Nuclear Magnetic Resonance Spectroscopy as a Tool to Distinguish between Irradiated and Non-Irradiated Meat

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***Abstract*— The aim of this study was to assess the usefulness of Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy *i)* to detect the occurrence of the irradiation marker 2-dodecylcyclobutanone (2-dDCB) in irradiated meat, as alternative to the European Standard EN 1785 method, and *ii)* to differentiate irradiated from non-irradiated meat by coupling spectral data with chemiometrics. ^{1}H NMR spectra of 2-dDCB (different concentrations in $CDCl_3$) and of the lipid fraction (50 mg in 0.8 ml of $CDCl_3$) extracted from ground beef irradiated at 0, 2.5, 4.5 and 8 kGy were acquired on a Varian INOVA600 NMR spectrometer. Irradiation was performed using a ^{60}Co γ-irradiator (1.17-1.33 MeV) on frozen vacuum-packed beef samples (25 g). The data set obtained integrating the whole spectra was statistically elaborated by multivariate analysis. The proton multiplet centred at 2.997 ppm, not overlapped by the intense signals of lipids, was used to identify 2-dDCB. The limit of detection of 2-dDCB was 50 mg/kg meat, much higher than the levels measured in beef irradiated up to 8 kGy. Discriminant analysis applied to NMR spectral data correctly classified 88.9% of originals samples according to the irradiation dose and 81.9% of cross-validated samples. In conclusion, ^{1}H NMR spectroscopy is not suitable to detect 2-dDCB at the levels expected in beef irradiated at doses for commercial purposes. However, coupled with multivariate analysis, it may be considered a promising tool for a rapid detection of irradiated meat.**

Keywords— **Irradiated meat, NMR spectroscopy, discriminant analysis.**

I. INTRODUCTION

The European Standard EN 1785 method [1] for the identification of irradiated lipid-containing food is based on the detection of 2-alkylcyclobutanones by solvent extraction followed by absorption chromatography and gas-chromatographic analysis. This method, validated for the determination of 2-dodecylcyclobutanone (2-dDCB) in different species of meat, is highly time consuming (72-84 h) and labour-intensive, and implies large organic solvent volumes. The application of Nuclear Magnetic Resonance (NMR) spectroscopy to the analysis and quality control of foods has shown great development in the last few years. The increase of new applications and the attention to this technique by scientists, official control institutions and food industries can be attributed both to the high specificity and versatility of the NMR techniques and to the improvement of the instrument performances and availability [2]. The aim of this study was *i)* to assess the usefulness of NMR spectroscopy to detect the occurrence of the irradiation marker 2-dDCB in irradiated meat, as alternative to the European Standard EN 1785 method, and *ii)* to differentiate irradiated from non-irradiated meat by coupling spectral data with chemiometrics.

II. MATERIALS AND METHODS

Ground beef (2 kg) was purchased at a local supermarket. Portions of about 25g were vacuum packed and stored at -18°C prior to irradiation. Twenty samples were randomly chosen for comparison purposes (non-irradiated control samples) and twenty aliquots were randomly allotted in each of three groups intended for treatment at irradiation dose 2.5, 4.5, and 8 kGy, respectively. The samples were arranged in polystyrene foam boxes able to keep their temperature in the range from -18°C to -13°C for all the treatment period. Irradiation was performed using a ^{60}Co γ-irradiator (1.17-1.33 MeV). Dosimeters were positioned to the top and bottom surfaces of each box and the absorbed dose was within ±5% of the targeted dose. Total fat was extracted by Soxhlet extraction

using diethyl ether from beef samples previously dried at 105°C for 24 h. ^{1}H NMR spectra of 2-dDCB (Fluka) were studied dissolving the standard in 0.8 ml of $CDCl_3$ in 5 mm glass NMR tubes both without and with fat (50-100 mg) extracted from non-irradiated control samples. ^{1}H NMR spectra of beef samples were recorded dissolving 50 mg of fat in 0.8 ml of $CDCl_3$. All the spectra were acquired on a Varian INOVA 600 MHz spectrometer, operating at 599.736 MHz for proton, equipped with a Nalorac probe. Spectra were acquired at 298 K, with 32K 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. To analyse the profiles by pattern recognition, ^{1}H NMR spectra were transferred to Amix 3.9.7 software (Brucker) and referenced to chloroform residual signal. An integration pattern was defined choosing buckets manually on all the considered spectra in the overlapped form. Buckets were chosen as large as to compensate the little chemical shifts fluctuation in each single spectrum. The defined pattern was used for the automatic integration of all the spectra and the integrals were referred to the glycerol signals in the region 4.3-4.1 ppm. The integral table obtained was statistically elaborated with SPSS 18.0 software package. Stepwise discriminant analysis was performed to determine the variables that best discriminate between irradiated and non-irradiated meat.

III. RESULTS

A. Part i)

The ^{1}H NMR spectrum of 2-dDCB (1 mg/ml in $CDCl_3$) with identification of the main peaks is presented in Figure 1. The ^{1}H NMR spectra of fat from a sample of non-irradiated beef and fat added with 2-dDCB are shown in Figure 2. The proton multiplet centered at 2.997 ppm (-C*H*$_2$-CO), not overlapped by the intense signals of lipids, was chosen to detect 2-dDCB in meat. The limit of detection (LoD) of 2-dDCB was 50 mg/kg meat, much higher than the levels measured in beef irradiated up to 8 kGy which, from literature data, are about 1 mg/kg. Indeed, 2-dDCB was under the LoD in all non-irradiated and irradiated beef samples submitted to analysis.

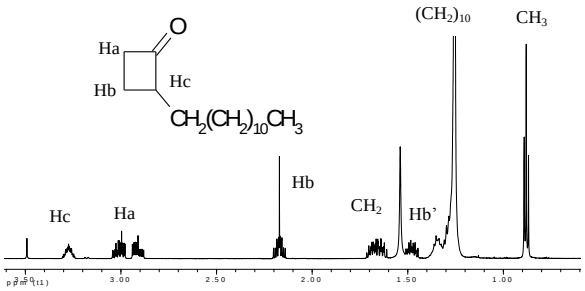

Fig. 1 ^{1}H NMR spectrum (0-3.5 ppm) of 2-dDCB

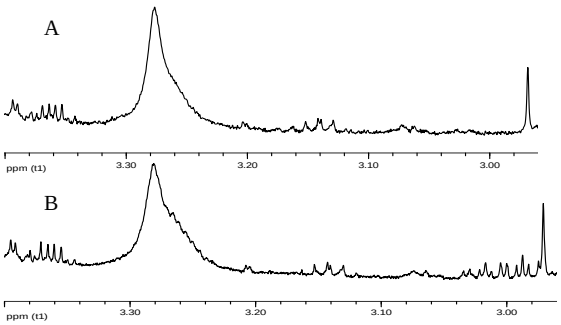

Fig. 2 Expanded region (2.96-3.40 ppm) of ^{1}H NMR spectra of a sample of fat (A) and fat added with 2-dDCB (B)

B. Part ii)

The discriminant analysis was performed on 72 cases (29 non-irradiated meat samples and 17, 18 and 8 samples irradiated at 2.5, 4.5 and 8 kGy, respectively) with the stepwise method, in order to reduce the initial number of variables (56 integrals from each spectrum) useed for the discriminant function construction: at each step the variable that contributes mostly to the separation of the groups is entered into the discriminant function (and the variable that adds the least discrimination power is removed). The statistical F function was used as criterion for variable selection.

At first, the analysis was performed by considering two groups, one for irradiated and one for non-irradiated meat. The final model selected 16 variables. The signals of the ^{1}NMR spectrum with the highest

discriminant power corresponded to those of allylic proton of unsaturated fatty acids (around 2 ppm) which decreased as a consequence of irradiation. All the other signals with discriminant power corresponded to minor compounds, which generally disappeared in the spectra of irradiated beef. As the classification table shows, 100% of the beef samples were classified correctly into "irradiated" or "non-irradiated" groups, respectively (Table 1), even in cross-validation.

Table 1 Classification of non-irradiated and irradiated beef by stepwise discriminant analysis of ^{1}H NMR spectral data

			Predicted group membership	
		Group	Non-irrad.	Irradiated
Original	Count	Non-irrad.	29	0
		Irradiated	0	43
	%	Non-irrad.	100	0
		Irradiated	0	100
Cross-valid.	Count	Non-irrad.	29	0
		Irradiated	0	42
	%	Non-irrad.	100	0
		Irradiated	0	100

Stepwise discriminant analysis was then performed by considering four groups: one for non-irradiated meat and three different groups based on dose (2.5, 4.5, and 8 kGy) for irradiated meat.

The scatterplot (Figure 3) relative to first two discriminant functions showed a good separation among the 4 groups of beef samples. The classification table showed that 88.9% of original samples and 81.9% of cross-validated samples were correctly classified.

IV. DISCUSSION

A. Part i)

Although the developments in equipment have extended sensitivity to the micromolar range, the still limited sensitivity of NMR spectroscopy makes difficult the determination of minor components. In this study the limit of detection of the irradiation marker 2-dDCB was much higher than that of the European Standard EN 1785 method and needed to differentiate non-irradiated meat from meat treated at doses allowed and currently in use n some countries.

B. Part ii)

The possibility of using high-resolution ^{1}H NMR as a "fingerprint" in the definition of geographical origin or authentication of oils, fish species and meat has been widely explored in the last decade. A similar approach was used in the present study whose aim was to assess the usefulness of NMR spectroscopy to differentiate irradiated from non-irradiated meat. Multivariate statistical analysis of ^{1}H NMR spectral data obtained from 72 beef samples indicated a possible contribution of NMR spectroscopy in the detection of meat submitted to irradiation treatment. The findings of this study showed that ^{1}H NMR analysis of fat from beef could be used as a rapid test to distinguish between irradiated and non-irradiated meat. Moreover, NMR spectroscopy has the potential to detect the irradiation dose applied.

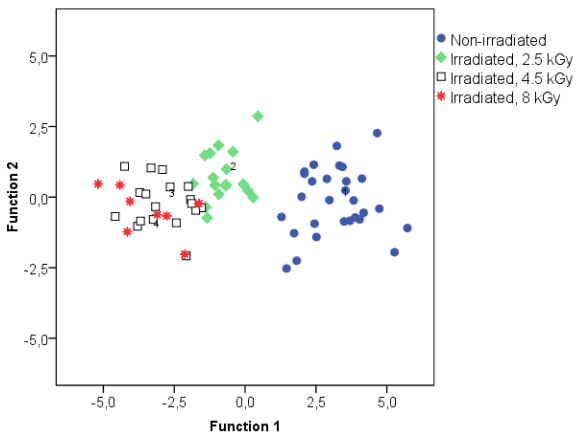

Fig 3 Graph of sample values divided by irradiation dose on the first two functions of the stepwise discriminant analysis

V. CONCLUSIONS

^{1}H NMR spectroscopy is not suitable to detect 2-dDCB at the levels expected in beef irradiated at doses for commercial purposes. However, coupled with multivariate statistical analysis, it may be considered a

promising tool for a rapid detection of irradiated meat. It has also the potential to identify the irradiation dose applied to meat.

ACKNOWLEDGMENT

The research was supported by the project PRIN2008 funded by the Italian Ministry of Education, University and Research.

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