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DETECTION OF 2-ALKYLCYCLOBUTANONES AS A MARKER OF IRRADIATED AVOCADO.

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The 2-alkylcyclobutanones are compound only formed in processed foods with ionizing radiations, which are used as markers to detect foods that have been irradiated and they have some content of fat. The samples were irradiated in a dose 7 kGy, the extraction was carried out for the Shoxlet method and the detection for the EN 1785 Standard. The results showed the utility of the 2- alkylcyclobutanone marker for the identification of irradiated foods. The obtained results showed that the avocado can be identification by the marked 2-TCB.

1. INTRODUCTION.

The irradiation technology is used in the treatment of foodstuffs, to retard the ripening of fruits and vegetables, in disinfesting products, to inhibit sprouting of tubers and bulbs and to reduce contamination of bacteria [1]. Since this is a technology adopted by the World Health Organization (WHO) and United Nations Organization for Food and Agriculture (FAO) for the processing of these products [2]. These organizations concluded in 1980 that food irradiation has not nutritional or toxicological problems for human health at doses up to 10 kGy. On the other hand, Health Authorities in 40 countries have adopted the irradiation of 60 food products for different purposes a variety of foods, according to their needs [3].

The use of irradiation as a preservation method requires the existence of assays capable of distinguishing one irradiated food is not irradiated in order to comply with international market regulations [4].

The European Committee for Standardization (CEN) has published 10 formal protocols for the detection of irradiated foods, within which is the EN 1785:2003, which is a method based on the detection of 2-alquilcyclobutanones (2-ACBs) tested for various irradiated foods, based on a preparation, extraction, purification, separation and detection by gas chromatography mass spectrometry (CG-MS) of the analyzed sample [5-8]. The 2-ACBs are compounds produced by irradiation of food products containing lipids, detected in 1990 in irradiated products as chicken, beef and pork, then in 2000, other researchers found the 2-DCBs in mango fruit pump, salmon and cheese irradiated [9].

The 2-CBAs are considered the only products radiolytic permanently present in irradiated foods containing fats, are formed depending on the fatty acid (palmitic, stearic, oleic etc.). Present in food, being recommended for detection markers 2-dodecylcyclobutanone (2-DCB), and 2-tetradecilciclobutanona (2-TCB) for their stability over time.

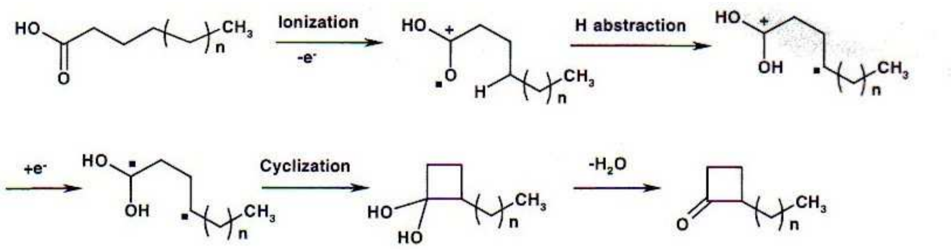

Figure 1: Chemical transformation of fatty acids at 2-ACBs by the action of ionizing radiation.

In recent years it has increased the export of tropical fruits in the area of Latin America and the Caribbean. These products are processed by irradiation with the objective of reducing microbial contamination before being consumed. Moreover, as avocado fruit is irradiated with quarantine purpose and delay the maturation. The aim of this work was to implement the method of detection of 2-ACB marker in irradiated fruits that contain fat.

2. MATERIALS AND METHODS.

2.1 Reagents

2-ciclohexylciclobutanona (2-CHCH), 2-DCB and 2TCB to 100 µg/ml as an internal standard for gas chromatography-mass spectrometry (GC-MS). The solvent used for the extraction and purification was n-hexane for use in chromatography PA (Sigma Aldrich). The matrix employed for purification was Florisil 60-100 mesh, and Na_2SO_4 anhydrous was heated for 5 h at 650 ^{o}C, it was allowed to cool in a desiccator before use, the Florisil was activated with 2% pure water.

2.2 Samples

The experiments were conducted with lyophilized avocado fruit and it was irradiated at 7 kGy in an electron accelerator.

2.3 Extraction and Purification

Approximately 20 g of anhydrodrous sodium sulphate and 15 g of sample were weighed into an extraction thimble, mixed and extracted with 200 ml of hexano in a shoxhlet apparatus type Solab, modelo SL-201/6/A. The purification was performed on Florisil columns, (3m x 15 cm) and eluted with 200ml n-hexane and 1% ether, with a flow of 4 ml / min. The latter fraction was reduced in volume to 5-10 ml by rotary-evaporation to 40 ^{o}C and 120 mbar volume approximately 3-5 ml. Typo Fisatom, modelo 801.
The concentration to dryness under a stream of nitrogen and resuspended in 200 µl of a solution containing the internal standard 2-CHCH. These samples were treated in the same manner as the irradiated and control sample.

2.4 Gas chromatograph mass spectrometer.

All samples were analyzed by CG-MS. In order to test the reliability of the methodology, the samples unirradiated spiked controls were prepared by addition 2-CBS standards of 100 µg/ml.
The volume of sample injected into the gas chromatograph was 1 µl, separation was performed on a column VF 5ms.

3. RESULTS AND DISCUSSION.

The 2-ACBs were measured using GC-MS the peaks corresponding to the internal standards 2-ciclohexilxiclohexanona (2-CHCH), 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB), as well as retention times, ion mode was used, the ion 98 and 112 m / z were used for monitoring the relationship between these two ions 98/112, it was 4:1 for markers 2TCB 2-DCB and as described in EN 1785. Figure 2 shows the peaks of the internal standard (2-CHCH, 2-DCB and 2-TCB) and their retention times to the conditions used and the mass spectra of ions.

There were clear peaks in the retention times of 2-CHCH (17.294±0.01), 2-DCB (18.75±0.01) y 2-TCB (22.34±0.01).

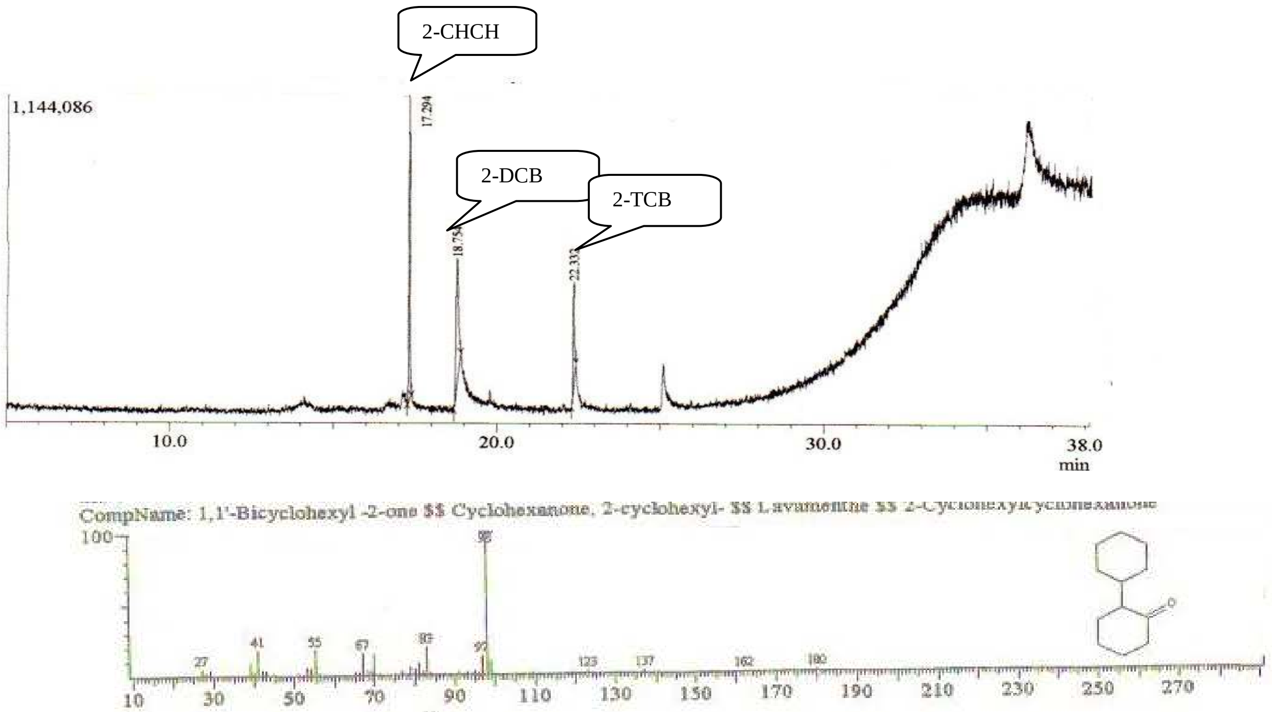

Figure 2: GC-MS Chromatograms the ions m/z 98 and m/z 112 of 2-CHCH, 2-DCB y 2TCB.
Electron impact mass spectrum of internal standard 2-CHCH.

The non-irradiated samples show no marker peaks these retention times on the analytical conditions used, this demonstrates that the 2-ACB is a specific marker of irradiated food containing fat.
The figure 3 shows the chromatogram of irradiated avocado at 7kGy, the typical chromatogram of 2- CHCH in internal standard was illustrated with peaks at 17.29 min, and 2-TCB at 22.3.min.

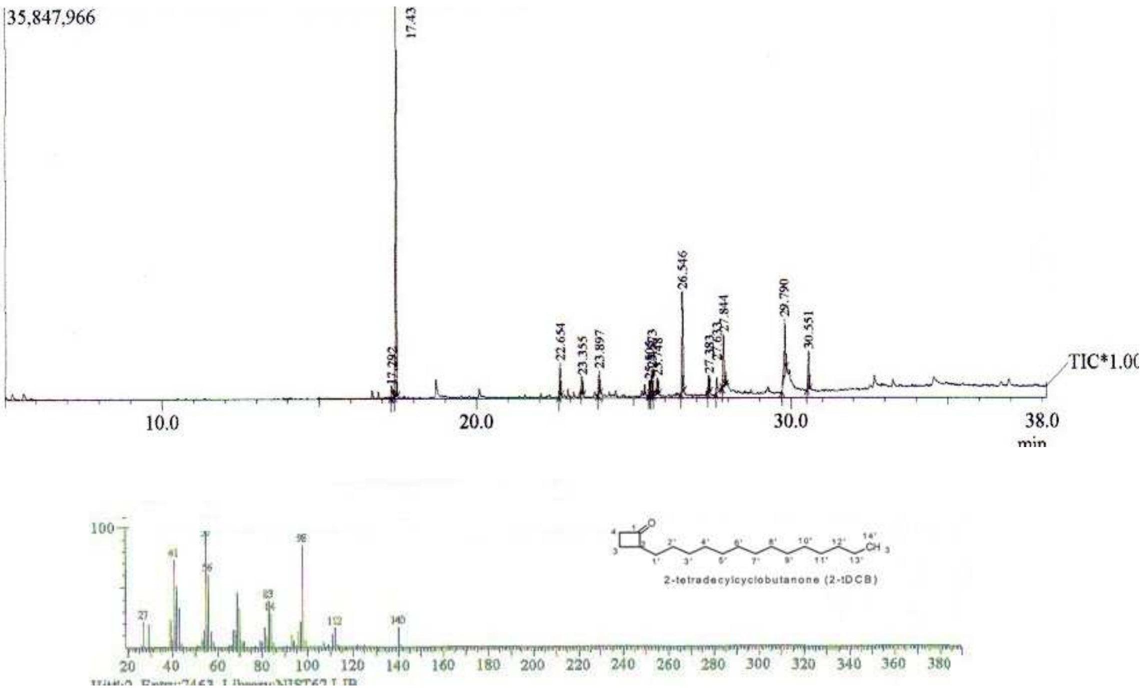

Figure 3: CG-MS Chromatograms the 7 kGy of irradiated avocado.
Electron impact mass spectrum of 2-TCB irradiated avocado.

The samples 2-DCB extracted from avocado produces peaks for the ions m/z 98 and 112 both are similar to standard solutions. The palmitic and oleic acid are predominant fatty acids in avocado, consequently the irradiation treatment of this fruit should lead to the formation of 2-DCB and 2-TCB, respectively, however it was observed the score of 2-TCB but not 2-DCB, these data correspond to the obtained results by other authors in identifying avocado irradiated at 0.5 kGy using the method of supercritical extraction [12].

4. CONCLUSIONS

The samples of irradiated avocado at 7 kGy were identified with the marker 2-tetradecilciclobutanona using the EN 1785. The assay of detection of foods irradiated with CG-MS with 2-alquilciclobutanonas was implemented in the IPEN.

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