**), FA (**B**), and ACT (**C**) from fructose (FR), glucose (GL), sucrose (SU), malic acid (MA), and starch (ST) solutions. Carbohydrate solutions (90 mg mL^{-1}) and malic acid (20 mg mL^{-1}) solution were irradiated with 3.0 kGy γ-rays at 5 °C. Aldehydes in the solutions were then measured on the day of irradiation. Bars with the same letter are not significantly different (LSD, $P < 0.05$).

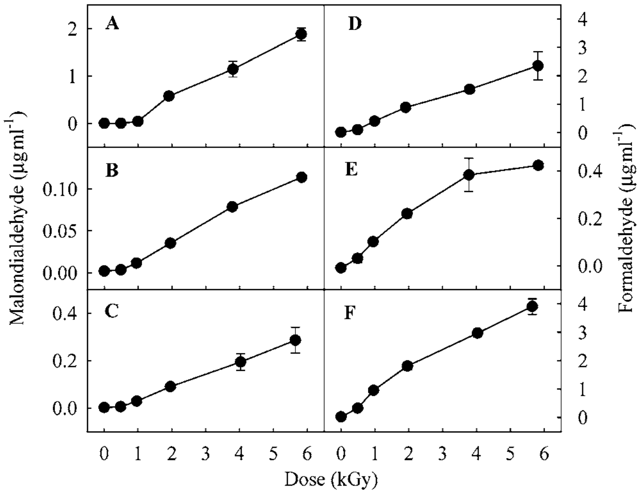

Figure 2. Effect of radiation dose on formation of MDA (**A**–**C**) and FA (**D**–**F**) from fructose (**A**, **D**), glucose (**B**, **E**), and sucrose (**C**, **F**) solutions. Sugar solutions (90 mg mL^{-1}) with a pH of 3.5 were irradiated with a series of doses of γ-rays at 5 °C. Aldehydes in the solutions were then measured on the day of irradiation. Vertical bars represent standard deviations of means (n = 4).

Dose−Response. MDA and FA formation from fructose increased with higher radiation dose (**Figure 2A**,**D**). The rate of increase in MDA formation was lower in the dose range of 0−1 kGy as compared to the range of 2−4 kGy. Overall, the increase was linear (R^2 = 0.97). The *G* values (number of molecules formed per 100 eV radiation) for MDA and FA formation from fructose were 0.042 and 0.134, respectively. The formation of ACT also increased with higher radiation dose, and the rate of increase was higher between 4 and 6 kGy (data not shown).

The formation of MDA from glucose increased linearly (R^2 = 0.99) with higher radiation dose (**Figure 2B**). The *G* value for MDA formation from glucose solution was 0.00261. Formation of FA increased linearly with dose from 0 to 4 kGy, and then, the rate of formation decreased between 4 and 6 kGy (**Figure 2D**). The *G* value for FA formation, calculated from the linear portion (0−4 kGy) of the plot was 0.0327. ACT formation increased linearly with increasing dose (data not shown), and the *G* value for ACT formation was 0.00811.

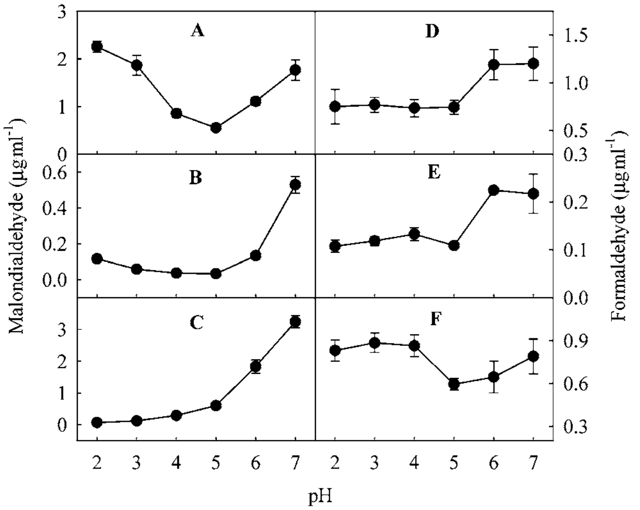

Figure 3. Effect of pH on irradiation-induced formation of MDA (**A**–**C**) and FA (**D**–**F**) from fructose (**A**, **D**), glucose (**B**, **E**), and sucrose (**C**, **F**) solutions. Sugar solutions (90 mg mL^{-1}) with different pH values were irradiated with 3.0 kGy γ-rays at 5 °C. Aldehydes in the solutions were then measured on the day of irradiation. Vertical bars represent standard deviations of means ($n = 4$).

MDA and FA formation from sucrose also increased linearly ($R^2 = 0.99$ and 0.98, respectively) with higher radiation dose (**Figure 2C**,**F**). The *G* values for MDA and FA were 0.0066 and 0.233, respectively. There was little ACT formed from irradiation of sucrose (data not shown).

Influence of pH. The formation of MDA from fructose was the lowest at pH 5 (**Figure 3A**,**D**). Higher or lower pH increased formation of MDA. The formation of FA from fructose was the highest at pH 6 and 7. Lowering pH from 6 to 5 decreased the formation of FA from fructose. A further decrease in pH did not affect ($P < 0.05$) the formation of FA from sucrose.

Formation of MDA from glucose was the lowest at pH 5 (**Figure 3B**,**E**). As pH decreased from 5 to 2, MDA formation increased. The formation of MDA from glucose was 3.4 times higher at pH 2 than at pH 5. As pH increased from 5 to 7, MDA formation increased from 33.8 to 529.6 ng mL^{-1}. The formation of FA from glucose was not affected by pH from 2 to 5. More FA formed at pH 6 and 7 than at other pH values. pH had a limited effect on the formation of ACT partially due to the relatively low amount of ACT formation from the carbohydrates. It appears that formation of ACT from fructose was the highest at pH 7 and 2.

The formation of MDA from sucrose increased with pH (**Figure 3C**,**F**). The MDA concentration increased 45-fold more at pH 7 as compared to pH 2 with the rate of formation increasing from pH 5−7. While pH had a limited effect on the formation of FA from sucrose, it appears that the formation of FA was the lowest at pH 5.

Concentration Effect. The rate of MDA and FA formation was maximal as concentrations of fructose (**Figure 4A**,**D**), glucose (**Figure 4B**,**E**), and sucrose (**Figure 4C**,**F**) increased from 0 to 9 mg mL^{-1}. At higher concentrations, the rate of formation for MDA and FA did not change or increased less, respectively. There was no consistent concentration effect on ACT formation, probably because the formation of ACT was low in all of these three solutions. Carbohydrates are major components of many fruits and their products, with a concentration commonly above 1% (*19*). These results suggest that the concentration of carbohydrates may not be a factor limiting for the formation of MDA and FA in fruit juice.

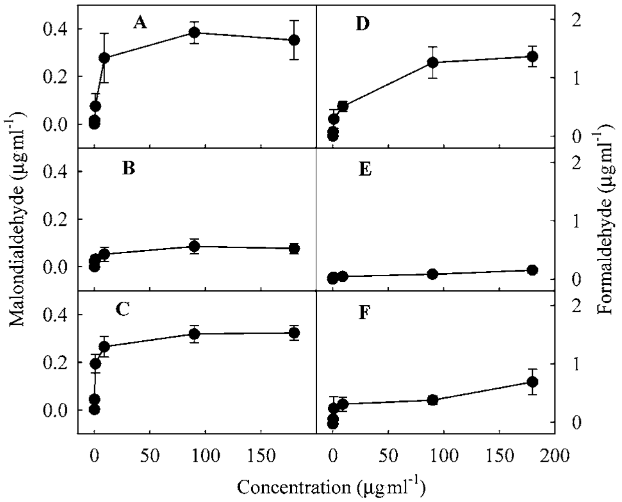

Figure 4. Effect of concentration on irradiation-induced formation of MDA (**A**–**C**) and FA (**D**–**F**) from fructose (**A**, **D**), glucose (**B**, **E**), and sucrose (**C**, **F**) solutions. Sugar solutions were irradiated with 3.0 kGy γ-rays at 5 °C. Aldehydes in the solutions were then measured on the day of irradiation. Vertical bars represent standard deviations of means ($n = 4$).

Irradiation-induced formation of MDA and FA from glucose was lower as compared to that from fructose or sucrose. For example, at pH 3.5, the *G* values for MDA formation from glucose solution were 2.5 and 16.1 times lower than from sucrose and fructose solutions, respectively. The *G* values for FA formation from glucose were 7.1 and 4.1 times lower than from sucrose and fructose, respectively. A lower amount of ACT was also formed from glucose than fructose although the amount of ACT from all of these sugars was low. Therefore, the formation of these compounds in processed food products intended for irradiation can be reduced by using glucose as the primary source of carbohydrates. Formation of MDA and FA from fructose and glucose, as well as FA formation from sucrose, is the lowest around pH 5, and MDA formation from sucrose solution decreases with decreased pH. To lower the formation of these compounds in products containing fructose and glucose as major carbohydrates, a pH of 5 should be employed. For products containing mainly sucrose, a pH as low as possible that does not significantly change product quality should be used. Most fruit juices have a pH of 3−4, and pH cannot be adjusted without significantly alternating quality characteristics. Therefore, it is impractical to change pH for fruit juices. Other means of reducing MDA and FA formation should be used, such as conducting irradiation at low temperature, suppression of radiation-induced reactions using antioxidants, and exclusion of oxygen during irradiation (*5*).

Although our results suggest that irradiation induced formation of MDA, FA, and ACT in pure carbohydrate and malic acid solutions, it should be stressed that accumulation of these aldehydes in foods will likely be dramatically reduced because of the protective nature of food matrix. For example, as compared to the formation of MDA from fructose (the major sugar in apple cider), *G* values were 4.9 times less in apple cider (*5*). Considering the simple composition of apple cider, the formation of these aldehydes in other more complex foods containing carbohydrates or organic acids will be even more reduced due to competitive reactions of other food components with irradiation-induced free radicals. Nevertheless, our results provided information for the food industry to further minimize the formation of these compounds.

Earlier results suggested that deoxycompounds and MDA were formed when carbohydrates were irradiated at high doses, particularly at alkaline pH (*12*, *13*). Exposure of carbohydrate solutions to UV radiation also induced formation of MDA (*20*), and MDA was induced by exposing deoxyribonucleosides to UV (*21*). A deoxy group on carbon 2 of the ribose moiety is required for the UV-induced formation of MDA. ACT can also be formed from organic acids such as L-threonic acid and L-ascorbic acid upon UV radiation (*22*). The formation of these compounds in carbohydrate solutions during exposure to ionizing radiation may be due to reaction of sugars with free radicals produced from radiolysis of water. These radicals include hydrated electron (e_{aq}), hydroxyl radical (${}^{\bullet}OH$), and hydrogen atom (${}^{\bullet}H$). ${}^{\bullet}OH$ radicals may be primarily responsible for the formation of MDA from glucose (*11*, *12*). Bucknall and others (*11*) have suggested that reaction of ${}^{\bullet}OH$ with C-5 and C-6 of glucose produces MDA. The results presented in the present study indicate that more MDA and FA are generated from fructose (a ketose) than glucose (an aldose), suggesting that the presence of a keto group at the sugar C-2 position is important.

pH (ranging from 2 to 7) has a profound effect on the irradiation-induced formation of MDA from sugars. Many early studies focused on effects of alkaline conditions (pH range 7−13) on the formation of MDA (*11*−*13*). Scherz (*13*) found formation of MDA increased sharply above pH 10. However, most foods are not strongly alkaline. At high pH, enolization and even fragmentation of sugar molecules may occur, followed by additional secondary reactions. At pH lower than 6, no MDA was found in sucrose or glucose solutions even at a dose of 50 kGy. Most of the earlier studies measured MDA using a nonspecific colorimetric method. Using a more sensitive and specific GC-MS method, this study shows MDA as well as FA and ACT can be generated even at low pH. Monosaccharides and oligosaccharides are fairly stable in solutions with a pH range of 3−7 (*23*). It is unclear how pH affects the radiation-induced formation of the aldehydes from carbohydrate. pH effect may be a consequence of protonation and deprotonation of various function groups of the carbohydrates (*18*). Furthermore, pH may affect the formation and amounts of the primary radicals generated from radiolysis of water and consequent secondary reactions. Under acidic conditions, the following reaction may occur, $e_{aq}^{-} + H^{+} = {}^{\bullet}H$ (*24*). Different sugars (i.e., aldose, ketose) may be differentially affected by pH due to structural differences.

In summary, MDA and FA were generated from carbohydrate solutions during irradiation while ACT was induced from malic acid solution. The formation of these compounds in carbohydrate solutions increased linearly with dose. Solution pH has a profound effect on the irradiation-induced formation of these aldehydes, especially MDA in carbohydrate solutions.

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LITERATURE CITED

(1) Besser, R.; Lett, S.; Weber, J. An outbreak of diarrhea and hemolytic uremic syndome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *J. Am. Med. Assoc.* **1993**, *169*, 2217−2220.

(2) Parish, M. Relevancy of Salmonella and pathogenic *E. coli* to fruit juices. *Fruit Process.* **2000**, *7*, 246−250.

(3) Buchanan, R. L.; Edelson, S. G.; Snipes, K.; Boyd, G. Inactivation of *Escherichia coli* O157: H7 in apple juice by irradiation. *Appl. Environ. Microbiol.* **1988**, *64*, 4533−4535.

(4) Niemira, B. A.; Sommers, C. H.; Boyd, G. Irradiation inactivation of four *Salmonella* species in orange juices with varying turbidity. *J. Food Prot.* **2001**, *64*, 614−617.

(5) Fan, X.; Thayer, D. W. Formation of malonaldehyde, formaldehyde and acetaldehyde in apple juice induced by ionizing radiation. *J. Food Sci.* **2002**, *67*, 2523−2528.

(6) Marnett, L. J.; Hurd, H. K.; Hollstein, M. C.; Levin, D. E.; Esterbauer, H.; Ames, B. C. Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. *Mutat. Res.* **1985**, *148*, 25−34.

(7) Mukai, F. H.; Goldstein, B. D. Mutagenicity of malonaldehyde, a decomposition of product of polyunsaturated fatty acids. *Science* **1976**, *191*, 868−867.

(8) Restani, P.; Galli, C. L. Oral toxicity of formaldehyde and its derivatives. *Crit. Rev. Toxicol.* **1991**, *21*, 315−328.

(9) Shamberger, R. J.; Andrione, T. L.; Willis, C. E. Antioxidants and cancer. 4. Malonaldehyde has initiating activity as a carcinogen. *J. Natl. Cancer Inst.* **1974**, *53*, 1771−1773.

(10) Hagenmaier, R. D.; Baker, E. A. Microemulsions and emulsions as citrus coating. *J. Agric. Food Chem.* **1994**, *42*, 899−902.

(11) Bucknall, T.; Edwards, H. E.; Kemsley, K. G.; Moor, J. S.; Phillips, G. O. The formation of malonaldehyde in irradiated carbohydrates. *Carbohydr. Res.* **1978**, *62*, 49−59.

(12) Kawakishi, S.; Okumura, J.; Namiki, M. γ-Radiaolysis of carbohydrate in aqueous solution. *Food Irradiation* **1971**, *6*, 80−86.

(13) Scherz, H. Formation of deoxycompounds and malondialdehyde in irradiated aqueous solution of carbohydrates and related compounds. *Radiat. Res.* **1970**, *43*, 12−24.

(14) Berry, R. J.; Hills, P. R.; Trillwood, W. Demonstration of a cytotoxic agent in gamma-irradiated carbohydrate solutions. *Int. J. Radiat. Biol.* **1965**, *9*, 559−572.

(15) Yeo, H. C.; Helbock, H. J.; Chyu, D. W.; Ames, B. B. Assay of malondialdehyde in biological fluids by gas chromatography−mass spectrometry. *Anal. Biochem.* **1994**, *220*, 391−396.

(16) Fan, X. Measurement of malondialdehyde in apple juice using GC-MS and a comparison to the thiobarbituric acid assay. *Food Chem.* **2002**, *77*, 353−359.

(17) ISO/ASTM, 51607:2002(E). Practice for the use of the alanine-EPR dosimetry system. *2002 Annual Book of ASTM Standards*; ASTM International: West Conshohocken, PA, 2002.

(18) Simic, M. G. Radiation chemistry of water-soluble food components. In *Preservation of Food by Ionizing Radiation*; Josephson, E. S., Peterson, M. S., Eds.; CRC Press: Boca Raton, FL, 1982; Vol. 2, pp 1−73.

(19) Scherz, H.; Senser, F. *Food Composition and Nutrition Tables*; CRC Press: Boca Raton, FL, 1994.

(20) Scherz, H. On the formation of malonaldehyde and deoxy compounds by photolysis of aqueous solutions of carbohydrates and related compounds. *Carbohydr. Res.* **1970**, *14*, 417−419.

(21) Miyake, T.; Shibamoto, T. Formation of malonaldehdye and acetaldehyde from the oxidation of 2′-deoxyribonucleosides. *J. Agric. Food Chem.* **1999**, *47*, 2783−2785.

(22) Miyake, T.; Shibamoto, T. Formation of acetaldehyde from L-ascorbic acid and related compounds in various oxidation systems. *J. Agric. Food Chem.* **1995**, *43*, 2783−2785.

(23) Belitz, H. D.; Grosch, W. *Food Chemistry*; Springer-Verlag: New York, 1986.

(24) Swallow, A. J. Chemical effects of irradiation. In *Radiation Chemistry for Major Food Components*; Elias, P. S., Cohen, A. J., Eds.; Elsevier: Amsterdam, Netherlands, 1977; pp 5−20.

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