# Detection of Irradiated Frozen Deboned Seafood with the Level of Radiolytic $H_2$ and CO Gases as a Probe

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A method to detect irradiated frozen shrimp without cuticle, cod slices, and deshelled oyster has been developed based on the fact that radiolytic $H_2$ and CO gases are retained in the irradiated samples for a certain period during the storage. These gases could be recovered from irradiated frozen samples in the headspace of a gastight glass screw vial by microwave heating. Gas chromatographic analysis of the headspace gas revealed that significant amounts of $H_2$ and/or CO gases were retained in the irradiated samples. Using those gases as a probe, irradiated frozen shrimp could be distinguished from non-irradiated shrimp for 3 months after 1.1−8.8 kGy irradiation, and irradiated frozen cod slices and oyster could be distinguished for at least 2 months at the dose ranges of 1.4−5.5 and 1.2−6.0 kGy, respectively.

**Keywords:** *Radiolytic $H_2$ gas; radiolytic CO gas; irradiation; detection; deboned seafood; food irradiation*

## INTRODUCTION

Fish and shellfish are often contaminated with pathogenic bacteria such as *Salmonella*, *Shigella*, *Staphylococcus aureus*, enteropathogenic *Escherichia coli*, *Vibrio cholerae*, or *Vibrio parahaemolyticus*, and *Vibrio vulnificus*, which cause serious disease in children (Thayer et al., 1996). For example, *V. vulnificus* may cause gastroenteritis or septicemia primary through ingestion of contaminated raw oysters, and their mortality rates are more than 50% in affected children (Thayer et al., 1996). Freezing technology has been successfully employed for preservation of fresh seafood products, but contamination with a high microbial load and pathogens often occurs after thawing because bacteria can survive during storage even under freezing temperatures although their growth is inhibited (Ito et al., 1989). Thus survival of contaminated microorganisms including pathogens in frozen seafood can jeopardize the safety of these products. Food irradiation has been recognized as an efficient technology to extend the shelf life of fresh foods and to decontaminate frozen foods because irradiation does not induce either heat or harmful substances which adversely effect the wholesomeness of the foods (Vajdi and Pereira, 1973). Because of possibility of initial microbial contamination including several species of pathogens from a variety of sources, a dose of 2−8 kGy is recommended for radicidation (Giddings, 1984; Monk *et al.*, 1995; Nerkar and Bandekar, 1990). Radicidation of seafood has already been cleared in various countries. In the case of frozen products, for example, India has cleared frozen seafood irradiation up to 6 kGy and irradiation of frozen shrimp has been cleared by Indonesia (up to 7 kGy) and Thailand (up to 5 kGy) (*Food Irradiation Newsletter*, 1995).

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Development of a detection method for irradiated foods is urgently required to control irradiation process and labeling of irradiated foods moving in trade in order to reinforce the consumer's confidence of the irradiation process (IAEA, 1989). Under these trends, the Coordinate Research Program on Analytical Detection Methods for Irradiation Treatment of Foods (ADMIT) examined various methods from 1990 through 1994 (Joint FAO/IAEA, 1990, 1994). So far, electron spin resonance (ESR) spectroscopy (Dodd et al., 1988) and thermoluminescence (TL) methods (Moriarty et al., 1988; Sanderson et al., 1989) turned out to be well developed for detection of bone- and/or shell-containing irradiated fish samples (Delincee, 1993; Schreiber, 1993). On the other hand, *deboned* samples including the flesh of fish seemed to be more difficult to identify. When these findings were presented, research on gas−liquid chromatography analysis of radiolytic products of fatty acids and analysis of DNA damage caused by irradiation was underway (Joint FAO/IAEA, 1994).

Considerable amounts of low molecular weight gases such as $H_2$, CO, $CH_4$, and $CO_2$ are produced by radiolysis of organic components in irradiated foodstuffs (Pratt and Kneeland, 1972; Simic et al., 1979). We found that the radiolytic $H_2$ and CO gases could be recovered from irradiated spices, grains, and frozen meat and poultry. Utilizing these gases as a probe for irradiation detection, we demonstrated that $^{60}$Co $\gamma$-irradiated pepper (Dohmaru et al., 1989; Furuta et al., 1995) and frozen meat and poultry (Furuta et al., 1992) could be detected for at least 2 months and over 1 year after irradiation, respectively.

In the present study, we extend this method to detection of irradiated *deboned* frozen seafood and show that irradiated frozen shrimp, cod, and oyster without cuticles or bones could be detected after at least 2 months of storage.

## MATERIALS AND METHODS

**Materials.** Frozen shrimp without cuticle, slices of cod, and oyster without shell were purchased from a local market in

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Sakai city. Each sample was stored in a freezer at ca. −20 °C before analysis.

**Irradiation.** Frozen seafood products were placed in a polyethylene bag, irradiated with $\gamma$-rays from a $^{60}$Co source (10 kGy/h) in the irradiation pool at the Research Institute for Advanced Science and Technology, Osaka Prefecture University (Furuta et al., 1970), at doses varying from 1 to 9 kGy at −19 °C. The dose was determined with Radiachromic dye film (Far West, FWT 60-00). The temperature of −19 °C was achieved using a NaCl−ice mixture as a refrigerant, and the samples were stored again under the same temperature. The bags were not sealed so that the headspace gases could go out freely during the storage period. Non-irradiated control samples were also stored under the same condition.

**Sampling of $H_2$ and/or CO Gases Retained in the Irradiated Frozen Products.** A 5 g amount of the sample and 10 mL of water were put in a glass screw vial (30 mL) with a hole cap and a rubber packing and then quickly heated in a microwave oven until the sample thawed. Quick heating was necessary to avoid additional gas production from unknown origins during the heating period. Water was necessary for uniform heating of the sample. After heating, 1 mL of headspace gas was taken out by a gas-tight syringe through the packing, and subjected to analysis by gas chromatography.

**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 13X molecular sieves (60−80 mesh) 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 the lower limit of detection of $H_2$ as 2.0 ppm and that of CO as 0.4 ppm in air when the sample amount is 1 mL. $H_2$ was measured by comparing its gas-chromatographic area with that of the authentic samples of known concentrations. CO was identified by a GC−MS (JEOL JMS-DX302).

$H_2$ and CO concentrations (ppm) 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 of the glass screw vial (20 mL) and dividing by the amount of the samples (5 g in the glass screw vial); 2.0 ppm of $H_2$ and 0.4 ppm of CO are then converted to 0.008 and 0.0016 $\mu$L/g, respectively.

## RESULTS

We observed neither detectable $H_2$ nor CO within non-irradiated samples, whereas considerable amounts of $H_2$ and/or CO were detected from all the irradiated samples. Figures 1−3 show the variations in the amount of $H_2$ and CO within the irradiated frozen shrimp, oyster, and cod samples, respectively, as a function of irradiation doses.

After less than 1 month of storage, both $H_2$ and CO were recovered from irradiated samples and their amounts showed an increase with increasing irradiation doses up to 5.2 and 6 kGy in the cases of shrimp (Figure 1) and oyster (Figure 2), respectively. After *ca.* 2 months, the $H_2$ content decreased to the detection limit but could still be distinguished from the non-irradiated samples at higher doses. A considerable amount of $H_2$ was detected from 1.2 kGy irradiated oyster samples after 70 days of storage (see Figure 2).

In contrast, the level of CO after *ca.* 2 months was still above detection limits in all the irradiated samples even at minimum doses. CO contents in shrimp and oyster samples still showed dose-dependent increase (Figures 1 and 2, bottom). Especially, in case of shrimp, as shown in Table 1, dose dependent increase of CO gas was observed even after 96 days of storage; also, 5.5 kGy irradiated cod samples could be distinguished from non-irradiated samples after 93 days of storage (Table 1).

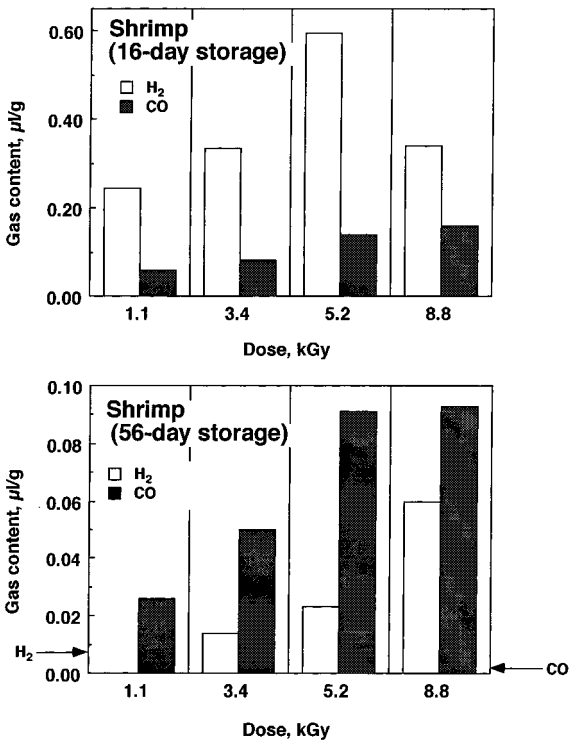


**Figure 1.** Amounts of $H_2$ and CO ($\mu$L) retained in 1 g of frozen shrimp without cuticle irradiated at various doses and stored for indicated days at −20 °C. Top: Storage for 16 days. Bottom: Storage for 56 days. Detection limits of $H_2$ and CO were indicated by arrows at both sides ($H_2$, left; CO, right) of the bottom graph.

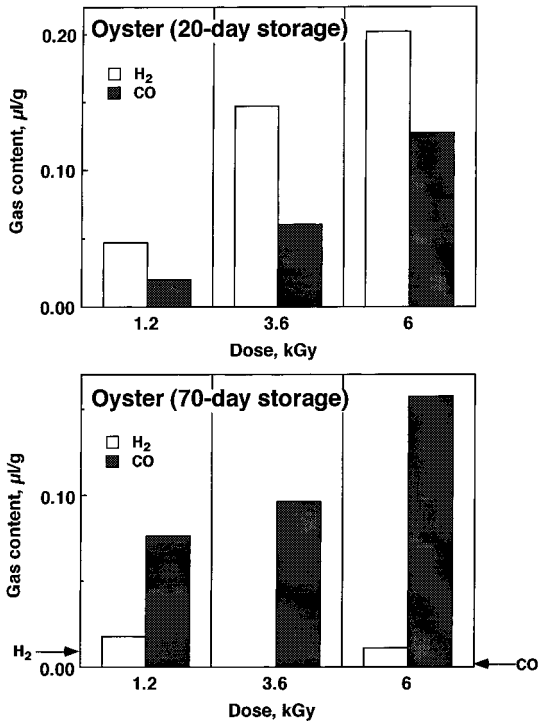


**Figure 2.** Amounts of $H_2$ and CO ($\mu$L) retained in 1 g of frozen deshelled oyster irradiated at various doses and stored for indicated days at −20 °C. Top: Storage for 20 days. Bottom: Storage for 70 days. Detection limits of $H_2$ and CO were indicated by the arrows in the bottom graph.

**Table 1. Level of CO Retained in Irradiated Frozen Shrimp and Cod after *ca.* 3 Months of Storage**

| item | days after irradiation | CO content (mL/g) after irradiation | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1.2 (1.4***) | 3.4 (3.2***) | 5.2 (5.5***) | 8.8 kGy |
| shrimp | 96 | ND* | 0.027 ± 0.004** | 0.052 ± 0.037 | 0.171 ± 0.010 | 0.196 ± 0.075 |
| cod | 93 | ND | ND | ND | 0.095 ± 0.056 | NP**** |

*Not detected. **Expressed as mean ± SEM from three measurements. ***Doses for cod samples. ****Not performed.

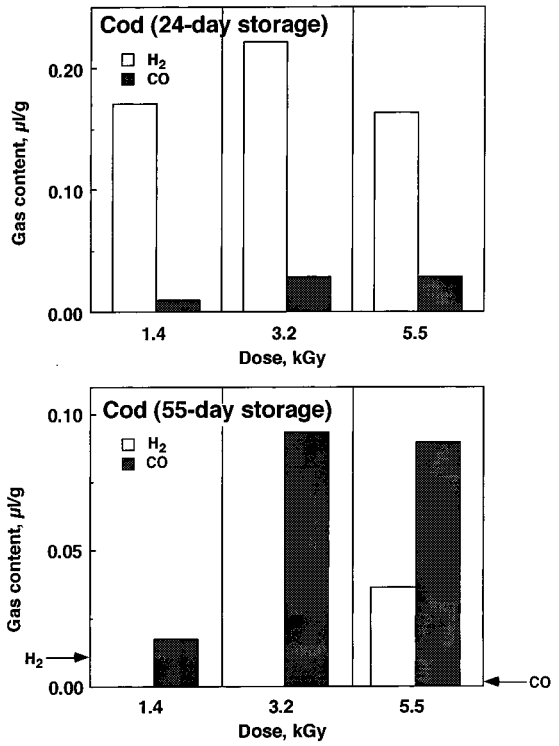


**Figure 3.** Amounts of $H_2$ and CO ($\mu$L) retained in 1 g of frozen cod slices irradiated at various doses after storage for indicated days at −20 °C. Top: Storage for 24 days. Bottom: Storage for 55 days. Detection limits of $H_2$ and CO were indicated by the arrows in the bottom graph.

## DISCUSSION

Data above clearly demonstrated that radiolytic $H_2$ and CO gases arising from irradiated seafood could be retained in the frozen sample for a considerable period. When these gases are used as a probe, irradiated frozen deshelled oyster and cod slices could be detected for at least 70 and 55 days, respectively, and, in the case of irradiated frozen shrimp, the detectable period was extended to 96 days even at the minimum doses (1.1−1.4 kGy). Although these periods are shorter than those of frozen meat and poultry (*ca.* 1 year) (Furuta et al., 1992), the dose dependent increase of retained CO in each sample even after 2 months of storage suggests a possible extension of detectable period in a higher dose range. Irradiation detection of frozen seafood has the advantages of practically no background gases from the non-irradiated samples and possible use of $H_2$ together with CO as a detection probe for nearly 1 month after irradiation.

The determination of radiolytic gases is a rapid screening method for irradiation detection with low cost and easy accessibility to gas chromatography. It also has wide applicability to irradiated frozen foods including deboned meat, poultry, and seafood as well as spices and grains.

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