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FORMATION AND SAFETY OF 2-DODECYLCYCLOBUTANONE, A UNIQUE RADIOLYTIC PRODUCT IN IRRADIATED BEEF

P. Gadgil and J. S. Smith

Introduction

Treating food with ionizing radiation improves product safety and helps maintain quality. The main selling point of irradiated foods is that it is microbially safe. Beginning in October 2002, companies could petition the FDA for permission to use terms like "electronic pasteurization" on the labeling for irradiated foods. Consumers are already familiar with pasteurization and they associate the term with a safe product. There needs to be a protocol in place to test for irradiation to verify that products meet regulatory requirements. Being able to differentiate between irradiated and non-irradiated food will aid in proving the authenticity and safety of irradiated products and in detecting mislabeled products. In November 2003, Excel Corporation (Dodge City, KS) voluntarily recalled 26,000 pounds of ground beef that was mislabeled as irradiated. The incident appears to be the first case of its kind, and it emphasizes the need for a method that can reliably distinguish between irradiated and non-irradiated foods.

At the doses currently approved for food irradiation, the only unique radiolytic products that have been identified are alkylcyclobutanones (2-ACBs). These are cyclic compounds formed by rearrangement of fatty acids when exposed to irradiation. They are found in a wide variety of lipid-containing foods and have been universally accepted as indicators of irradiation exposure.

Recent studies have raised the possibility of 2-ACBs being weak genotoxins or cancer promoters when tested at high concentrations.

Numerous long-term and short-term toxicity tests have demonstrated the safety of irradiated foods. In spite of these reports, some claim that irradiated foods are unsafe and have used the previous studies as proof that alkylcyclobutanones are carcinogenic. Therefore, more studies evaluating the toxicity of these chemicals at high and low concentrations are needed to conclusively prove their safety. Accordingly, the objectives of this research were to evaluate the formation of 2-dodecylcyclobutanone (2-DCB), the alkylcyclobutanone formed from palmitic acid, in irradiated ground beef, and to assess its toxicity.

Experimental Procedures

Formation of 2-DCB in Irradiated Beef.

Quarter-pound ground beef patties with 15% or 25% fat were made and irradiated at an electron beam facility at 4 doses: 1, 2, 3 and 4.5 kGy. The data obtained from these samples were used to construct the dose response curves in Figure 1.

One lb chubs of commercially irradiated ground beef were obtained from two sources. Two samples of Brand X (ground round with 7% fat and ground chuck with 20% fat) and two samples of Brand Y (ground round with 10% fat and ground chuck with 20% fat) were evaluated. The 2-DCB was extracted by supercritical fluid extraction and analyzed by gas chromatography-mass spectrometry.

Toxicity and Mutagenicity. The Ames assay uses special strains of *Salmonella* to detect chemical substances that lead to gene mutations. Incubating these strains with a

mutagenic chemical will cause an increase in the number of bacterial colonies compared to the same strains incubated without the mutagen. The number of colonies usually increases with an increase in concentration of the mutagen. Some chemicals are transformed into mutagens by the body's metabolic processes. Therefore, a liver enzyme extract was used in this assay to check for this possibility. We evaluated five *Salmonella* test strains with and without liver enzyme activation, as well as four concentrations of 2-DCB.

The Microtox system is a screening tool used for a variety of toxicity testing applications. The assay utilizes *Vibrio fischeri*, a marine bioluminescent bacterium. The inhibition of light production by *V. fischeri* in the presence of toxins forms the basis of this assay. Acute toxicity of 2-DCB was evaluated by the Microtox acute toxicity system and compared with cyclohexanone and 2-nonenal (both GRAS additives).

Results and Discussion

Formation of 2-DCB in Irradiated Beef.

2-DCB was detected in all the irradiated samples and its concentration increased linearly with dose, as illustrated in the response curve shown in Figure 1. There was no significant difference in the amount formed between the two fat levels. There might be an upper threshold beyond which the amount of fat does not effect 2-DCB formation. This indicates that the amount of fat may not be a factor affecting 2-DCB formation, at least at these fat levels. Thus, the absorbed dose can be estimated for commercial samples with a wide range of fat levels. In a commercial setting where there is considerable variation in product composition, this would be an advantage.

The 2-DCB was detected in all the commercial samples and the absorbed doses were calculated from the dose response curves. The estimated doses applied to the commercial

samples ranged between 1.38 kGy and 1.55 kGy—values consistent with doses normally used in the industry (1.0 to 2.0 kGy)

Lab samples were irradiated at a Sure-Beam facility that also irradiates ground beef for retail sale. The samples were processed in much the same way as commercial samples would be and were suitable for estimating applied dose. It should be noted that the absorbed dose values are estimates. There were no true controls for the commercial samples and there was no information about when the samples were irradiated. Therefore, the effect of storage conditions and/or time, if any, was unknown. However, these values are within the range of 1.0 to 2.0 kGy normally used in the industry indicating that this method was able to approximate the dose applied.

Mutagenicity and Toxicity: The 2-DCB did not increase the number of colonies compared to controls with or without S9 addition. Therefore, 2-DCB was non mutagenic in the Ames assay and was not activated by liver enzymes, indicating that it was not biotransformed into mutagenic by-products.

The Microtox assay measures the relative toxicity of each chemical by calculating concentration of a chemical that reduces the light production of the microorganism *V. fischeri* by 50% (EC_{50}). The lower the EC_{50} value is, the higher the toxicity. The dose-response curves for the three compounds tested are shown in Figure 2. As the EC_{50} value for 2-DCB was between that of nonenal and cyclohexanone, 2-DCB would not represent a greater risk compared to nonenal, a Generally Recognized as Safe additive.

The maximum number of cells of *V. fischeri* affected by 2-DCB was $65 \pm 4\%$, while it reached 90-100% for the other two compounds. When comparing toxicity of chemicals, two parameters can be examined; potency and efficacy. Potency is the range of doses over which toxicity is observed and ef-

ficacy is the elicited by the chemical. Compared to the other chemicals, 2-DCB had the lowest maximum toxic effect.

The amount of 2-DCB found in commercial ground beef patties ranged from 0.03 to 0.05 $\mu\text{g/g}$ of ground beef. For a patty weight of about 4 oz (115g), this amounts to about 3.5 to 5.8 μg per patty. The total exposure to 2-DCB by eating one of these irradiated patties would be 0.08 $\mu\text{g/kg}$ body weight for a 154 lb adult. Thus, the amount consumed would be

so small that any potential risk of 2-DCB would be minimal.

Implications

The amount of 2-DCB formed in irradiated beef can be used to monitor the irradiation dose and is too low to pose a significant health risk. Therefore, irradiation is a safe method to ensure quality and safety of ground beef.

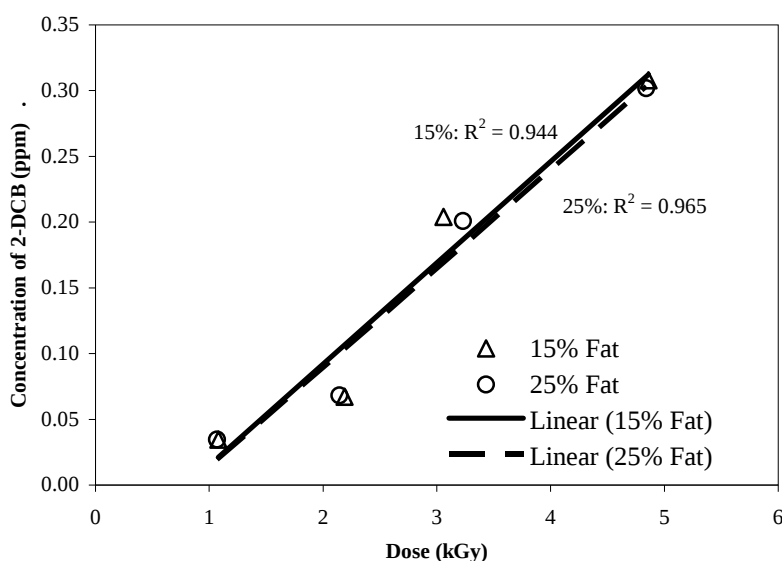


Figure 1: Response of 2-DCB (μg of beef) with Increasing Irradiation Dose.

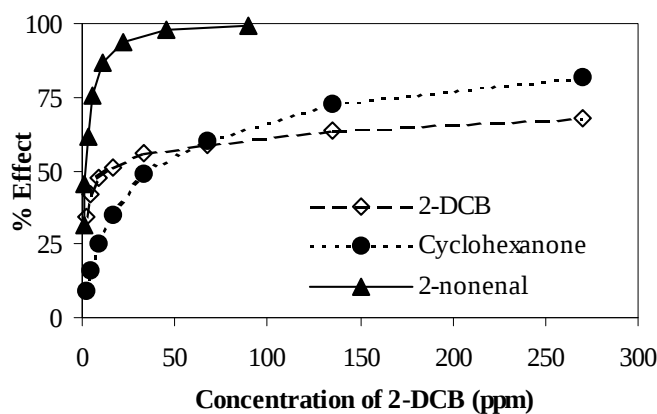


Figure 2: Effect of Concentration of 2-DCB, Cyclohexanone, and 2-nonenal on Light Emission by *V. fischeri*. Concentrations tested were between 90 ppm to 270 ppm for 2-DCB and cyclohexanone, and 90 ppm for 2-nonenal.