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Detection of irradiated beef by nuclear magnetic resonance lipid profiling combined with chemometric techniques

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ABSTRACT

The combination of ^{1}H NMR lipid profiling with multivariate analysis was applied to differentiate irradiated and non-irradiated beef. Two pattern recognition chemometric procedures, stepwise linear discriminant analysis (sLDA) and artificial neural networks (ANNs), provided a successful discrimination between the groups investigated. sLDA allowed the classification of 100% of the samples into irradiated or non-irradiated beef groups; the same result was obtained by ANNs using the 1 kGy irradiation dose as discriminant value suggested by the network. Furthermore, sLDA allowed the classification of 81.9% of the beef samples according to the irradiation dose (0, 2.5, 4.5 and 8 kGy). ^{1}H NMR lipid profiling, coupled with multivariate analysis may be considered a suitable and promising screening tool for the rapid detection of irradiated meat in official control of food.

1. Introduction

Irradiation of food is used to improve the safety and extend the shelf life by inactivating pathogenic bacteria and decreasing spoilage microbial load. Food irradiation involves the exposure of food to ionizing radiation under controlled conditions (Cleland, 2006). The treatment, when performed at absorbed doses lower than 10 kGy, induces biochemical changes that could affect the nutritional adequacy of food (Giroux & Lacroix, 1998).

The effects of ionizing radiation on lipids have been extensively studied because they were suspected to degrade the flavor of irradiated food (Nawar, 1986). The lipids are subjected to non-oxidative radiolysis which involves cleavage at preferential locations in the lipid molecule, formation of free radicals which mainly add hydrogen to other molecules or, to a lesser extent, lose a hydrogen or combine with other free radicals. Stable radiolytic products are thus formed with their composition being related to the composition of the initial lipid molecule (Giroux & Lacroix, 1998). The main reported radiolytic products derived from the major fatty acids are 2-alkylcyclobutanones (2-ACBs) which can be specifically used as markers of irradiation (Le Tellier & Nawar, 1972; Stevenson, Crone, Hamilton, & McMurray, 1993). Moreover, irradiation has been shown to initiate or promote lipid oxidation (Thakur & Singh, 1994; Zanardi et al., 2009). Oxidative and non-oxidative lipid changes induced by irradiation depend on several parameters such as food lipid concentration and composition, environmental conditions, storage conditions and irradiation dose (Delincée, 1983).

A confirmatory analytical method based on the determination of 2-ACBs is applicable to food whose triglyceride content is not negligible (>1%) provided that the absorbed dose of irradiation is higher than 0.5 kGy. This protocol was validated for the determination of 2-dodecylcyclobutanone (2-dDCB) and 2-tetradecylcyclobutanone (2-tDCB) in raw meat and liquid whole egg by large scale interlaboratory studies for suitability for use in official food control, and was adopted by the European Committee for Standardisation as European Standard EN 1785 (Anonymous, 2001) to differentiate between irradiated and non-irradiated food containing fat. The Standard EN 1785 is based on lipid extraction, cleanup by absorption chromatography prior to separation and detection by gas-chromatography coupled to mass-spectrometry. It has the twofold disadvantage of being time consuming and labor-intensive, and uses large organic solvent volumes, with a consequent low number of samples to be analyzed per day (Marchioni, 2006). In Italy most of the laboratories appointed for official control of irradiated food are limited to screening procedures and only a few of them have started monitoring plans due to the difficulties in setting up the European Standards (ISTISAN, 2007). Within this frame, the European Commission promotes the development of new techniques and the setting up of new protocols aimed to simplify or improve existing procedures (Boniglia, 2004; Califano, 2009).

High resolution nuclear magnetic resonance (NMR) spectroscopy is a suitable technique for the characterization of complex systems

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such as foods because it allows the simultaneous determination of a high number of compounds. Actually, NMR profiles provide a quite exhaustive representation of the chemical composition of the sample without the need of extensive manipulation. High resolution NMR spectroscopy has been used for the compositional study of complex mixtures of lipids providing both qualitative and quantitative information (Pollard, 1986; Sacchi, Addeo, & Paolillo, 1997; Sacchi, Savarese, Falcigno, Giudicianni, & Paolillo, 2006). The use of NMR in lipid science includes the application of ^{1}H NMR to vegetable lipids to assess fatty acid composition and positional distribution on triacylglycerols, and to evaluate the presence of markers to assess oil quality (Miyake, Yokomizo, & Matsuzaki, 1998; Sacchi, 2001; Sacchi, Addeo, Giudicianni, & Paolillo, 1992; Sacchi et al., 1996; Wollemberg, 1990). High resolution NMR has been also successfully applied to the analysis of fish oils and lipids for the determination of polyunsaturated fatty acid composition (Gunstone, 1991; Sacchi, Medina, Aubourg, Paolillo, & Addeo, 1993; Sacchi et al., 1993), and in fish-processing quality control by monitoring lipolysis and oxidative degradation of fish lipids (Falch, Sørseth, & Aursand, 2005; Guillén & Ruiz, 2001; Saito & Nakamura, 1990). Besides the individual signal assignment to the corresponding molecular species, the recent approach of NMR spectroscopy applied to food science is the exploitation of the spectra as a whole metabolic profile to be analyzed by chemometrics. NMR profiling has been successfully applied to the identification of botanical, zoological and geographical origin of different foods. This approach has been employed for the authentication, geographic origin and varietal traceability of virgin olive oil (Mannina, Patumi, Proietti, Bassi, & Segre, 2001; Sacchi, 2001) the discrimination of virgin olive oil from olive-pomace oil and refined olive oil (Zamora, Gomez, & Hidalgo, 2002), and in the characterization of monocultivar binary wine mixtures (Imparato, Di Paolo, Braca, & Lamanna, 2011). In food of animal origin, there is the possibility of using NMR spectra as a fingerprint in recognition of wild and farmed fish (Aursand & Alexon, 2001; Aursand, Mabon, & Martin, 2000), and in the detection of adulteration by undeclared mixing of offal to ground beef muscle (Al-Jowder et al., 2001).

The aims of this study were *i)* to assess the usefulness of NMR spectroscopy to detect the occurrence of 2-dDCB in irradiated meat as an alternative to the European Standard EN 1785, and *ii)* to apply NMR spectroscopy in combination with supervised chemometric techniques to lipid extracts from beef to achieve the distinction of irradiated and non-irradiated specimens and to classify them according to the irradiation dose.

2. Materials and methods

2.1. Chemicals

All reagents and solvents were of analytical grade. 2-dodecylcyclobutanone (2-dDCB), 4-cyclohexylcyclohexanone (4-CCH), and deuterated chloroform ($CDCl_3$, 99.8 atom % D) were purchased from Sigma-Aldrich (Milan, Italy). *n*-hexane and diethyl ether were supplied by Lab-Scan (Gliwice, Poland). Florisil (60/100 mesh) was supplied by Supelco (Milan, Italy).

2.2. Preparation of samples and irradiation

Ground beef (one batch of about 3 kg) was prepared from the forequarter, overwrapped in food paper and provided by a local butcher. 120 portions of about 25 g were vacuum packed and stored at −20 °C prior to irradiation (7 days). Thirty samples were randomly chosen for comparison purposes (non-irradiated control samples) and thirty aliquots were randomly allotted to each of three groups intended for treatment at irradiation doses of 2.5, 4.5, and 8 kGy, respectively. The samples were arranged in polystyrene foam boxes able to keep their temperature in the range 18 °C to −13 °C for all the treatment period. Irradiation was performed using a ^{60}Co γ-irradiator (1.17–1.33 MeV) at the Gammatom S.r.l. facilities (Guanzate, Italy). Alanine dosimeters were positioned at the top and bottom surfaces of each box and the absorbed dose was within ±5% the targeted dose. The samples were stored for 5 days at 5 ± 1 °C prior to analysis.

2.3. Extraction of muscle lipids

25 g-aliquots of non-irradiated ($n = 30$) and irradiated ($n = 90$) beef were dried at 105 °C for 24 h in an oven (Carbolite, Hope, England). Lipids were extracted from dried samples by an Extraction Unit E-816 Soxhlet (BUCHI Italia S.r.l., Assago, Italy) using 120 mL of *n*-hexane. At first the solvent was removed by a Heidolph VV2000 rotavapor (Heidolph Instruments GmbH & Co, Kelheim, Germany), and then by a stream of nitrogen (99.9995% purity). Total lipids were measured gravimetrically. After extraction the lipids were stored at −20 °C.

2.4. Determination of 2-dDCB

2-dDCB was determined in non-irradiated ($n = 30$) and irradiated ($n = 90$) beef samples according to the European Standard EN 1785, following the procedure described by Zanardi et al. (2007). Briefly, the extracted muscle lipids (200 mg) were fractionated by Florisil column chromatography with 1% diethyl ether in *n*-hexane, and gas chromatography/mass spectrometry analysis in the selected ion monitoring (SIM) mode was carried out. 4-CCH was used as an internal standard.

2.5. ^{1}H NMR analysis

2.5.1. Part i)

Solutions (1 mg/mL) of 2-dDCB in $CDCl_3$ were prepared and transferred into a 5 mm glass tube for preliminary ^{1}H NMR spectra acquisitions. ^{1}H NMR spectra of non-irradiated beef were recorded dissolving 50 mg of extracted muscle lipids in 0.8 mL of pure $CDCl_3$ or 2-dDCB-spiked $CDCl_3$ to assess the effect of the matrix on the 2-dDCB signals. The limit of detection (LOD) of 2-dDCB, defined as the amount giving a signal-to-noise ratio of 3, was determined on spiked muscle lipids extracted from non-irradiated beef. NMR spectra were registered on an INOVA 600 MHz (Varian, Milan, Italy) spectrometer operating at 599.736 MHz for ^{1}H observations using a HCN probe maintained at 300 K. Spectra were acquired at 298 K, with 32 K complex points, using a 45° pulse length and 1 s of relaxation delay (d1). 128 scans were acquired with a spectral width of 9595.8 Hz and an acquisition time of 1.707 s.

2.5.2. Part ii)

^{1}H NMR spectra of 72 beef samples (29 non-irradiated and 17, 18 and 8 irradiated at 2.5, 4.5 and 8 kGy, respectively) were acquired. For the NMR study 72 out of 120 meat samples subjected to the determination of 2-dDCB were considered because this investigation was intended as a preliminary test to evaluate the possibility of using ^{1}H NMR spectroscopy for the detection of irradiated beef. Only a few samples irradiated at 8 kGy were considered in this part of the study; this high irradiation dose is not employed for commercial purposes but was taken into account to explore the NMR performances in a sufficiently broad range of irradiation dose. Although treatment with ionizing radiation of meat is not authorized in the European Union (EC, 1999), a few national authorizations exist in some member states for the irradiation of poultry meat at a maximum dose of 7 kGy. The same maximum value is allowed for the treatment of frozen red meat in the USA. Extracted muscle lipids (50 mg) were diluted in 0.8 mL of $CDCL_3$ in a 5 mm glass tube for NMR analysis. NMR spectra were registered by the spectrometer operating at the conditions previously reported. 128 scans were acquired with a spectral