Retention of Radiolytic CO Gas in Irradiated Pepper Grains and Irradiation Detection of Spices and Dry Grains with the Level of Stocked CO Gas

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The release of radiolytic CO gas from ^{60}Co γ-irradiated pepper seeds was unexpectedly slower than that of radiolytic H_2 gas during a storage period after irradiation. These gases were retained in the grains and could be recovered by pulverization under gastight condition. Using this procedure, 10-kGy-irradiated pepper grains could be distinguished from nonirradiated samples for more than 2 months by the level of CO and H_2 gases. The patterns of CO change at 10, 20, and 30 kGy were similar, and the CO amounts were proportional to irradiation doses at any point of the storage period after irradiation. ^{60}Co γ-irradiated grains of allspice, cinnamon, cumin, polished rice, and wheat could be distinguished from nonirradiated ones by the level of retained CO gas even after 2 months of storage at room temperature. Thus, radiolytic CO gas could be an effective probe for rapid screening of irradiated pepper and dry grains.

Keywords: *Radiolytic CO gas; irradiation; detection; pepper grains; spices; dry grains*

INTRODUCTION

Spices and grains are often contaminated with pathogenic bacteria, fungi, or insects which deteriorate their safety and quality. These products have been treated by postharvest pesticides, such as ethylene dibromide, ethylene oxide, and methyl bromide, for decontamination. However, toxicity and carcinogenicity of residual fumigants recognized in the food products have been revealed as a new concern for public health (Vajdi and Pereira, 1973). Food irradiation has been recognized as a new alternative technology for decontamination of spices and grains without heating because irradiation induces neither heat, causing deterioration of aromatic quality, nor substances harmful to food safety. Irradiation of spices has been cleared in more than 25 countries including the United States, where irradiation of up to 30 kGy was approved (*Federal Register*, 1986), and commercial irradiation has already been started at several facilities (Japanese Research Association for Food Irradiation, 1992).

At the Joint FAO-WHO-IAEA-ITC/UNCTAD-GATT Conference on Acceptance, Control of and Trade in Irradiated Food, Geneva, December 1988, it was agreed that to encourage successful commercialization of irradiated foods and reinforce the consumer's confidence of irradiation process control, administrative control of irradiation and labeling of irradiated food moving in trade are essential, and therefore analytical detection methods for irradiated foods are urgently required (IAEA, 1989). Under these trends, the Co-ordinated Research Program on Analytical Detection Methods for Irradiation Treatment of Foods (ADMIT) was started in 1990 (FAO/IAEA, 1990), and several methods for irradiation detection have been proposed for spices (Moriarty et al., 1988; Bögl and Heide, 1985; Farkas et al., 1990; Heide et al., 1990; Uchiyama et al., 1990; Suzuki et al., 1988); so far, thermoluminescence (Moriarty et al., 1988) has been well developed as a standard method to detect clearly an irradiation treatment of spices (Sanderson et al., 1989a,b), which was proved in interlaboratory studies (Schreiber et al., 1993a,b).

Considerable amounts of low molecular weight gases such as H_2, CO, CO_2, and CH_4 are produced by radiolysis of organic components in irradiated foodstuffs (Pratt and Kneeland, 1972; Simic et al., 1979). However, there is insufficient information on the possibility of using them for irradiation detection because they have been considered to be readily released from irradiated products immediately after irradiation (Bögl et al., 1988).

We previously demonstrated that radiolytic H_2 could be recovered from irradiated pepper grains for 2 months after 10-kGy irradiation with ^{60}Co γ-rays (Dohmaru et al., 1989). During the course of the experiments, we found that CO gas was retained in irradiated pepper grains unexpectedly longer than H_2.

In this paper, we report that retained CO gas could be recovered together with H_2 and it could become a more effective probe for detection for irradiated pepper and other grains.

MATERIALS AND METHODS

Materials. Grains of black and white pepper, allspice, cinnamon, cumin, turmeric, nutmeg, ginger, red pepper, sage, paprika, garlic, and onion were purchased from Lion Co. Ltd. and Yasuma Co. Ltd. Pelletted feeds for experimental animals were purchased from Oriental Yeast Industry Co. Polished rice grains were purchased from a local market in Sakai. Wheat grains were a gift from Saitama Agricultural Experimental Station. Each sample was stored at room temperature.

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Analysis of Radiolytic H_2 and CO Gases Released from the Pepper Grains Contained in Glass Vessels. Fifty grams each of black pepper grains and powder were separately placed in a gastight glass vessel (730 mL) with a rubber septum and irradiated at a dose of 10 kGy with γ-rays from a ^{60}Co source (10 kGy/h) in the irradiation pool at the Research Institute for Advanced Science and Technology, University of Osaka Prefecture (Furuta et al., 1970). The vessels were not evacuated before irradiation. The dose was monitored with Radiachromic dye film (Far West, FWT 60-00). After irradiation, the vessels were stored under room temperature. To analyze H_2 and CO gases released from the samples, 1 mL of the headspace gas was taken out using a gastight syringe through the septum at a given time after irradiation and subjected to analysis by gas chromatography. In the case of heating, each vessel was put in an oven and heated for 25 min at indicated temperatures before gas sampling. To recover those gases retained in the sample, 20 g of each sample was put in a ceramic mill together with a ceramic grinding rod (Dohmaru et al., 1989) and ground for up to 3 min (particle size mostly less than 16 mesh) using a vibrating apparatus (Heiko TI-100). One milliliter of the headspace gas was taken out using a gastight syringe through an opening otherwise plugged with a pin and subjected to analysis by gas chromatography.

Analysis of Radiolytic H_2 and CO Gases Retained in Irradiated Pepper and Other Grains Stored in Paper Bags. Two hundred grams of each sample was placed in a paper bag, irradiated as described above, and stored at 25 °C with relative humidity of 50%. The paper bags were not sealed so that the headspace gases could escape freely during the storage period. Nonirradiated control samples were also stored under the same condition. After each storage period, 20 g of pepper sample was pulverized by the same ceramic mill as described and 40 g of the other grains was pulverized to the same particle size as pepper by a coffee mill (Mellita, CG-1) with an opening; 1 mL of the headspace gas was sampled for analysis.

Gas Chromatography. Each sample was analyzed with a gas chromatograph (HP5890A) equipped with a methanizer (Gasukuro MT-221), a thermal conductivity detector (TCD), and a flame ionization detector (FID) on a 3 mm o.d. × 2.3 m SUS column of 60–80 mesh Molecular Sieve 13X at 350 °C, using Ar as a carrier gas. H_2 gas was detected by TCD. CO gas was reduced to CH_4 with the methanizer and detected by FID. H_2 and CO peak areas were measured by Shimadzu C-R3A and HP3890 integrators, respectively, which, on the basis of the computed slope sensitivities, gave lower limits of detection of H_2 of 2.0 ppm and CO of 0.4 ppm in air when the amount of sample is 1 mL. H_2 was identified by comparing its retention time with that of the authentic sample. CO was identified by a GC–MS (JEOL JMS-DX302). H_2 and CO gases were not detected in the ambient air under this condition.

H_2 and CO concentrations (parts per million) thus obtained were converted to H_2 and CO amounts in microliters at 25 °C and 1 atm per gram of samples by multiplying by the effective volume of the headspace (ceramic mill, 80.3 mL; coffee mill, 322 mL) and dividing by the amount of sample (grains, 20 g in the ceramic mill, 40 g in the coffee mill); 2.0 ppm of H_2 and 0.4 ppm of CO are, then, converted to 0.00803 and 0.00161 μL/g for grains pulverized by the ceramic mill and to 0.0161 mL/g and 0.00322 μL/g for grains pulverized by the coffee mill, respectively.

RESULTS

Comparison of H_2 and CO Gas Liberated from the Pepper Grains. When we analyzed radiolytic H_2 and CO gases liberated from grains in the glass vessels after 10-kGy irradiation, we found that the amounts of H_2 and CO gases were unexpectedly small in comparison with those in the powder-containing vessels. During the 12-day storage at room temperature, the released H_2 gas gradually increased in amount to the same level as the powder. On the other hand, CO gas remained only one-fourth as much as that of the powder (Figure 1). Heating the vessels at 40 °C did not accelerate either H_2 or CO gas release from the irradiated grains. At 60 °C, H_2 gas was gradually released from the irradiated grains but only a slight amount of CO gas escaped. From these results, we assumed that radiolytic CO must be retained more strongly in the irradiated pepper grains than H_2.

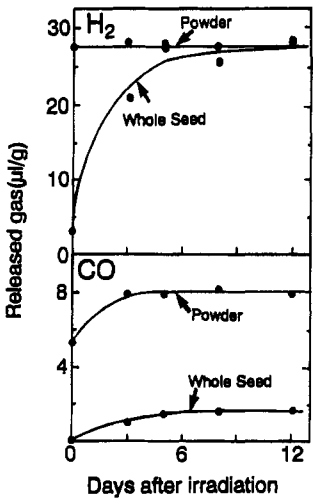

Figure 1. Release of H_2 and CO gases from whole seeds and powder of black pepper irradiated at 10 kGy with ^{60}Co γ-rays. Samples were stored in gastight vessels.

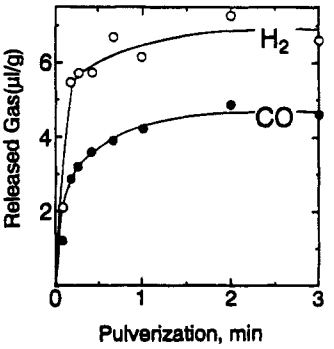

Figure 2. Release of radiolytic H_2 and CO gases by pulverization of γ-irradiated black pepper seeds. The amounts of H_2 and CO are expressed in terms of the volume of H_2 and CO at 25 °C and 1 atm liberated from 1 g of sample. Samples were stored in gastight vessels.

To confirm this hypothesis, 20 g of the irradiated grains was sampled from the glass vessel after 12 days of storage and H_2 and CO gases were analyzed by pulverization. The amount of CO gas rapidly increased together with H_2 with grinding time, and, after 3 min, its amount reached a level almost (ca. 80%) making up for the difference in amounts of CO gas released between grains and powder (see Figure 2).

Detection of Irradiated Pepper Grains Using Radiolytic CO Gas as a Probe. The above results clearly indicate that the irradiated pepper grains contain radiolytic CO as well as H_2. We examined the level of CO in the irradiated pepper grains during storage. Figure 3 shows the decrease of the stocked CO gas in comparison with H_2 within the black pepper grains in a paper bag after 10-kGy irradiation. CO gas decreased exponentially with time during a few weeks after irradiation, but the decreasing rate was slower than that of H_2. Even after 2 months, the CO level remained at a little less than half of the initial content in each irradiated sample, although H_2 went out from it. Figures 4 and 5 illustrate the change of CO contents of

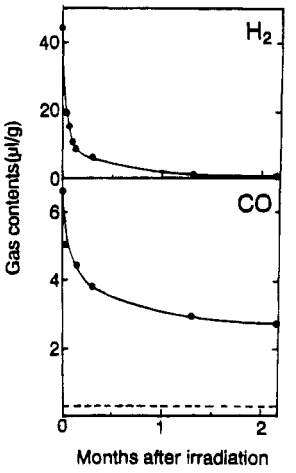

Figure 3. Decrease of stocked CO gas in comparison with H_2 within the black pepper grains after 10-kGy irradiation. Samples were stored in paper bags. The storage temperature and relative humidity are 25 °C and 50%, respectively. CO content (μL/g) in nonirradiated sample is indicated by a dotted line.

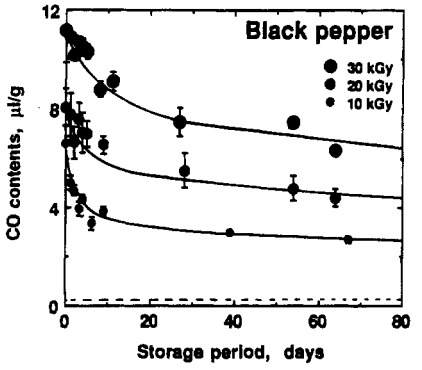

Figure 4. Amount of CO (μL) retained in 1 g of black pepper *vs* days after irradiation at 10, 20, and 30 kGy. Samples were stored in paper bags. The storage temperature and relative humidity are 25 °C and 50%, respectively. Error bars depict 1 standard deviation calculated from three measurements. CO content (μL/g) in nonirradiated sample is indicated by a dotted line.

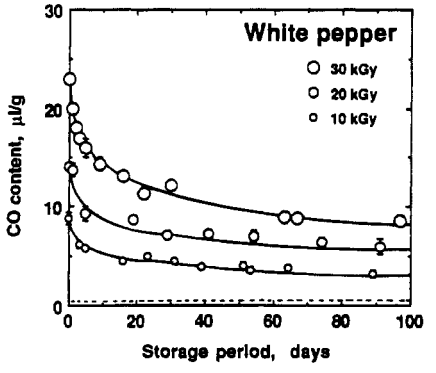

Figure 5. Amount of CO (μL) retained in 1 g of white pepper *vs* days after irradiation at 10, 20, and 30 kGy. Samples were stored in paper bags. The storage temperature and relative humidity are 25 °C and 50%, respectively. Error bars depict 1 standard deviation calculated from three measurements. CO content (μL/g) in nonirradiated sample is indicated by a dotted line.

the black and white pepper grains, respectively, after irradiation at 10, 20, and 30 kGy. The patterns of CO change obtained for black and white peppers were almost identical. The CO content observed in the irradiated pepper was proportional to irradiation dose up to 30 kGy at any point of analysis. Amounts of CO within nonirradiated samples were small, as indicated in Figures 4 and 5; the average yield was 0.4 μL/g for both black and white peppers, which was less than $^1/_{10}$ of the value obtained for 10-kGy-irradiated pepper after 2 months of storage.

Table 1. Level of CO Retained in the Irradiated Spices and Cereals after 2 Months of Storage

irradiation (kGy)	CO contents (μL/g)				
	allspice	cinnamon	cumin	rice	wheat[a]
0	0.12	0.20	0.09	0.02	0.07
10	0.22	0.39	0.44	0.10	2.64

[a] 42 days of storage.

Detection of Other Spices and Grains Using CO Gas as a Probe. To test the feasibility of this method for other dry foodstuffs, we analyzed released CO gas from 10-kGy-irradiated turmeric, nutmeg, allspice, cumin, ginger, red pepper, cinnamon, sage, paprika, garlic, onion, rice (whole seed and polished), wheat, and pelletted feeds for experimental animals after pulverization in the coffee mill. Although CO gas was detected in nonirradiated samples together with irradiated ones, the irradiated grains of allspice, cinnamon, cumin, rice, and wheat could be distinguished from nonirradiated ones even after 2 months of storage at room temperature. Unexpectedly, the CO content of wheat irradiated with 10 kGy (2.64 μL/g) after 42 days of storage was the 6–10-fold the other value (Table 1). In the case of turmeric, nutmeg, ginger, red pepper, sage, paprika, garlic, onion, and pelleted feeds, CO contents decreased to the same level as nonirradiated ones in a few days.

DISCUSSION

There are very few studies on the behavior of radiolytic gas in irradiated foods. In the early 1960s Phillips and Baugh observed that more than 70% of radiolytic H_2 gas was retained in irradiated glucose crystals but it was reduced to 50% in freeze-dried amorphous glucose and immediately released by addition of water (Phillips and Baugh, 1963). More recently, Hitchcock demonstrated that the irradiation of water (>0.1 kGy) generated H_2 gas that could be quantified by headspace gas analysis using an electric sensor, but he concluded the potential application of the hydrogen-specific detector for monitoring irradiated food is limited to packaged or solid foods because of the rapid diffusion of H_2 away from unsealed samples (Hitchcock, 1993).

In contrast with H_2, Furuta et al. observed that radiolytic CO gas was stably retained in irradiated frozen chicken and meat for more than 1 year (Furuta et al., 1992). Also, in the present study, we demonstrated that radiolytic CO gas was also retained in the irradiated pepper grains longer than H_2. These observation can be explained by the fact that larger molecules diffuse much more slowly than smaller molecules in a solid. In the case of frozen foods and solid foods such as dry grains, the ice matrix structure or rigid crystallized structure might contribute as an effective barrier against release of the radiolytic gases. Therefore, CO gas could be a more effective probe than H_2 in this "gas detection method" for irradiation treatment of foods because of the wider applicability to various food items such as grains of spices and cereals, in addition to frozen foods.

Thermoluminescence measurements of mineral contamination of spices indicate clearly an irradiation treatment for at least 9 months after irradiation. The

technique can be applied to all foods that are contaminated by minerals (spices, herbs, fruits, vegetables, shellfish). Doses of 1 kGy and above can be detected. In contrast, the determination of CO gas is a rapid screening method for the detection of irradiated foods with low cost and easy accessibility to gas chromatography. However, CO gas was also detected in unirradiated samples, and therefore positive identification should be confirmed by one of the other methods applicable to the concerned food.

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