

# Detection of 2-alkylcyclobutanones in Irradiated Hamburgers

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## Abstract

Food irradiation is the process of exposing food to ionising irradiation. This technology is used for food hygiene, spoilage reduction and extension of shelf-life. Although properly irradiated food is safe, commercialization of irradiated food requires the available methods to identify irradiated foods. The detection of 2-alkylcyclobutanones (2-ACB's) is a standard method used for irradiated food whose fat content is higher than 1g %. However, 2-ACB's are not detected in nonirradiated food samples.

In this study, hamburger samples with 0.8 % NaCl content were produced from beef meat and 1.2 kGy were applied to samples. Unirradiated (control) and 1.2 kGy irradiated samples were stored for 8 and 30 days at two different storage temperature (+4 °C and -12 °C). The analysis of 2-ACB's formed in fat containing irradiated foods with gas chromatograph-mass spectrometer was evaluated to detect the irradiation on hamburgers. The effect of storage temperatures on formation and diagnosis of 2-dodecycyclobutanone (2-dDCB) of irradiated samples was not significant. 2-dDCB was not determined on the control sample regardless of storage time. As a consequence, 2-ACB's can be used as an irradiation indicator in foods.

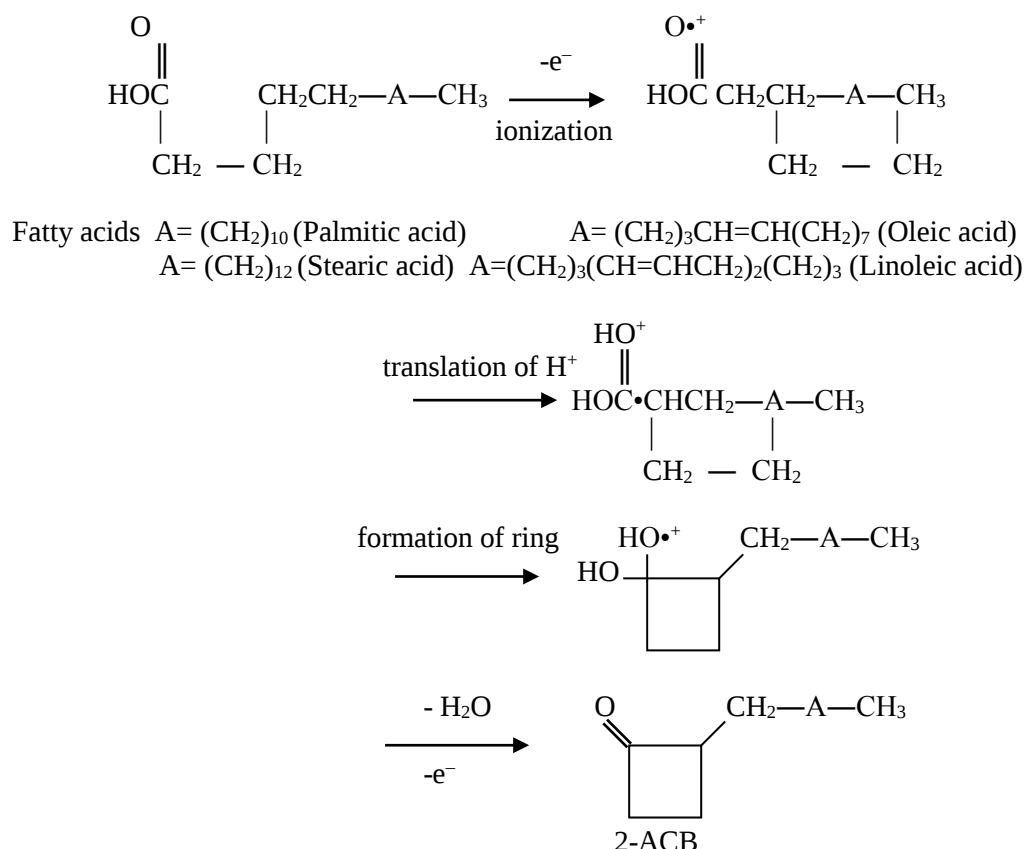
## Introduction

Interest in use of irradiation for the treatment and preservation of foods has increased throughout the world, and need for the development of a detection method for irradiated food was recognized (Lee et al., 2000). The irradiation treatment of foodstuffs is used essentially to reduce bacterial contamination and to eliminate pathogenic bacteria from meat, fish, shellfish, eggs and spices (at doses included between 2 and 10 kGy), disinfest and slow down the senescence of fruit and vegetables (at doses usually lower than 1 kGy), disinfest cereals and leguminous plants (at doses included between 0.1 and 0.8 kGy) and inhibit the germination of bulbs and tubers (at doses lower than 0.1 kGy). Although properly irradiated food is safe and wholesome, consumers should be able to make their own free choice between irradiated and non-irradiated. For this purpose labelling is indispensable. In order to check compliance with existing regulations, detection of radiation treatment by analysing the food itself is highly desirable (Delincee, 1998).

2-Alkylcyclobutanones are the same number of carbons as the parent fatty acid and substituted with an alkyl group located in the 2 position (Figure 1). Table 1 shows that radiation-induced 2-alkylcyclobutanones from fatty acids (Lee et. al., 2000).

**Table 1.** Radiation-induced 2-alkylcyclobutanones from fatty acids (Lee et. al., 2000).

| Fatty acid     | Radiation-induced 2-alkylcyclobutanones     |
|----------------|---------------------------------------------|
| Palmitic acid  | 2-Dodecylcyclobutanone                      |
| Stearic acid   | 2-Tetradecylcyclobutanone                   |
| Oleic acid     | 2-(5'-Tetradecenyl)cyclobutanone            |
| Linoleic acid  | 2-(5',8'-Tetradecadienyl)cyclobutanone      |
| Linolenic acid | 2-(5',8',11'-Tetradecatrienyl)cyclobutanone |



**Figure 1.** Basic structure and side chains of the four most common alkylcyclobutanones found in irradiated lipid-containing foods (Haire et al., 1997)

## Material and Methods

In this study, hamburger samples with 0.8 % NaCl content were produced from beef meat and 1.2 kGy were applied to samples in Co<sup>60</sup> Gamma-cell (Tenex, Issledovatel, dose rate 0,79 kGy/h in July 2010, Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center). The analysis of the 2-alkylcyclobutanones is performed following EN 1785 standard method in Sarayköy Nuclear Research and Training Center Laboratories (Anonymous 2005). Weight 5 g of anhydrous sodium sulfate and 3 g of well mixed homogenized hamburger sample into an extraction thimble, mix and plug with cotton wool. Samples are placed semi-automatic fat extraction unit (Soxtec Avanti – 2055 Soxtec, Foss Tecator) and fat extraction was performed on samples with n-hexane. Transfer mixture of the fat and solvent from the flask to a 100 ml-stoppered cylinder and adjust the volume to 100 ml with more solvent. Add 5 g of anhydrous sodium sulfate, stopper, mix and leave overnight. This mixture of n-hexane using a rotary evaporator and evaporated to give fat extract was prepared so that the Florisil column.

Prepare a Florisil column (20 – 21 cm) using deactivated Florisil and n-hexane. Take a volume of the extract which provides approximately 200 mg of lipid and rinse the flask with 5 ml of n-hexane and apply to the column. Place 150 ml n-hexane in the separating funnel on the top of the column, elute 2 – 5 ml/min and collect eluent. When the separating funnel is empty, change the collection flasks and place 150 ml of 1% diethyl ether in n-hexane and elute 2 – 5 ml/min. Rotary evaporate the 1% diethyl ether fraction at 40 °C, using minimum reduced pressure, to 5 – 10 ml and transfer to a test tube. Concentrate to dryness under a stream of nitrogen at 40°C ensuring that the sample is not left under nitrogen flow once it is dry. Resuspend in 200 µl of a solution of 2-cyclohexylcyclohexanone.

2-ACB's were separated using a Varian Factor Four , VF 5MS column, 30 m x 0,25 mm internal diameter with a 0,25 µm stationary phase (95 % dimethyl, 5 % polysiloxane) in a Varian CP 3800 gas

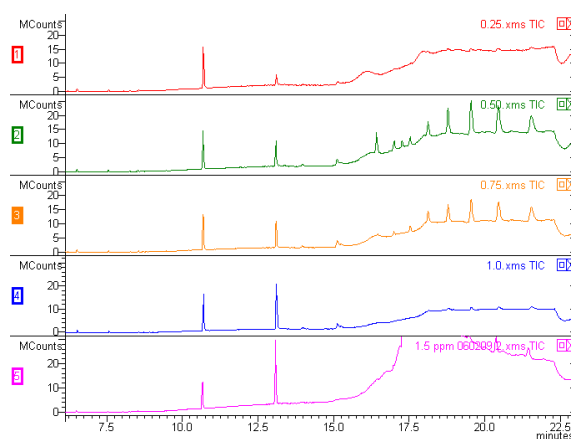
chromatograph directly linked to a Varian 1200 L quadrupole mass selective detector. Conditions used were as follows: injector temperature, 250 °C; column temperature programme; 55 °C (1 min), first ramp, 15 °C/min to 300 °C, first temperature hold, 300 °C (5 min); injection volume, 1 µl; injection mode, splitless; carrier gas He, 1 ml/min; MSD, selected ion monitoring of ions m/z 98 and m/z 112.

Samples are considered to be irradiated when:

- at least one 2-ACB has been positively identified and
- the estimated concentration exceeds the concentration equivalent to a signal to noise ratio of 3 to 1 in the least sensitive ion.

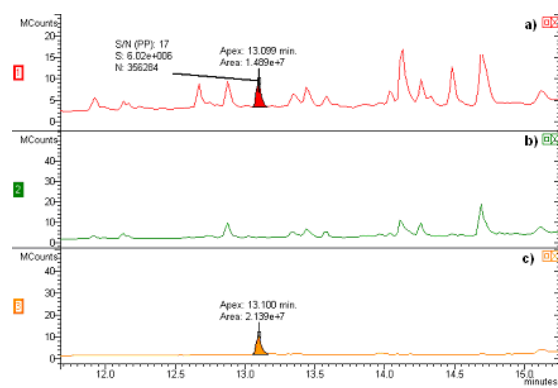
## Result and Discussion

As for GC/MS analysis, it was preliminary carried out on the 2-dDCB (induced by irradiation of palmitic acid, the most abundant saturated fatty acids in beef hamburgers) standard to evaluate its retention time, that was found to be between 13.0 – 13.25 min. Figure 2 shows that GC/MS chromatogram of different concentrations (0.25; 0.50; 0.75; 1.0 and 1.5 ppm) 2-dDCB standard. Since the cyclobutanones are at present considered to be radiation specific, no background levels having been found in unirradiated samples, this method shows great potential (Delincee, 1998). Moreover, the extract ions chromatogram of unirradiated sample of hamburgers did not show any specific signal at the retention time attended for 2-dDCB the first day of irradiation. On the other hand, the 2-dDCB, expected from hamburgers lipids after irradiation, was clearly detected at expected retention time between 13.0 – 13.25 min (Figure 3).

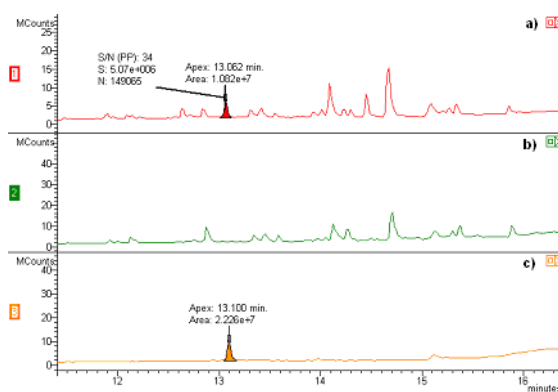


**Figure 2.** GC/MS chromatogram of 2-dDCB standards

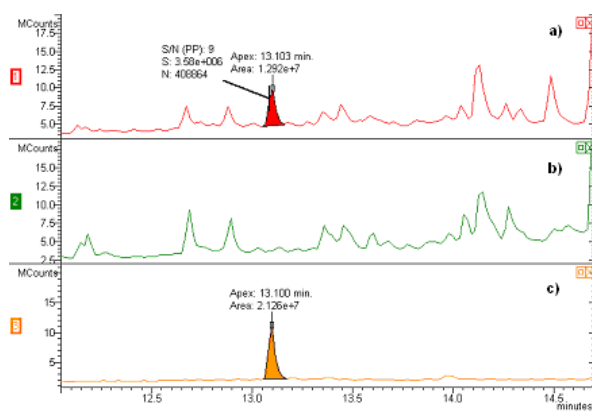
The chemical stability of 2-alkylcyclobutanones in food is quite good and the moderate losses observed during storage do not reduce the validity of the standard method (Marchioni 2006). The 2-dDCB in irradiated hamburgers was relatively stable during 8 and 30 days of two different storage temperature (+4 °C and -12 °C). After 8 day storage at +4 °C (Figure 4) and -12 °C (Figure 5), the 2-dDCB was clearly detected at retention time respectively 13.062 min and 13.103 min. Similarly, after 30 days storage at -12 °C (Figure 6), while unirradiated sample of hamburgers did not show any specific signal at the retention time, 2-dDCB was detected at 13.116 min retention time at 1.2 kGy irradiated sample.



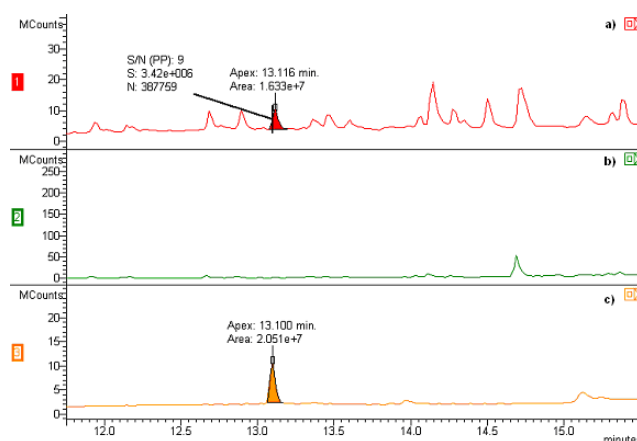
**Figure 3.** Immediately after irradiation 1.2 kGy irradiated and control sample chromatograms  
a) 1.2 kGy irradiated sample; b) control; c) 0.50 ppm 2-dDCB standard



**Figure 4.** After 8 day storage at +4 °C 1.2 kGy irradiated and control sample chromatograms  
a) 1.2 kGy irradiated sample; b) control; c) 0.50 ppm 2-dDCB standard



**Figure 5.** After 8 day storage at -12 °C 1.2 kGy irradiated and control sample chromatograms,  
a) 1.2 kGy irradiated sample; b) control; c) 0.50 ppm 2-dDCB standard



**Figure 6.** After 30 day storage at -12 °C 1.2 kGy irradiated and control sample chromatograms,  
a) 1.2 kGy irradiated sample; b) control; c) 0.50 ppm 2-dDCB standard

## Conclusions

Many researchers state that the duration of storage has not significant effect the amount of 2-ACB's (Obana et al., 2006; 2007a; 2007b; Parlato et al., 2007). Furthermore, food process such as cooking, freezing, microwave, UV radiation, high pressure applications and packaging techniques have not effect on the formation mechanism and amount of 2-ACB's (Hartwig et al., 2007). The main conclusion of this work is that the GC/MS technique can be successfully applied on hamburgers irradiated at 1.2 kGy. As 2-ACB's are formed only during irradiation of foods containing lipids, these compounds provide a good way to confirm if food is irradiated or not.

## References

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