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# Effect of gamma-ray irradiation on the fatty acid profile of irradiated beef meat

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ABSTRACT

The effect of γ-ray irradiation on the fatty acid profile of beef meat was examined at doses of 2.5, 5.0, 7.5, 10.0 and 15.0 kGy by means of $^{1}$H NMR spectroscopy. NMR results revealed a clear trend toward an increase in the amount of saturated fatty acids and a decrease in the amount of polyunsaturated fatty acids in the triacylglycerol composition of the irradiated samples compared to the unirradiated sample with increasing the irradiation dose.

The observed changes in the fatty acid profile were confirmed by gas chromatography analysis of the samples irradiated at doses of 7.5, 10.0 and 15.0 kGy.



## 1. Introduction

Treatment of food with ionising energy is a well known method aimed at improving the safety of a wide range foods and extending their shelf-life (Arvanitoyannis, Stratakos, & Mente, 2009; Diehl, 2001; Farkas, 1998, 2006; O'Bryan, Crandall, Ricke, & Olson 2008) while maintaining wholesomeness (WHO, 1981, 1991, 1994, 1999). In particular, irradiation is an effective way to eliminate or reduce pathogenic and spoilage microorganisms including *Listeria monocytogenes, Salmonella* and *Staphylococcus aureus*, *Escherichia coli O157:H7, Campylobacter, Yersinia enterocolitica*, yeast and mould in meat products, therefore it can increase the hygienic quality of raw meats to prevent possible public health hazards.

The European Committee for Standardisation (CEN) has published as European Standards the analytical methods EN 1784 and 1785 for the detection of the volatile hydrocarbons and 2-alkylcyclobutanones induced by the radiolysis of triacylglycerols treated with γ-ray radiation ($^{60}$Co or $^{137}$Cs) (Anonymous, 2003a, 2003b). However, these reference analytical methods have disadvantages of being quite time consuming and requiring the use of considerable amounts of organic solvents due to a sample preparation and analysis. Furthermore, simultaneous cleanup of the radiolytic products – hydrocarbons and 2-alkilcyclobutanones – on the Florisil column is not possible owing to their polarity differences. Some of the hydrocarbons, such linear alkanes $C_{14}$, $C_{15}$, $C_{16}$ and $C_{17}$ to detect the radiation treatment are occurred in both control and irradiated samples. This means that linear alkanes are non-dose related indicators, therefore the analytical method EN 1784 is not radiation specific. 2-Substituted alkylcyclobutanones have been stated that they are unique radiolytic products as they have not been found as natural constituents in unirradiated foods so far. Therefore, their presence in the foodstuffs should be used to identify irradiated lipid-containing food.

It is worth pointing out, however, that recently, Variyar, Chatterjee, Sajilata, Singhal, and Sharma (2008) for the first time reported evidence of the natural existence of 2-dodecylcyclobutanone and 2-tetradecylcyclobutanone in commercial unirradiated as well as fresh cashew nut samples and 2-decylcyclobutanone as well as 2-dodecylcyclobutanone in unirradiated nutmeg samples. 2-Tetradecenylcyclobutanone was also detected in both commercial and irradiated cashew nuts. These observations contradict the claim that 2-alkylcyclobutanones are unique radiolytic products.

The traditional methods described in the literature for quantitative determination of the composition of acyl chains in fats are related to the chemical transformation of lipids by hydrolysis of the triacylglycerol and esterification of the acyl groups with methanol or by transesterification (Christie, 1990a, 1990b) and a subsequent gas chromatographic analysis (Craske & Bannon, 1987).

Nuclear magnetic resonance (NMR) spectroscopy has been successfully used to determine quantitatively the fatty acid components of the lipid composition. Numerous papers have reported the application of $^{1}$H and $^{13}$C NMR mainly for analysis of fish oils

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(Igarashi et al., 2000; Sacchi, Medina, Auborg, Paolillo, & Addeo, 1993), vegetable oils (Guillen & Ruiz, 2003; Knothe & Kenar, 2004; Retief, McKenzie, & Koch, 2009; Sacchi, Addeo, & Paolillo, 1997), in particular olive oil (Manina, Sobolev, & Segre, 2003; Mavromoustakos et al., 1997; Vlahov, 1999; Vlahov, Schiavone, & Simone, 2001), and seed oils (Ketshaywang, Holmback, & Yeboah, 1998), however very seldom for analysis of animal fats.

The aim of the present study was to use NMR spectroscopy in conjunction with gas chromatography (GC) to assess the effect of irradiation doses on the fatty acid profile in the triacylglycerol composition of beef meat. It was demonstrated in this work that the fatty acid contents can be measured with $^1$H NMR spectroscopy as accurately as with GC.

In addition, a dose-dependent effect of irradiation on the fatty acids components of the triacylglycerol composition was revealed and confirmed by these techniques.

To the best of our knowledge, no study on the application of the $^1$H NMR technique for the identification of irradiated meat has been conducted up to now. This is the first report on the $^1$H NMR detection of the irradiation treatment of meat from changes in a fatty acid composition point of view.

## 2. Materials and methods

### 2.1. Samples and materials

Beef meat was purchased from the local market. It was sliced and packaged in the presence of air in polypropylene bags, and stored at −20 °C to avoid changes in the chemical composition. Meat was defrosted immediately prior to irradiation and exposed to a 10.0 kGy/h Cobalt-60 source to absorbed doses of 2.5, 5.0, 7.5, 10.0 and 15.0 kGy by using a Gammacell 220-EXCEL (MDS Nordian, Ottawa, ON, Canada) irradiation facility at an average sample temperature of 20 °C. The absorbed dose was calculated with respect to water, following a standard procedure (National Institute of Standards and Technology, Washington). All the food samples were stored at −20 °C after the radiation treatment until subsequent analysis.

In addition to the control beef sample (unirradiated) and test beef samples (irradiated with doses of 2.5, 5.0, 7.5, 10.0 and 15.0 kGy), one imported unknown commercially obtained from the supermarket, designed as "UN" was investigated.

n-Hexane was a Fluka product. Chloroform-d (CDCl3) with 0.03% ml tetramethylsilane (TMS) as an internal standard was an Aldrich product.

2-Dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB) were synthesised according to the method described by Miesch et al. (2002).

### 2.2. Soxhlet extraction procedure

Food samples (20 g of an irradiated and unirradiated meat, representing test and control sample, respectively) finely ground and anhydrous sodium sulphate (20 g) were placed into an extraction thimble and extracted gently with n-hexane in a Soxhlet apparatus 6 h (four cycles per 1 h) according to the reference method (Anonymous, 2003b). The lipid extract was transferred from the flask to a 100 ml glass measuring cylinder. The volume was adjusted to 100 ml with n-hexane and then 5–10 g of anhydrous sodium sulphate was added. After mixing the solution was left overnight. The lipid extracts of the test and control samples were evaporated to dryness on a rotary vacuum evaporator (200 mbar, 35 °C) to constant weight and stored at the abovementioned conditions until they were measured by NMR spectroscopy.

### 2.3. FAME preparation

Prior to GC analysis the triglycerides were transformed to fatty acids methyl esters (FAME) by a base-catalysed method (Christie, 1982).

### 2.4. Gas chromatography

Fatty acids methyl esters (FAME) were analysed by gas chromatography (GC) to identify and quantify the fatty acid composition in the fat. GC was performed by using Thermo Finnigan Trace GC Ultra equipped with a split/splitless capillary injector. FAME were separated by a capillary DB-225 column (60 m length, 0.25 mm ID, and 0.25 μm film thickness) and detected with a flame ionisation detector (FID). The FID and injector temperature were held at 260 °C. The temperature programming was 120 °C for 8 min, then increased to final temperature of 240 °C at a rate of 5 °C/min with a final isothermal period of 25 min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Injection volume of FAME dissolved in n-hexane was 1 μl. Peak retention times and area percentage were determined by comparison with the retention times and area intensities of known standards. The content of fatty acids was calculated from their corresponding integration data.

### 2.5. NMR spectroscopy

Lipid extracts (100 mg of the fats) and methyl esters of fatty acids (FAME, 30 mg) were dissolved in 0.6 ml chloroform-d. $^1$H NMR spectra were recorded on a Bruker FT spectrometer Avance II + 600 operating at a proton frequency of 600 MHz. The following acquisition parameters were used: temperature 200 °C, 32/64 K time domain data points, spectral width of 9615 Hz, acquisition time 3.4 s, pulse width 300, number of scans 16 or 32, acquisition time 1.70 s, relaxation delay (D1) 60 s. The FID was zero filled up to 64 K point prior to FT, therefore the final spectral resolution (Hz/Point) was 0.15 Hz. Processing was accomplished by the software TOPSPIN, which was also used for automatic integral calculation on predefined spectral areas.

For quantitative purpose a precise baseline correction (manual or automatic) was performed and the integrals were normalised to the integral of the methylene protons of the glycerol moiety and methoxyl protons, respectively.

### 2.6. Gas chromatography–mass spectrometry (GC/MS)

The GC/MS analysis of 2-alkylcyclobutanones of the unknown commercially sample ("UN") isolated by adsorption chromatography on the Florisil® column according to the reference method EN-1785 (Anonymous, 2003b) was performed on a Thermo Finnigan Trace GC Ultra gas chromatograph coupled with a Finnigan Trace DSQ mass selective detector and an autosampler injector AI 3000. The GC conditions were as follows: capillary column Rtx® – 5MS (5% diphenyl and 95% dimethylpolysiloxane) with dimensions of 15 m length, 0.25 mm ID and 0.25 μm film thickness; column temperature program, the initial temperature of 50 °C maintained for 2.10 min, following by an increase of 20 °C/min to a temperature of 200 °C and of 30 °C/min to a final temperature of 270 °C held for 12 min; carrier gas, helium with a flow rate of 1 ml/min; injector temperature, 250 °C; injection volume, 1 μl; injection mode, splitless. The MS were measured in selected ion monitoring (SIM) mode at the following conditions: the source and transfer line temperatures, 220 and 270 °C, respectively; filament current, 0.3 mA. The ion currents at $m/z$ 98 and 112 were monitored for 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TCB). The 2-alkyl substituted cyclobutanones of the sample "UN" were identified by comparison with