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Effectiveness of Ionizing Radiation in Reducing Furan and Acrylamide Levels in Foods

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Furan and acrylamide are two possible carcinogens commonly found in many thermally processed foods. The possibility of using ionizing radiation to reduce the levels of thermally induced furan and acrylamide in water and selected foods was investigated. Aqueous furan solutions, and foods (frankfurters, sausages, infant sweet potatoes) that contained furan were irradiated to various doses of γ-rays. Water and oil spiked with acrylamide and potato chips (a known acrylamide-containing food) were also irradiated. In addition, possible irradiation-induced formation of acrylamide in glucose and asparagine solutions was analyzed. Results showed that irradiation at 1.0 kGy destroyed almost all furan in water. In frankfurters, sausages, and infant sweet potatoes, the rate of irradiation-induced destruction of furan was much lower than the rate in water, although significant reductions in furan levels were observed in all foods. Irradiation at 2.5−3.5 kGy, doses that can inactivate 5-log of most common pathogens, reduced furan levels in the food samples by 25−40%. Similarly to furan, acrylamide in water was also sensitive to irradiation. After 1.5 kGy of irradiation, most of the acrylamide was degraded. Irradiation, however, had a very limited effect on acrylamide levels in oil and in potato chips, even at a dose of 10 kGy. No detectable acrylamide was formed in the mixture of asparagine and glucose upon irradiation. These results suggest that a low dose of irradiation easily destroys furan and acrylamide in water. In real foods, however, the reduction of furan was less effective than in water, whereas the reduction in acrylamide was minimal.

KEYWORDS: Furan; acrylamide; ionizing radiation; reduction

INTRODUCTION

Both furan and acrylamide are classified as "reasonably anticipated to be a human carcinogen" by the U.S. Department of Health and Human Services and as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (*1*−*3*) on the basis of laboratory animal studies showing that furan and acrylamide cause a variety of tumors in rats and mice. Both potential toxic compounds have been found in a number of foods that are thermally processed. A recent survey by the U.S. Food and Drug Administration (FDA) (*4*) found that furan was present in many canned foods such as infant foods, soups, and meat products that underwent retort process. The highest levels of furan found in sausages were about 40 ng/g, whereas some baby foods such as sweet potatoes had furan levels as high as 108 ng/g. The presence of furan in many infant foods is of particular concern due to infants' weak immune systems and higher risks of health complications. It has been shown that furan is induced by thermal treatments from simple carbohydrates, ascorbic acid, amino acids, fatty acids, or a mixture of these above compounds (*5*−*8*). Acrylamide has been found in foods that have been cooked or processed at temperatures over 120 °C (*9*). Foods such as potato chips and French fries generally contained relatively high levels (60−1800 ng/g) of acrylamide (*10*−*12*). It is believed that acrylamide is formed by Maillard reaction from asparagine and reducing sugars (*7*, *13*−*16*). Because of the wide presence of the two compounds in many foods and their potential hazards to human health, means to reduce levels of the two compounds or to minimize the formation of these compounds are being investigated by many researchers (*17*−*19*).

Ionizing radiation is a nonthermal processing technology that is being used to prolong shelf life, inactivate foodborne pathogens, and control insects and parasites in various foods (*20*−*22*). Our earlier study demonstrated that irradiation induced furan formation in carbohydrate-rich foods such as fruit juice (*23*). However, irradiation did not induce, and even slightly reduced, furan formation in meat products (*24*). Irradiation has been studied to reduce toxic compounds such as nitrosamine and polyamines in some model foods (*25*, *26*). The objective of this study was to investigate the possibility of using irradiation to reduce furan and acrylamide in water solutions and in selected foods that contain furan or acrylamide.

MATERIALS AND METHODS

Chemicals and Materials. Furan (99%), d_4-furan (99%), acrylamide (99%), D-glucose (ACS reagent grade), anhydrous $MgSO_4$, and NaCl were purchased from Sigma-Aldrich (St. Louis, MO). L-Asparagine

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was from Eastman-Kodak (Rochester, NY) and 2,3,3-d_3-acrylamide (98%) from Cambridge Isotope Laboratories, Inc. (Andover, MA). Primary secondary amine (PSA) sorbent was obtained from Varian (Harbor City, CA). All solvents (acetonitrile, methanol, ethanol, and hexane) were a high-purity grade for residue analysis from Burdick & Jackson (Muskegon, MI). Deionized water was prepared by a Barnstead (Dubuque, IA) water purification system. Sausages, frankfurters, canned infant sweet potatoes, canola oil, and potato chips were purchased from a local supermarket.

Reduction of Furan in Water. Furan and d_4-furan solution (50 ng/mL each) was prepared in deionized cold water. Aliquots (5 mL) of the solution were equilibrated in 15-mL vials at 5 °C for 3 h before being irradiated (in two replicates per dose) at 0, 0.2, 0.4, 0.6, and 1.0 kGy at 5 °C. Furan was then analyzed as described earlier (*6*).

Reduction of Furan in Frankfurters, Sausage, and Infant Sweet Potatoes. Beef and turkey frankfurters and sausage were diced into 3 × 3 mm cubes on a stainless steel pan, which was placed on ice. Ten grams of the diced samples was placed into 40-mL chilled vials and sealed immediately with septa and caps. Chilled infant sweet potatoes (10 g) from plastic containers were placed into 40-mL vials. The vials were incubated at 5 °C for 16−18 h before being irradiated at 0, 2.5, 5, 7.5, and 10 kGy at 5 °C. After irradiation, 10 mL of 20 ng/mL d_4-furan solution in deionized water was injected into the vials through the septum. The vials were vortexed for 30 s on high speed and equilibrated at 5 °C for 2 h before the analysis.

Test of Acrylamide Formation from Glucose and Asparagine. A mixture of D-glucose (10 mM) and L-asparagine (10 mM) was prepared in deionized water. Aliquots (12 mL) of the solution were irradiated in 15-mL vials (in four replicates per dose) at 0, 0.5, 1.0, 2.0, 5.0, and 10 kGy at 20 °C. Acrylamide content was then measured.

Reduction of Acrylamide in Water and Oil. Acrylamide solution (1000 ng/mL) was prepared in deionized water. Canola oil samples (1 g) were spiked with acrylamide at 1000 ng/g by adding 50 μL of 20 μg/mL acrylamide solution in ethanol, followed by vortexing for 1 min. The aqueous solutions (1.5 mL) and oil samples (1 g) were equilibrated in 2-mL vials at 5 °C overnight before being irradiated (in two replicates per dose) to 0, 0.5, 1.0, 1.5, 2.0, and 2.5 kGy of γ-rays at 5 °C. Acrylamide content was then measured.

Reduction of Acrylamide in Potato Chips. Crushed potato chips (1 g) were placed into 15-mL vials. Ten milliliters of deionized water was added to half of the vials, whereas the other half of the vials were without the addition of water. The vials with water were vortexed. Both samples with and without water were irradiated in three replicates at 10 kGy of γ-rays at 20 °C. Acrylamide content was then measured.

Irradiation and Dosimetry. Irradiation was conducted using a self-contained, Lockheed Corp. (Marietta, GA) ^{137}Cs γ-radiation source. The dose rate was between 0.093 and 0.089 kGy/min depending on the time of experiments. Actual doses were typically within ±5% of targeted doses. Temperature was maintained by injecting the gas phase from a liquid nitrogen tank into the radiation chamber. 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. 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.

Measurement of Furan. The analysis of furan and d_4-furan has been described earlier (*6, 23*). Briefly, samples in 40-mL vials were incubated at 35 °C for 25 min before a solid-phase microextraction (SPME) fiber (75 μm Carboxen-PDMS from Supelco, Bellefonte, PA) was inserted into the headspace of a vial. After 20 min of extraction time, the SPME fiber was inserted into the GC injection port at 240 °C and held for 5 min to desorb volatile compounds. Volatile compounds were separated by a Hewlett-Packard 5890/5971 GC-MSD (Agilent Technologies, Palo Alto, CA) equipped with a 3.5-m GasPro capillary column (0.32 mm i.d.) connected to a DB-5 column (30 m × 0.32 mm i.d., 0.1-μm film thickness; J&W Scientific, Folsom, CA). The temperature program of the GC oven was set to 50 °C for 2 min, increased to 130 °C at 10 °C/min and then to 250 °C at 15 °C/min, and held for 2 min at the final temperature. Helium was the carrier gas at a constant linear velocity of 39 cm/s. The MS transfer line was held at 250 °C. Furan and d_4-furan were identified by comparison of the MS spectra and retention times of the sample compounds with those of standards. Furan was quantified using a standard curve established in the individual matrixes and corrected using the internal standard (d_4-furan).

Measurement of Acrylamide. Acrylamide content was determined by liquid chromatography−tandem mass spectrometry (LC-MS/MS) using a high-performance LC Agilent 1100 system with a binary pump, autosampler, column heater (kept at 25 °C), and degaser (Agilent Technologies) interfaced to an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Toronto, ON, Canada). A Phenomenex Aqua C18 column (150 × 3 mm; 5-μm particle size, 125-Å pore size) coupled to a C18 4 × 3 mm guard column (both from Phenomenex, Torrance, CA) was employed for the LC separation (*27*). The mobile phase was 99.5:0.5 water−methanol (at 200 μL/min for 8 min) for elution of acrylamide and 0.1% formic acid in acetonitrile−methanol (50:50, v/v) for the postanalysis wash (at 500 μL/min for 7 min) of retained matrix components on the column followed by equilibration to initial conditions. The internal standard d_3-acrylamide was added to all samples (at 50 ng/mL of solution or 500 ng/g of potato chip sample) prior to each analysis (sample extraction). The MS determination of acrylamide and d_3-acrylamide was performed in electrospray positive mode (using the optimized MS instrument parameters obtained by the tuning) combined with monitoring of the most abundant MS/MS (precursor → product) ion transitions. For acrylamide, transitions m/z 72 → 55, 54, 44, and 27 were monitored, whereas transitions m/z 75 → 58, 44, and 30 were used for d_3-acrylamide. Relative responses (peak areas) of acrylamide versus d_3-acrylamide (transitions m/z 75 → 55 and m/z 75 → 58, respectively) were used for calibration and quantification purposes.

Water samples were analyzed directly by injecting 20 μL of the tested solutions. The oil samples (1 g) were extracted with hexane (2 mL) and water (3 mL). After vortexing (for 1 min) and centrifugation (at 3450 rcf for 5 min), 20-μL aliquots of the water layer were analyzed by LC-MS/MS. In the case of potato chip samples and solutions of asparagine and glucose, a sample preparation method developed by Mastovska and Lehotay (*27*) was employed prior to the LC-MS/MS analysis. Ten milliliters of deionized water was added to dry potato chips (1 g), and 5 mL of hexane was added to all potato chip samples for defatting. Ten milliliters of the tested asparagine and glucose solutions were taken for the extraction; no hexane was added in this case. The rest of the procedure was practically identical for both sample types: 10 mL of acetonitrile, 4 g of $MgSO_4$, and 0.5 g of NaCl were added to the samples placed in 50-mL centrifuge tubes (Corning, Corning, NY). After vortexing (1 min), centrifugation (at 3450 rcf for 5 min), and removal of the hexane layer (potato chip samples), a 1-mL aliquot of the acetonitrile layer was placed in a 2-mL centrifuge tube. The extract was cleaned using a dispersive solid-phase extraction (SPE) procedure involving the addition of 50 mg of primary secondary amine (PSA) sorbent and 150 mg of $MgSO_4$ (*28*). The mixture was vortexed for 30 s and then centrifuged for 1 min at 3450 rcf. The supernatant was placed in an autosampler vial, and 10 μL of the final extract was analyzed by the above-described LC-MS/MS method with typical quantification limits of 5 ng/g in the potato chips and 0.2 ng/mL in the aqueous solutions.

Statistical Analysis. Each experiment was repeated twice with multiple vials for each replicate. Data were analyzed using SAS version 8.2 (SAS Institute, Cary, NC). The least significant difference test was used to analyze the difference among the treatments. Curve fitting was performed using the Regression Wizard of SigmaPlot version 6.0 (SPSS Inc., Chicago, IL).

RESULTS

Reduction of Furan in Water. Furan in water was very sensitive to irradiation. As the dose increased, the concentration of furan decreased rapidly (**Figure 1**). It appears that there were two phases of furan reduction as a function of radiation dose: a rapid decrease between 0 and 0.4 kGy followed by a slow decrease between 0.4 and 1.0 kGy. At 0.4 kGy, approximately

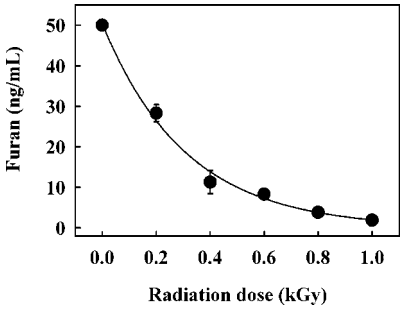

Figure 1. Furan concentration in water as a function of irradiation dose. Furan (50 ng/mL) solutions were irradiated at doses of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 kGy at 5 °C. Vertical bars represent standard deviations ($n = 4$).

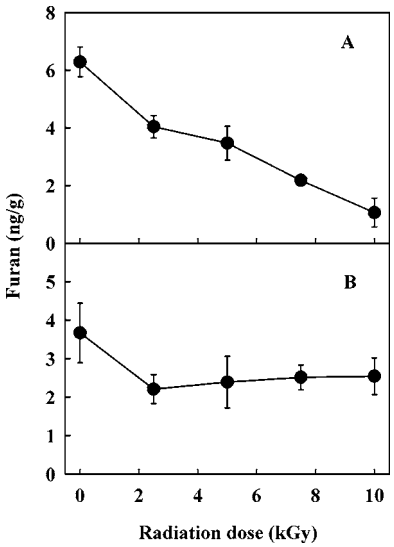

Figure 2. Effect of irradiation on furan levels in beef (**A**) and turkey (**B**) frankfurters. Frankfurters were irradiated at doses of 0, 2, 4, 6, 8, and 10 kGy at 5 °C. Vertical bars represent standard deviations ($n = 4$).

78% of furan was destroyed by irradiation. The decrease in furan levels as a function of dose could be expressed as an exponential curve. Irradiation-induced chemical reactions are generally linear if the amount of precursors is not limited (*29*). It is unclear why the overall response of furan reduction in response to radiation dose is not linear. The availability of furan may be a factor. The initial concentration of furan had an effect on the rate of furan reduction. At the initial concentration of 10 ng/mL, no detectable furan (limit of detection = 0.5 ng/mL) was observed at 0.6 kGy (*23*), whereas at 50 ng/mL, furan was destroyed by only 84% at the same radiation dose. Nevertheless, our results suggested that furan in water was sensitive to irradiation.

Reduction of Furan in Frankfurters, Sausages, and Infant Sweet Potatoes. Beef frankfurters purchased from a local supermarket had about 6.3 ng/g furan, whereas turkey frankfurters had about 3.7 ng/g (**Figure 2**). Furan concentrations of beef frankfurters decreased linearly with increasing radiation dose. At 10 kGy, only 1.1 ng/g furan remained in the samples. For turkey frankfurters, irradiation at 2.5 kGy significantly ($P < 0.05$) reduced furan formation compared with the non-irradiated control. However, as the dose increased, there was no further reduction in furan concentration. It is unclear why the response of the two frankfurters was different in terms of furan reduction, perhaps reflecting the difference in composition of the two frankfurters. Our earlier results showed that low levels of furan could be induced by irradiation in some foods, such as juice

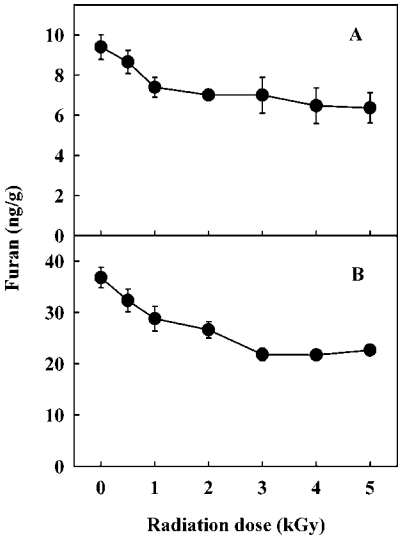

Figure 3. Effect of irradiation on furan levels in sausages (**A**) and infant sweet potatoes (**B**). The foods were irradiated at doses of 0, 1, 2, 3, 4, and 5 kGy at 5 °C. Vertical bars represent standard deviations ($n = 4$).

(*23*), but in most of the meat and meat products, no significant increase in furan due to irradiation was observed (*24*). In turkey and beef frankfurters, irradiation at 4.5 kGy significantly reduced the levels of furan (*24*). Irradiation is used to inactivate human pathogens in meat and meat products. One of the major pathogens in ready-to-eat products such as frankfurters is *Listeria monocytogenes*. To achieve a 5-log reduction of *L. monocytogenes*, doses between 2.5 and 3.5 kGy are required (*22*). At those doses, furan levels can be reduced by about 37−40%.

Sausages made from chicken and pork had 9.4 ng/g furan (**Figure 3**). As the dose increased from 0 to 1 kGy, furan levels decreased to approximately 7.4 ng/g, a significant reduction ($P < 0.05$). At 3 kGy, furan was reduced by 26%. However as the dose further increased, the rate of the decrease became less. At 5 kGy, there was still 6.4 ng/g furan remaining in the sausages. Furan concentration in infant sweet potatoes was about 37 ng/g, which was a much higher level compared to the other foods tested in this study but not surprisingly high for this type of sample. An FDA survey (*4*) reported furan content in 18 tested infant sweet potato samples ranging between 58 and 108 ng/g. The furan level decreased as radiation increased from 0 to 3 kGy. At 3 kGy, furan was reduced by 41%. However, as the dose further increased, there was no further decrease in furan levels.

Formation of Acrylamide from Glucose and Asparagine. Many earlier studies have demonstrated that acrylamide is formed from a mixture of a reducing sugar (e.g., glucose) and asparagine at elevated temperatures (*13*−*16*). To test whether acrylamide formation can be induced by ionizing radiation, we irradiated an aqueous mixed solution of glucose and asparagine at 0, 0.5, 1.0, 2.0, 5.0, and 10 kGy at 20 °C. Results showed that irradiation of the solutions did not produce a detectable level of acrylamide. It appears that, unlike thermal treatment, irradiation did not induce acrylamide formation in the mixture of glucose and asparagine.

Reduction of Acrylamide in Water and Oil. As the dose increased from 0 to 1.5 kGy, acrylamide in water decreased from 1000 ng/mL to approximately 20 ng/mL, a 98% reduction (**Figure 4**). Similarly to furan, lower initial acrylamide concentrations in the irradiated solutions resulted in even faster degradation rates (data not shown). Thus, an irradiation treatment

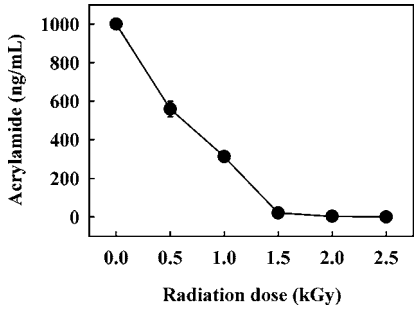

Figure 4. Effect of irradiation on acrylamide in water. Acrylamide solutions (1000 ng/mL) were irradiated with 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 kGy of γ-rays at 5 °C. Vertical bars represent standard deviations ($n = 4$). Only error bars larger than the size of the symbols are shown.

easily reduced acrylamide levels in water. In oil, however, irradiation had very little effect on acrylamide reduction. Even at 10 kGy, only about 5% of acrylamide was destroyed (data not shown). The results suggest that irradiation was not effective in an oil system, although very effective in the water system.

Reduction of Acrylamide in Potato Chips. To test whether irradiation reduces acrylamide in real foods, we irradiated potato chips, a product with high levels of acrylamide (*14*). We found that irradiation even at 10 kGy did not significantly reduce acrylamide in the dry chips. When water was added to the chips, irradiation at 10 kGy destroyed acrylamide by only about 15%. Our results indicate that irradiation would be effective in destroying acrylamide only in foods containing mostly water. In foods with limited water content, irradiation at doses that do not cause significant changes in flavor and nutrition will have little effect on acrylamide levels.

DISCUSSION

The results in the present study and earlier studies (*23, 24*) suggest that irradiation can induce as well as degrade furan in many foods. Whether irradiation reduces actual levels of furan in a specific food will depend on the balance between the rate of synthesis and the rate of degradation. Factors such as food composition, water content, and irradiation dose may play important roles in the rates of synthesis and degradation. For foods rich in carbohydrates and ascorbic acid, irradiation will likely increase furan levels because furan is formed from carbohydrates and ascorbic acid (*6*). For foods that mainly contain meats, irradiation should decrease furan content because irradiation induces little furan formation from fatty acids or proteins (*24*). The effect of irradiation on furan levels will also depend on the irradiation dose. The present study showed that irradiation significantly reduced furan levels in the food samples by 25−40% at doses of 2−3 kGy. A further dose increase did not result in an additional significant decrease in furan content. It is possible that irradiation at low doses (1−3 kGy) favored the reduction of furan over the formation of furan. However, as the dose increased, the rate of furan reduction decreased while the rate of furan formation remained at a similar level, resulting in overall accumulation of furan in the foods. Studies are needed to investigate the mechanism for the reduction of furan levels by irradiation in complex food products. Unlike furan, irradiation did not induce acrylamide formation from its precursors. However, the effectiveness of irradiation to reduce acrylamide levels was very limited.

Irradiation exerts its effects directly or indirectly (*30, 31*). In the direct action, ionized particles or rays directly target food components such as proteins and DNAs. In the indirect mechanism, irradiation exerts its effect through radiolysis of water, which results in the production of radicals such as hydrated electrons, hydroxyl radicals, and hydrogen atoms. These radicals then attack food compounds. It appears that irradiation destroys furan and acrylamide through the indirect reaction as it was very effective in reducing the two compounds in water, but had a very limited effect in foods tested in our experiments. Irradiation would be effective only in foods with a very high water content, for example, potential reduction of acrylamide in coffee. In complex foods, free radicals from water radiolysis will react not only with furan or acrylamide but also with other food components. Thus, unlike in pure water, the toxic compounds need to compete against the high concentrations of other food components for the primary radicals formed via radiolysis of water, lowering the effectiveness of furan and acrylamide reduction in real foods. In addition, all samplers were irradiated in the presence of oxygen (air). Whether exclusion of oxygen or modifying atmosphere will affect the effectiveness of irradiation in reducing furan and acrylamide is unclear.

Because of the limited effectiveness in most foods, other factors (such as the possibility of nutrient loss and off-odor compound formation) (*32*), and economical aspects, irradiation is unlikely to be used for the sole purpose of reducing the two toxic compounds. Currently, irradiation is employed for destroying harmful microorganisms and extending shelf life and for disinfestation purposes (*21*−*23*). When used for the above purposes, irradiation will likely also reduce the levels of furan in many foods, except for those rich in carbohydrates and ascorbic acid, such as orange juice (*23*), in which the furan formation rate may exceed the reduction rate. The irradiation technology, when used as a terminal processing step, that is, after thermal processing (if any) and packaging, may reduce furan in foods accumulated as a result of heat treatments.

In summary, our results suggest that irradiation even at doses of <2 kGy was very effective in destroying furan and acrylamide in water. However, in real foods that contained high levels of furan and acrylamide, radiation at doses of up to 10 kGy only partially reduced the levels of the two compounds. Irradiation did not induce acrylamide formation from a mixture of asparagine and a reducing sugar. The degree of furan and acrylamide reduction in any particular food will very likely depend on its composition, with water content being one of the most crucial factors.

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