2-アルキルシクロブタノンを指標にした放射線照射食肉,魚肉およびその調理品の冷凍保存後の検知

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著者
尾花, 裕孝
古田, 雅一
田中, 之雄

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Detection of Irradiated Meat, Fish and Their Products by Measuring 2-Alkylcyclobutanones Levels after Frozen Storage

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Hirotaka Obana1,*, Masakazu Furuta2 and Yukio Tanaka1

1 Osaka Prefectural Institute of Public Health: 1-3-69 Nakamichi, Higashinari, Osaka 537-0025, Japan;
2 Graduate School of Science, Osaka Prefecture University: 1-2 Gakuen-cho, Naka, Sakai, Osaka 599-8570, Japan;
*Corresponding author

2-Alkylcyclobutanones, such as 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone, were analyzed to assess the irradiation history of irradiated meats or fish, and cooked foods with irradiated ingredients, which had been stored frozen for up to one year. The purpose of the study was to show that irradiated meats could be detected even after having been stored in the distribution system. 2-Alkylcyclobutanones showed a small decrease in irradiated raw meats that had been stored frozen for one year. The 2-alkylcyclobutanones became undetectable in highly dried samples, such as feed for lab animals, during the same period.

Key words: 2-alkylcyclobutanone; food irradiation; meat; cooked food; frozen storage

Introduction

Food irradiation is accepted as one of the available procedures to control microorganisms and extend product shelf life. The Japanese food sanitation law allows such irradiation only for potato, to prevent sprouting. Providing the irradiation history of foods would encourage consumers' acceptance of food irradiation, and may help in the enforcement of labeling regulations.

Among the known markers of food irradiation, 2-alkylcyclobutanones are uniquely found only in irradiated samples. The radicals induced by irradiation generate various radiolytic compounds, including 2-alkylcyclobutanones, from fatty acids and their esters in fatty foods. 2-Dodecylcyclobutanone (2-DCB), formed from palmitic acid, and 2-tetradecylcyclobutanone (2-TCB), formed from stearic acid, are recommended as markers for irradiation in the European official method EN1785. We have developed a new analytical method to detect food irradiation in meat and fish samples, which is similar to EN 1785 in accuracy and sensitivity, and is quicker.

Monitoring the irradiation history of foods in the distribution system is important, because raw meats and their cooked products, designed to be ready for eating after appropriate re-heating, are often stored frozen for long periods before consumption. Whether or not irradiated ingredients exist in commercial frozen ready-to-eat foods and if so, whether the foods are appropriately labeled are matters of serious concern to consumers.

The stabilities of 2-DCB and 2-TCB in irradiated samples were studied to assess the suitability of these markers for evaluating the irradiation history of samples. Boyd et al. reported that 2-DCB in chicken showed a moderate reduction during chilled storage for 20 days. A similar reduction was observed in irradiated chicken stored at 4°C for 18 days. 2-DCB and 2-TCB were reduced by about 50% in irradiated chicken and about 20% in irradiated sardine, stored at 4°C for one month. Those reports concluded that the two compounds could be used as markers to assess irradiation history. However, there is little information about the fate of the two compounds during prolonged storage. Victoria et al. analyzed chicken samples that had been irradiated with 57 kGy of γ-rays and 58 kGy of electrons and stored for 12 years at room temperature after having been canned, and found substantial amounts of 2-DCB, although these irradiation doses are too high for food. Information on cooked foods containing irradiated ingredients after long storage is not available.

The objectives of this study were to assess the suitability of 2-DCB and 2-TCB as markers to determine: (1) the radiation history of raw meats stored at −20°C for up to one year, (2) the radiation history of cooked foods stored at −20°C for up to one year, and (3) the effect of...
storage temperature. We wished to determine whether 2-DCB and 2-TCB remain useful as markers of the irradiation history of foods throughout transfer along the distribution system.

**Materials and Methods**

**Samples**

Meat and egg samples were purchased at a local market in Osaka. Meat samples were irradiated with \( \gamma \)-rays from a \(^{60}\)Co source (15 kGy/hr) in a frozen state at Osaka Prefecture University, as previously described\(^2\). The irradiation doses were set within the maximum levels recommended by the European Union or USA\(^2\). The irradiation samples were stored at \(-20^\circ\text{C}\) and analyzed within two weeks after irradiation. The remaining portion after the initial sampling were further stored at \(-20^\circ\text{C}\) for up to one year.

**Cooking**

At room temperature, a whole egg irradiated at 3.1 kGy was mixed with 80 g flour, 8 g butter, 30 g sugar and 70 g milk in a food processor to obtain a homogeneous mixture. The mixture was heated on an electric cooking plate at 180°C for 2 min on each side to cook a pancake. Five pancakes were analyzed on the following day and the remaining portion of each pancake was stored at \(-20^\circ\text{C}\) until re-analysis.

Pieces of chicken thigh irradiated at 3.0 kGy were sprinkled with spices and fried in cooking oil at 180°C for 4 min. Five pieces of the fried chicken were analyzed on the following day and the remaining pieces were stored at \(-20^\circ\text{C}\) until analysis.

**Feed**

Feed for laboratory animals, which was distributed as MF from Oriental Yeast, Tokyo, was irradiated at 5.3 kGy. About 20 g of the irradiated feed was put in a small polyethylene bag, which was sealed, and stored in a drawer in the lab at room temperature or in a freezer at \(-20^\circ\text{C}\) until analysis.

**Sample preparation for analysis**

Sample preparation for the analysis of 2-DCB and 2-TCB was performed as described previously\(^2\),\(^3\),\(^7\). Briefly, the procedure was as follows. The sample was extracted with ethyl acetate at 100°C in an accelerated solvent extraction system. Fat was removed from the extract by precipitation at \(-20^\circ\text{C}\), followed by cleanup with a silica gel cartridge. The test solution, after the addition of 2-cyclohexylcyclohexanone as an internal standard, was examined by gas chromatography linked with a mass spectrometer (GC/MS). The GC was performed with RTX-5 ms (Restek Bellefonte, PA, USA). RTX-1701 was used for the feed samples. The monitored ions for 2-alkylcyclobutanones were the \(m/z\) 98 ion and 112 ion, and the \(m/z\) 98 ion was selected for quantitation. The limits of detection of 2-DCB and 2-TCB were 2.5 ng/mL in the test solution.

**Results and Discussion**

Figure 1 shows the concentrations of 2-DCB and 2-TCB in irradiated raw samples after frozen storage for one year. Irradiation doses: beef intestine, 5.0 kGy; minced beef, 5.1 kGy; beef thigh, 4.8 kGy; pork thigh, 4.5 kGy; minced pork, 4.7 kGy; salmon, 4.4 kGy; chicken thigh, 4.2 kGy. Values were the averages of three trials.

![Fig. 1.](image-url)

The changes of 2-DCB and 2-TCB concentrations in irradiated raw samples after frozen storage for one year. Irradiation doses: beef intestine, 5.0 kGy; minced beef, 5.1 kGy; beef thigh, 4.8 kGy; pork thigh, 4.5 kGy; minced pork, 4.7 kGy; salmon, 4.4 kGy; chicken thigh, 4.2 kGy. Values were the averages of three trials.

The irradiated egg and chicken were cooked as food ingredients and the cooked foods were analyzed after storage in a frozen state. Pancakes and fried chicken are often distributed frozen, and thus these two foods...
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We chose to examine the stability of 2-DCB and 2-TCB in frozen foods after cooking. The storage period was set at up to one year since the shelf life of many frozen foods in the distribution chain is one year.

Pieces of pancake, which contained egg irradiated at 3.1 kGy, were analyzed just after cooking and after storage for seven months or one year at -20°C. Levels of 2-DCB and 2-TCB decreased as the storage period increased (Fig. 3A). 2-DCB fell by 17% over 7 months and by 32% over one year, while 2-TCB fell by 23% and 53% during the respective periods.

The fried chicken also showed decreases of 2-DCB and 2-TCB as the storage period was increased (Fig. 3B). 2-DCB showed a 46% reduction and 2-TCB showed a 30% reduction during storage for one year after cooking. Nevertheless, they remained readily detectable.

The pattern of decrease of 2-alkylcyclobutanones in cooked samples during frozen storage seemed to be different from that of raw foods, which did not show any obvious reduction. Ndiaye et al. speculated that the reduction of 2-alkylcyclobutanones during storage of foods might be caused by oxidation of the lactone ring in 2-alkylcyclobutanones. Cooked samples would contain more air inside, because of loss of water and oil with cooking. The infiltrated air in the foods might increase the loss of the markers during storage.

The storage temperature might also affect the stability of the two compounds. Most cooked foods can not be stored for a long time at room temperature. However, feed for laboratory animals, which has been dried by heating, can be stored at room temperature and contains a good balance of nutrients including fat, so it was selected for study. Irradiated feed has been used as sterilized food for toxicological study.

The feed contained 2-DCB at 0.54 µg/g fat and 2-TCB at 0.1 µg/g fat just after irradiation, and the concentrations of the two compounds decreased as the storage period increased (Fig. 4). Typical chromatograms of 2-alkylcyclobutanones in irradiated feed are shown in Fig. 5. The decrease was faster at room temperature than in the frozen state. The concentrations of the two compounds halved during storage for 3 months at room temperature, while it took six months for a similar reduction in the frozen state. The compounds were judged as not detected (ND) when the peak became indistinguishable from background peaks, i.e., when the signal-to-noise ratio fell belows. Irradiation history could be detected for up to six months in terms of 2-DCB at room temperature. The corresponding periods were twelve months and six months for 2-DCB and 2-TCB, respectively, in the frozen state.

The decrease of 2-DCB was about 80% in the frozen feed, although it was less than 50% in pancakes and fried chicken under the same storage conditions. The reduction of 2-alkylcyclobutanones in the feed was

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*Fig. 3. Reduction of 2-DCB and 2-TCB concentrations in cooked samples containing irradiated egg and chicken after frozen storage. Values were averages of five trials with standard deviations. (A), pancake; (B), fried chicken.

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http://www.oyc-bio.jp/lfeed_ray.htm
faster at room temperature than in frozen state. Higher temperature appears to facilitate the reduction, even though the dried feed contained far less water than the other samples tested.

Our results show that 2-alkylcyclobutanones in irradiated meats and fish and in foods with irradiated ingredients could be detected even after samples had been stored frozen for up to one year. The results indicate that the irradiation history of foods containing fat can be detected by using 2-DCB and 2-TCB as markers for as long as the foods are likely to remain in the distribution system.

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