in part, higher values of $^{14}C$ in the milk of [$^{14}C$]-*gem*-dimethyl-treated cows.

Radiocarbon reesidues in various tissues were generally very low except for liver, kidney, and abdominal and subcutaneous fats. These data are similar to those reported for cows given cypermethrin (Croucher et al., 1985), permethrin (Gaughan et al., 1978), and deltamethrin (FAO 1981) and with goats (Ivie and Hunt, 1980). About 30–50% of the total $^{14}C$ in liver and kidney was unmetabolized deltamethrin. In abdominal fat about 90% of $^{14}C$ from the [$^{14}C$]benzyl preparation was the parent insecticide. Kidney and liver also contained $Br_2CA$ and PBacid as both free and as conjugate(s).

About 78–82% of the total extractable $^{14}C$ from feces was in the unchanged deltamethrin. Croucher et al. (1985) have also identified cypermethrin as the only radioactive compound in the feces of cows treated with [$^{14}C$]cypermethrin. Urine of [*gem-dimethyl*-$^{14}C$]deltamethrin-treated cows contained $^{14}C$ metabolites that could be produced by a number of pathways as shown in Figure 1. The pathway appears to resemble closely that observed in laying hens (Akhtar et al., 1985).

The data suggest that even when deltamethrin is ingested by dairy cows at very high levels, the pesticide is poorly absorbed. Metabolism by rumen microflora and/or enzymes present in the gastrointestinal tract occurs to only a minor extent. Most of the ingested deltamethrin is excreted as unchanged insecticide in the feces. Consequently, residues are secreted in milk at trace levels and do not accumulate in edible tissues to any extent. Therefore, the data to date suggest that residues at levels that are of toxic concern may not be present in the milk and other dairy food products when deltamethrin is used around dairy cattle at much lower recommended levels. In addition to this short-term feeding trial, a longer term study is required at levels reflecting likely residues in feeds, to confirm our observations reported in this paper.

ACKNOWLEDGMENT

We thank Claude Danis for his technical assistance. We are also grateful to Floyd Fisher, Lorraine Robinson, and John Shackelton in caring for the cows on test.

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Received for review December 12, 1985. Accepted May 8, 1986. This work was supported in part by Hoechst Canada, Ltd. Contribution No. 1367. This study was part of a coordinated program of research under the sponsorship of the Joint Food and Agricultural Organization and the International Atomic Energy Agency, Vienna, Austria.

# High-Performance Liquid Chromatographic Determination of D-*arabino*-Hexos-2-ulose (D-Glucosone) in Irradiated Sugar Solutions: Application of the Method to Irradiated Mango

Laetitia Den Drijver,* Cedric W. Holzapfel, and Hendrik J. van der Linde

A strongly basic anion-exchange HPLC column coupled with an amperometric detector was used for the direct detection of a mutagenic compound, D-*arabino*-hexos-2-ulose, in γ-irradiated sugar solutions. The method was applied to an irradiated model fruit system as well as an irradiated whole fruit. Although D-*arabino*-hexos-2-ulose could be detected in the model system, no indication of its presence was found in the real fruit. This study represents the first direct analysis for radiolysis products in a whole fruit.

The γ irradiation of aqueous sugar solutions has been studied as part of our research on the wholesomeness of irradiated foodstuffs. A number of researchers have shown that γ-irradiated sugar solutions exhibit mutagenic activity (Schubert and Sanders, 1971; Schubert, 1969; Kesavan and Swaminathan, 1971; Kito et al., 1981; Schubert, 1973; Namiki et al., 1973; Aiyar and Subba Rao, 1975, 1977; Niemand et al., 1983), warranting further investigation since sugars are the main components of subtropical fruits. D-*arabino*-Hexos-2-ulose (D-glucosone) is one of the major radiolytic products of glucose and fructose (Dizdaroglu et al., 1975; Dizdaroglu and Von Sonntag, 1973; Von Sonntag, 1980; Kawakishi et al., 1973; Den Drijver, 1979.), the most common sugars in subtropical fruit. In an earlier investigation by Niemand et al. (1983), mutagenicity of glucosone toward *Salmonella* TA 100 was demonstrated. It was, therefore, necessary to develop a reliable and simple method for establishing the presence or absence of this compound in irradiated food.

The methods most commonly used for the determination of alduloses and diuloses in irradiated sugar solutions suffered from several disadvantages. We now report a simple, sensitive, high-performance liquid chromatographic method that allows the direct detection of D-glucosone in γ-irradiated sugar solutions and its application to irradiated fruit. As far as can be ascertained, this study represents the first analysis of an actual irradiated fruit for mutagenic compounds.

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0021-8561/86/1434-0758$01.50/0 

## EXPERIMENTAL SECTION

**Instrumentation.** Liquid chromatography was performed on a Dionex System 2011 ion chromatograph (all equipment and columns from Dionex Corp.). The system consisted of a pump, a chromatography module, and a pulsed amperometric detector. Separations were performed on a Dionex HPIC-AS6 anion-exchange column (250 mm × 4 mm) at 31 °C. Flow rate used was 0.9 mL/min.

**Samples.** The eluant consisted of sodium hydroxide (0.15 N) in deionized water. Sugars used were purchased from Merck and made with triple-distilled water to a concentration of 2% (w/v). All solutions were irradiated in a AECL Gammabeam-650 facility at a dose rate of 16.64 kGy/h. Glucosone was synthesized according to the method of Bayne et al. (1952), while all other standards were synthesized by using the methods described by Den Drijver (1979). The model mango was constituted after the method of Basson et al. (1979). Mangoes were subjected to irradiation for different times to accumulated doses of 0.75, 1.5, 5, and 20 kGy. After peeling and stoning, 100 g of flesh was treated according to the method of Chan et al. (1975). After addition of saturated lead acetate and centrifugation of the resulting suspension, 5 mL of clear supernatant was decanted and analyzed directly after suitable dilution.

## RESULTS AND DISCUSSION

In an earlier study on the mutagenicity of irradiated sugar solutions, Niemand et al. (1983) found oxygenated sugar solutions to be mutagenic toward *Salmonella* TA 100. The mutagenic activity was ascribed to the presence of compounds with a 1,2-diketo structure and was specifically demonstrated with D-glucosone. However, no mutagenicity could be illustrated in either irradiated model mango or in irradiated real fruit. It was thus important to develop an analytical method to study this phenomenon and to monitor the presence or absence of possible mutagenic compounds in irradiated foodstuffs.

**Methods Previously Used for Analysis of Irradiated Sugar Solutions.** A number of methods have been developed for the determination and detection of alduloses and diuloses in $\gamma$-irradiated sugar solutions. GC–MS is the method most widely used (Dizdaroglu et al., 1975; Kawakishi et al., 1975a,b; Schuchmann and Von Sonntag, 1977), but it involves time-consuming derivatization procedures. Another problem with some methods is the loss of information, for example when sodium borohydride reduction precedes derivatization. In this case, for example, D-gluconic acid, D-glucuronic acid, and D-*gluco*-hexodialdose are all reduced to D-glucitol (Schuchmann and Von Sonntag, 1977). In addition, the separations of the various components of the derivatized total irradiated product are generally not sufficient to allow direct analysis in the presence of large amounts of unreacted starting material. It is, therefore, often necessary to remove much of the unchanged sugar by preliminary column chromatography (Dizdaroglu and Von Sonntag, 1973). This problem can, at least in part, be solved by a method that involves benzyloximation, partitioning between solvents, and silylation followed by GC analysis (Den Drijver, 1979). The major disadvantage of this method, however, is that, as in the case of methoximation (Schuchmann and Von Sonntag, 1977), it often gives rise to more than one peak (due to syn–anti isomerization) for a single compound.

**High-Performance Liquid Chromatographic Methods for Sugar Analysis.** Carbohydrates can also be analyzed directly by high-performance liquid chromatography, using either strongly acidic cation-exchange columns in the metal ion form with water as eluant (Fett et al., 1980) or microparticulate silica with an amino-bonded phase (Yang et al., 1981). The latter method has been used quite successfully for the separation of glucosone from glucose and fructose (Geigert et al., 1980). However, in our hands problems were encountered with the analysis of irradiated samples. In these cases a serious problem is that, even at high doses, the main radiolysis products, formed in yields of less than 1%, are totally obscured by the much larger peak due to unreacted sugar. This is especially true since the resolution factor ($R$) for starting material (glucose, fructose) and some of the products can be as low as 0.2 on this type of column. Another problem with conventional HPLC for the direct analysis of carbohydrates is that the detection methods generally used are rather insensitive. Due to the absence of a strongly absorbing UV chromophore, detection by refractive index is generally preferred. This method is hampered by extreme dependence on accurate temperature control as well as by a lack of sensitivity. UV detection at low wavelength (192 nm) is another possibility, but the need for ultrapure solvents and the high sensitivity to interference by other substances limits its use. An alternative method of separation involves anion-exchange chromatography (Rocklin and Pohl, 1983). Carbohydrates have p$K$ values in the range of 12–14, making possible separation on a strongly basic anion-exchange column (hydroxide form) using highly alkaline eluants. This method gives excellent separation for carbohydrates (Rocklin and Pohl, 1983). Its success can be ascribed mainly to the application of a sensitive pulsed amperometric detector (Rocklin and Pohl, 1983; Edwards and Haak, 1983), which was developed by Dionex Corp. With this method the detection limit for monosaccharides is reported to be 30 ppb (Rocklin and Pohl, 1983).

We used the commercially available instrument and carbohydrate column, as described above, for the efficient separation of glucosone from fructose and glucose (Figure 1). Detector response for glucosone was found to be linear between 10 and 1000 ppm. When a pure solution of glucosone was analyzed, it was possible to detect 1 ppm accurately and reproducibly (±1%). The reproducibility of the method was also excellent in the case of the complex mixtures obtained by the irradiation of sugars. In this case, however, the lower limit of detection for glucosone was approximately 40 ppm, due to the column being overloaded by (the largely) unreacted sugars. Tailing of the unreacted material necessitated using the detector at the less sensitive end of the scale.

A series of alduloses and diuloses, synthesized for mutagenic testing (Niemand et al., 1983) and for use as standards for irradiated sugar analysis, were readily separated in the same manner (Figure 2). These preliminary results suggested that the method may be suitable for the direct analysis of irradiated sugars. In particular, it may be noted that each individual compound gives rise to a single sharp peak. This suggests that the alduloses and diuloses, which are known to be labile toward strong bases (Kucar et al., 1979; Niemand et al., 1983), do not decompose under the conditions used. Aldonic and uronic acids, which are often encountered in irradiated sugar solutions, give rise to single peaks, although these are rather broad.

**Analysis of Irradiated Sugar-Containing Solutions.** The analysis of a 2% fructose solution, irradiated (20 kGy) while oxygen was being bubbled through, showed glucosone to be the major radiolysis product formed in an amount (800 ppm) corresponding to that found by previous investigators (Kito et al., 1981). The chromatogram (Figure 3) shows that although the unreacted sugar peak probably

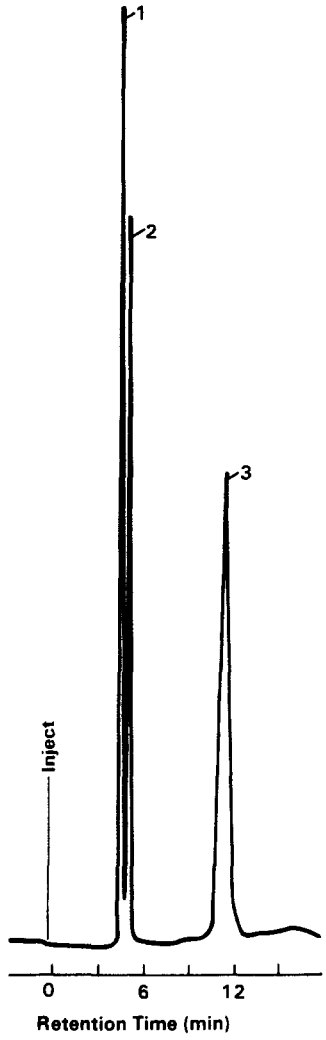


**Figure 1.** Separation: (1) glucose; (2) fructose; (3) glucosone.

obscures some of the diuloses, the important glucosone peak is clearly separated. Removal of much of the unchanged sugar by chromatography of the irradiated solution over silica in ethyl acetate–acetone–water (4:5:1) was not required for the unambiguous detection of glucosone, although it does allow the detection of the obscured components. All subsequent irradiations were carried out in open flasks on solutions presaturated with oxygen. The experimental conditions were chosen to simulate the actual condition in the fruit (oxygenated, changing to deoxygenated as oxygen is depleted during irradiation). Irradiation (20 kGy) of a 2% fructose solution under these conditions gave rise to the same pattern as in Figure 3, but a slightly lower yield of glucosone (ca. 650 ppm) was found. In the course of this investigation it was noted that the relative yield of glucosone, particularly in the case of fructose, was directly proportional to the sugar concentration (see Figure 4). This phenomenon, which may be due to a concentration dependence of competitive reactions (e.g., hydrogen abstraction by hydroxyl radicals vs. self-combination of the latter to give hydrogen peroxide) has, as far as we can ascertain, not been reported before, probably because previous studies were mainly confined to fixed concentration solutions.

On the basis of the results obtained with single sugar solutions we proceeded with the analysis of the radiation-induced changes in a model mango system (Basson et al., 1979). This is an aqueous solution of components found in the mango, mainly fructose and glucose, some sucrose and maltose, as well as some amino acids, fatty

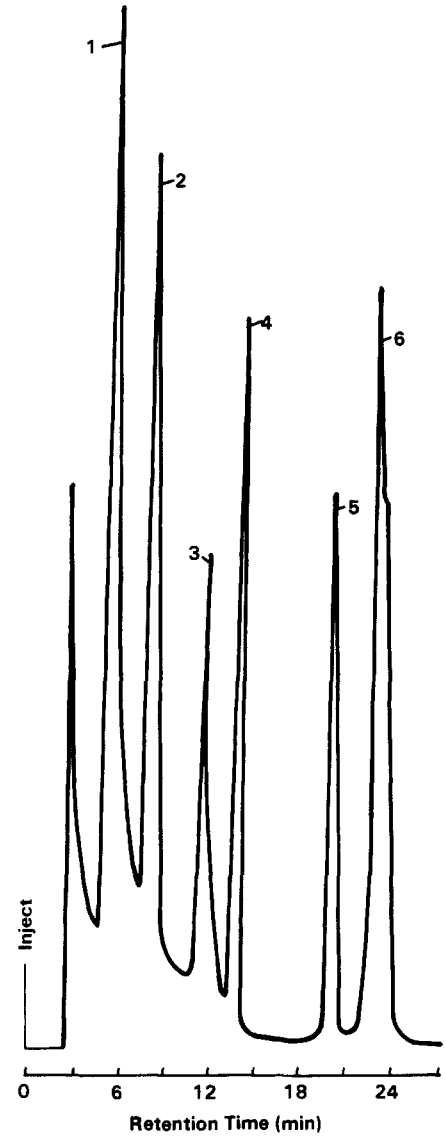


**Figure 2.** Separation of synthetic alduloses and diuloses: (1) D-*erythro*-hexo-2,3-diulose and D-*threo*-hexo-2,5-diulose; (2) D-*ribo*-hexos-3-ulose; (3) D-glucosone; (4) D-*gluco*-hexodialdose; (5) D-*threo*-hexo-2,4-diulose; (6) D-*lyxo*-hexos-5-ulose.

acids, and other minor constituents. Initially, an aliquot of the diluted irradiated (20–kGy) solution was directly subjected to analysis. However, interpretation of the chromatogram was complicated by the partial obscuring of the glucosone peak by the unreacted sucrose peak. This problem was readily solved by adding (30 min before analysis) invertase to the solution, which resulted in the conversion of sucrose into glucose and fructose, thus simplifying interpretation of the chromatogram. The chromatogram so obtained (Figure 5) shows the presence of glucosone, which, in agreement with GC studies (Kito et al., 1981), is a discernible radiolysis product. Another major radiolysis product of the mixture corresponds to the main radiolysis product of glucose, which has been identified as D-*gluco*-hexodialdose (Schuchmann and Von Sonntag, 1977).

To determine the amount of glucosone formed on irradiation, known quantities of pure glucosone was added to a series of irradiated solutions and the mixtures analysed as before. A graph (Figure 6) of ppm glucosone added plotted against peak area was obtained and extrapolated to zero peak area, which corresponds to the amount of glucosone originally formed. The amount of glucosone found in the synthetic mango was considerably less (<-

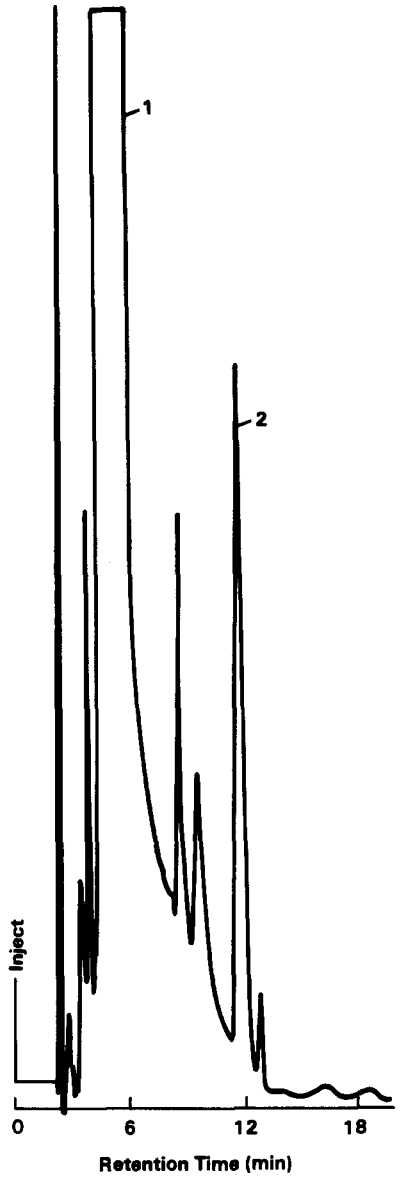


**Figure 3.** γ-Irradiated (20-kGy) D-fructose (2% solution): (1) fructose; (2) glucosone.

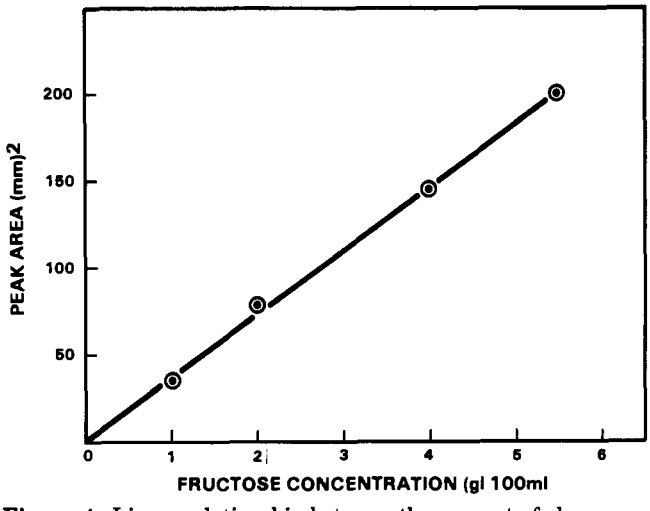


**Figure 4.** Linear relationship between the amount of glucosone formed at a given radiation dose (40 kGy) and sugar concentration.

50%) than that obtained when a solution of glucose and fructose, in concentrations corresponding to that of the model mango, was irradiated to the same doses. This suggests that the other components of the model mango

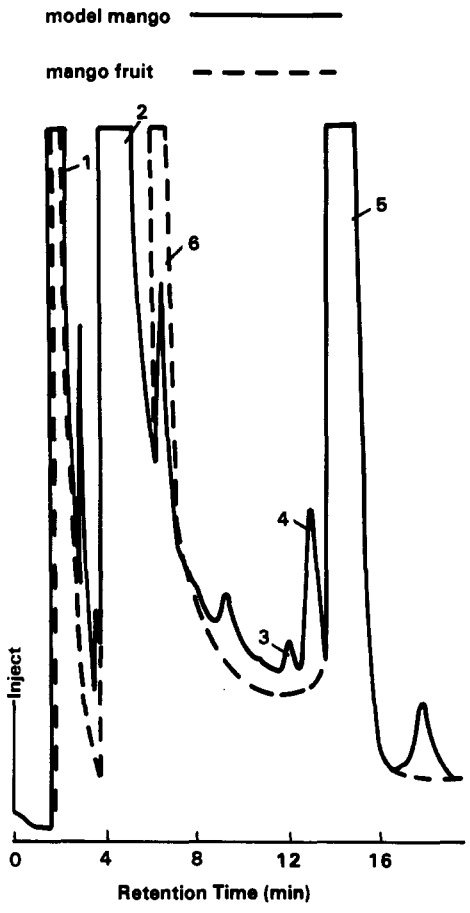


**Figure 5.** Irradiated (20-kGy) (a) model mango after treatment with invertase (—) and (b) whole mango (- - -): (1) smaller than 6-C chain radiolysis products; (2) glucose and fructose; (3) glucosone; (4) D-*gluco*-hexodialdose; (5) maltose; (6) sucrose.

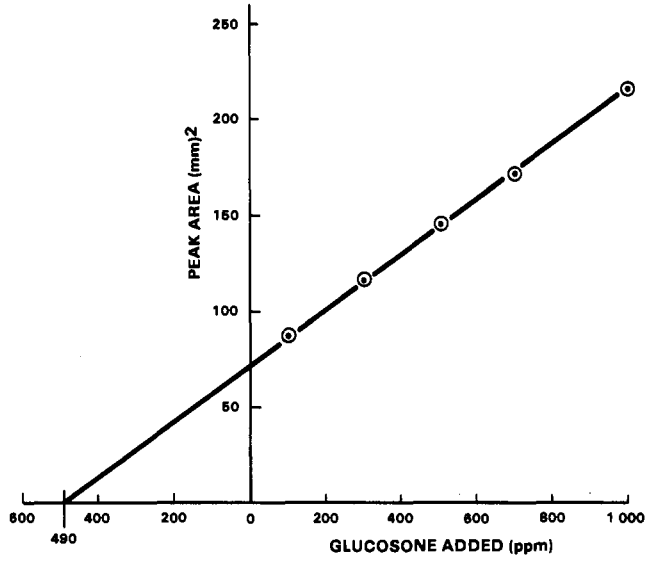


**Figure 6.** Irradiated (20-kGy) glucose plus fructose (model mango concentration) spiked with known quantities of glucosone. The $x$ intercept represents the amount of glucosone present in the original irradiated solution.

act as competitive scavengers for the hydroxyl radicals and other reactive species formed on irradiation and involved in the reactions that ultimately yield glucosone. Studies on the effects of additives showed that this "inhibition" of glucosone formation was, at least in part, due to the presence of the disaccharides that mainly react with hydroxyl radicals to yield their component monosaccharides

(Schubert, 1973). Other components of the model fruit also added to the total inhibitory effect, although it was impossible to ascribe the effect to a single compound or even a group of compounds.

It may be noted that, despite the presence of the mutagen glucosone (250 ppm), irradiated (20-kGy) model mango showed no mutagenicity toward *Salmonella* TA 100 (Niemand et al., 1983). This is in agreement with an earlier report (Niemand et al., 1983) that showed the mutagenic dose-response of glucosone to become meaningful only at doses higher than 1000 μg/plate (500 ppm).

**Analysis of Irradiated Mango.** This investigation was completed by the analysis of irradiated mango fruit. Whole Kent mangoes were irradiated up to doses of 0.75 (the commercial dose recommended for the preservation of subtropical fruit), 1.5, 5, and 20 kGy, treated as described in the Experimental Section, and analyzed. No glucosone could be detected, even at the extreme dose of 20 kGy. In this case analysis did not require the addition of invertase, which is present in the ripe fruit and starts its action on sucrose immediately after the fruit is homogenized (Chan et al., 1975). In order to establish at what level glucosone can be detected in irradiated fruit using pulsed amperometric detection, an aliquot of homogenized unirradiated fruit was spiked with various amounts of glucosone (10–100 ppm) and subjected to the same treatment as the irradiated samples. Glucosone could be detected as a clearly discernible peak when added at levels at and above 40 ppm, confirming the validity of the method. On the assumption that unirradiated food contains no glucosone, the detection limit for this compound in irradiated fruit would therefore be 40 ppm by pulsed amperometric detection.

These results indicate that components of the whole fruit provide considerable protection against the radiolytic formation of glucosone. This effect may be due, at least in part, to the presence of fiber and other insoluble material that may, for example, function as catalysts in the quenching of hydroxyl radicals and other reactive species. This suggests that the model mango should not be used without qualification to simulate the radiolysis behavior of actual fruit. The fact that glucosone could not be detected is in accord with the lack of mutagenicity of irradiated whole fruit as illustrated with the use of the Ames test (Niemand et al., 1983) as well as by animal feeding studies (International Food Irradiation Project, 1979).

**Conclusion.** No glucosone could be detected in irradiated fruit (20 kGy). It can therefore be expected that less than 1 ppm of glucosone will be formed in fruit irradiated at the commercial dose (0.75 kGy), a level of glucosone that is totally insignificant from a toxicological perspective. This finding validates the results of earlier mutagenicity tests and animal feeding studies.

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Received for review November 27, 1985. Accepted April 9, 1986.