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Characterization of animal products according to geographic origin and feeding diet using nuclear magnetic resonance and isotope ratio mass spectrometry. Part II: Beef meat

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Abstract

Meat samples from Charolais steers bred at different geographical sites in France and fed on either maize silage, indoors, or grass were analyzed using ^{18}O IRMS and ^{1}H, ^{2}H and ^{13}C NMR spectroscopy. Some parameters showed significant differences according to the production site or feeding. A discriminant factorial analysis allowed the selection of four parameters to identify the production site and diet of the animals. All grazing steers were well classified, as were 94% of the steers fed on maize ensilage.

Keywords: Meat; Lipids; Water; Fatty acids; Nuclear magnetic resonance; Isotopic ratio mass spectrometry; Authentication; Geographic origin; Feeding diet

1. Introduction

Recent health concerns (BSE, Foot and Mouth) in the beef meat industry have polarized consumer preferences with respect to geographic origin and agricultural practices and fuelled a demand for accurate labelling of food products. All retails of fresh meat have now to be labelled with the production site. Beyond conforming to the regulatory constraints, this traceability can be an advantage, by both responding to the consumer demand and by raising profits for the producers. The link to the soil is based on three criteria: the production site, diet and expertise.

The origin of food products can now be determined by some new analytical methods. Isotope analysis as isotope ratio mass spectrometry (IRMS) is recognized as an official method for guaranteeing the authenticity of batches of food products (Martin, 1995). However, the IRMS technique gives only an average value for the isotopic content of pure molecular species. $^{2}H/^{13}C$ magnetic resonance spectroscopy (MRS) has the advantage of simultaneously measuring different resonances, which gives a multi-isotopic fingerprint for characterizing the product (Martin & Martin, 1995).

A previous study, dealing with milk characterization according to geographic origin, revealed that ^{18}O IRMS results are strongly influenced by the production site and diet of the animals (Renou et al., 2003). With respect to the influence of the feeding protocol on meat composition, analyses of ^{15}N content have shown that $\delta^{15}N$ values are not indicative of a specific region (Piasentier, Valusso, Camin, & Versini, 2003). $\delta^{15}N$ values can be indicative of animal diets (Delgado & Garcia, 2001), but the level of $\delta^{15}N$ also depends on fertilizer application (Rhodes, Kelly, & Brereton, 2001). The $\delta^{13}C$ ratio was found to be highly dependent on diet composition, particularly with regard to maize, a C4 cycle plant. Moreover, the $\delta^{13}C$ enrichment varies in different meat

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tissues (Piasentier et al., 2003). On the other hand, meat is composed of 75% water, the isotopic content of which is influenced by the geographic origin and diet of the animal (Ritz, Cole, Couet, & Coward, 1996; Ritz, Cole, Davies, Goldberg, & Coward, 1996). ^{13}C NMR spectra also exhibit specific resonances characteristic of the unsaturated carbons of polyunsaturated or mono-unsaturated fatty acids (Ng and Ng, 1983). Hence the composition in triglycerides and the positions of fatty acids on glycerol can be monitored according to the diet of the animal and/or the location of the fatty tissue by using ^{13}C MRS. The variations of triglyceride composition observed by NMR are confirmed by those obtained using gas chromatography (Bonnet, Denoyer, & Renou, 1990), while the variations of the signal intensities characterize the feeding diet (Cunnane et al., 1993; Fan, Clifford, & Higashi, 1994; Thomas, Cunnane, & Bell, 1998).

Therefore, the aim of this study was to analyze the influence of the site where animals were bred and the diet they consumed on the isotopic composition of the water and fat in their meat.

2. Materials and methods

2.1. Samples

The experiment included a total of 36 Charolais steers raised on three distinct INRA (Institut National de la Recherche Agronomique) sites which differ in their geographical situation and altitude (Fig. 1): Le Pin is located in Normandy (altitude 50 m), Mirecourt in the Vosges (altitude 280 m) and Theix in the Massif Central (altitude 850 m). The diet was completed by grazing or maize silage, indoors, during the last 6 months before slaughtering, the intake being adjusted to achieve similar net energy intakes between the animals. The steers were slaughtered at 30 months old. On each site, six animals were in pasture and six others on maize ensilage and the feeding procedures were similar in all locations. Maize was cropped in each site and silaged as whole plant (30–35% dry matter, 70–80 g crude protein/kg DM). Pastures are based on natural grasslands : *Lolium perenne* and *Trifolium repens* in Normandy (Le Pin), *Lolium perenne*, *Poa*, *Trifolium repens*, *Agrostis tenuis* in Vosges (Mirecourt) and *Agrostis tenuis*, *Poa and Lolium perenne* in Massif Central (Theix).

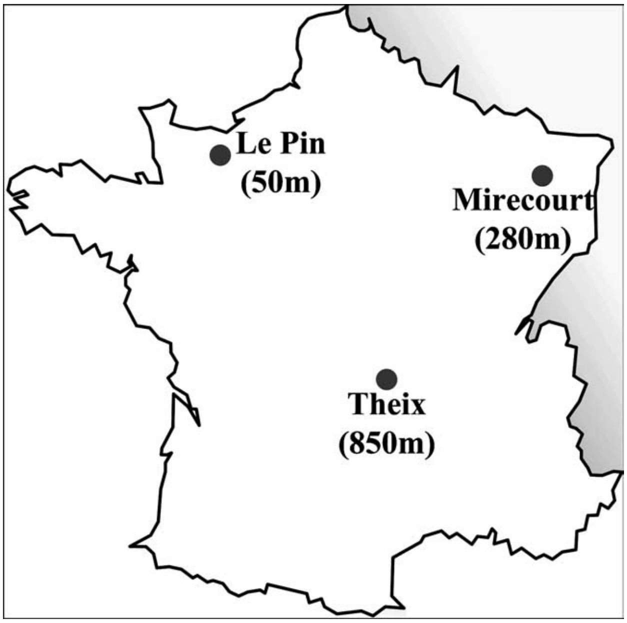

Fig. 1. The location in France of the three production sites.

2.2. NMR measurements

Triglycerides (TG) were extracted from adipose tissue by fusion in a microwave oven. For ^{1}H and ^{13}C NMR studies, 400 μl aliquots of triglycerides dissolved in $CDCl_3$ were put in 5 mm glass NMR tubes (Aldrich, USA). For ^{2}H NMR studies, triglycerides were dissolved in $CHCl_3$ and C_6F_6 was added for the lock.

All NMR spectra were recorded at 298 K on a Bruker AM400 spectrometer (Bruker Analytik, Rheinstetten, Germany) and the experimental conditions are summarized in Table 1. The spectra were processed conventionally using XWINNMR software (version 1.3, Bruker). Areas of interest in the ^{13}C spectra were identified and the proportions of fatty acids determined according to previous results (Bonnet et al., 1990).

To analyze the profiles by pattern recognition, ^{1}H NMR spectra were data-reduced using the software programme are AMIX (Bruker). The spectra were separated into 0.04 ppm spectral ‘buckets’ and the regions between 0.7 and 5.62 ppm automatically integrated,

Table 1
Experimental parameters for NMR acquisition

	Sweep width (Hz)	Memory size (k)	Excitation pulse (μs/°)	^{1}H Decoupling during acquisition	Acquisition time (s)	Recycling time (s)	Scans
^{1}H	5000	32	5/90°	–	3.276	0.1	16
^{13}C	20 000	32	5/60°	on	0.786	1	
^{2}H	445	4	14.5/90°	on	4.6	0.4	16 000

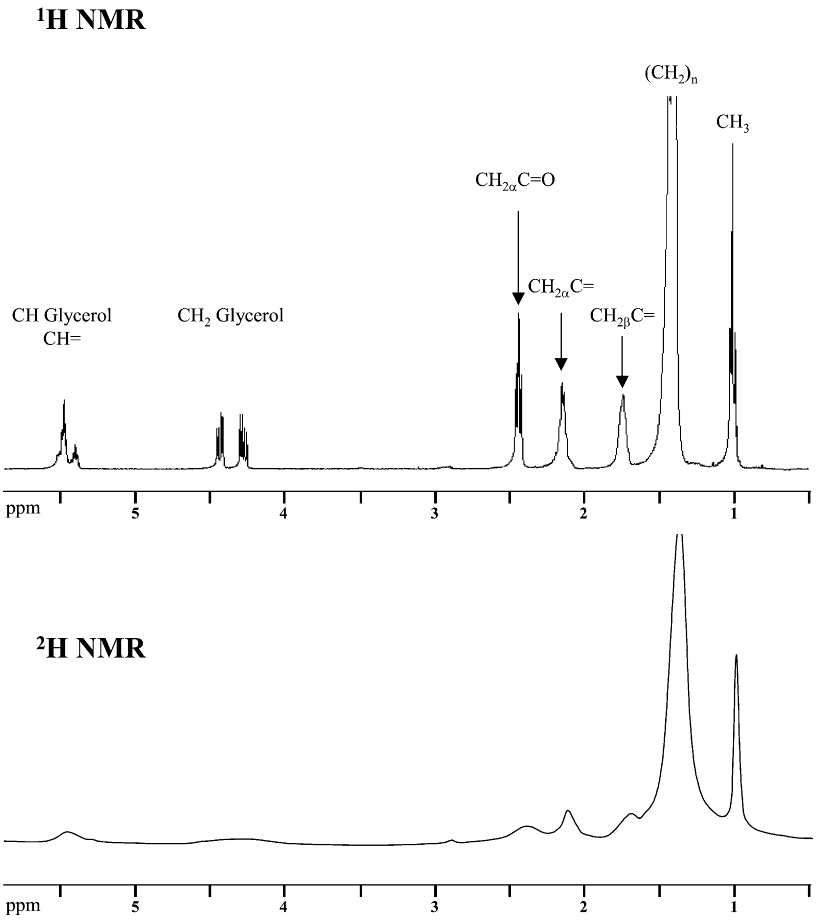

Fig. 2. H and ^{2}H NMR spectra from a sample of adipose tissue.

each integral region being represented as a ratio relative to the total integral over all individual regions. ^{2}H NMR spectra were less complex and integration was performed using PeakFit™ 4.11.

Fig. 2 shows the ^{1}H and ^{2}H NMR spectra from fatty tissue with the assignment of the different resonances. The ^{2}H spectrum displays lower resolution due to the magnetic properties of the deuterium nucleus. However, no J_{DD} coupling is observed in this spectrum, whereas the ^{1}H spectrum is complicated by numerous J_{HH} couplings. In these conditions, an accurate quantitative determination of the different fatty acids was impossible. The specific isotope ratio [D/H] of a spectral region was determined as the ratio of the integral calculated from the ^{2}H NMR spectrum to the integral calculated from the ^{1}H NMR spectrum.

2.3. IRMS measurements

After thawing at 5 °C for 24 h, the meat was ground and meat water was extracted by ultra-centrifugation at 25 000 rpm and 30 °C for 6 h.

The ^{18}O enrichment was determined by gas chromatography-isotope ratio mass spectrometry (VG Isochrom-μgas, VG Isotech, Cheshire, UK). A 1 ml sample was introduced into a 10 ml vacutainer (100 × 16 mm, Becton Dickinson sterile vacutainer with no additive) previously filled with a 5% CO_2/He gas mixture at atmospheric pressure. The vacutainers were then placed in a shaker and equilibrated at 25 °C for a minimum of 10 h, by which time at least 99% equilibrium had been reached. Results are expressed as the isotopic ratio in ppm relative to that in International Standard Vienna Mean Ocean Water (SMOW):

$$\delta = \left(\frac{R_S}{R_{\mathrm{SMOW}}} - 1\right) \times 10^3, \tag{1}$$

where R_S and R_{SMOW} are the heavy/light isotope ratios in the sample and SMOW, respectively. The isotopic ratio for $^{18}O/^{16}O$ in SMOW is 2005.2 ppm.

2.4. Data analysis and statistics

The factors studied in this work were the production region and feeding diet. For each factor, the results obtained by NMR spectroscopy and IRMS were considered as variables for the data analysis.

3. Results and discussion

3.1. IRMS

The $\delta^{18}O$ data (Table 2) differentiate the feeding (pasture or maize silage) ($p < 0.005$). On the Theix and Le Pin sites, the $\delta^{18}O$ enrichments were significantly different according to diet. At Mirecourt, no significant difference was observed, but these data displayed great variability within the group of animals at pasture. As expected, no significant differences were observed between the production sites. However, according to previous work (Renou et al., 2003; Ritz et al., 1996; Ritz et al., 1996), the enrichment varies with time. In this study, the animals were not slaughtered at the same time, as three months elapsed between the first slaughtering in August and the last in November. A linear decrease in $\delta^{18}O$ values with time was observed within each group ($p < 0.01$). In these experimental conditions, IRMS $\delta^{18}O$ values were not discriminant for the production site but were discriminant for the diet (Table 2).

3.2. NMR

In ^{13}C NMR spectroscopy, the relative contributions of polyunsaturated (PUFA), mono-unsaturated (MUFA) and saturated (SFA) fatty acids are shown in Table 2. The diet induced a variation in the degree of saturation of fatty acids. The MUFA percentage was significantly higher ($p < 0.005$) for animals fed on maize silage than for grazing steers (Table 2), while the inverse was observed for PUFA and SFA ($p < 0.05$) (Table 3). These results agree with those reported for the fatty acid composition of intramuscular fat (Bauchart et al., 2001). As in milk (Renou et al., 2003), the PUFA content of Theix samples was greater ($p < 0.1$) than that of samples from Le Pin, whereas MUFA and SFA did not display any significant differences. This variation in PUFA content is supported by the botanical composition of the diet, which leads to a greater proportion of PUFA in mountain milks than in plain milks (Chilliard, Ferlay, & Doreau, 2001).

In ^{1}H and ^{2}H NMR spectroscopy, the integral of each resonance area is shown in Table 3. The values

Table 2
$\delta^{18}O$ of meat water and relative proportions of PUFA, MUFA and SFA in meat according to the site and diet

		Pasture			Maize silage			Effect		
		Le pin	Mirecourt	Theix	Le Pin	Mirecourt	Theix	Site	Diet	Site × Diet
IRMS	$\delta^{18}O$	−3.75 ± 0.98	−5.76 ± 1.76	−4.55 ± 0.91	−5.84 ± 0.52	−5.20 ± 0.81	−6.09 ± 0.60	NS	**	**
^{13}C NMR	PUFA	2.9 ± 0.9	4.6 ± 1.6	5.9 ± 2.8	2.7 ± 0.6	3.1 ± 1.4	3.7 ± 0.9	*	*	NS
	MUFA	38.1 ± 3.9	38.2 ± 2.5	34.2 ± 1.7	42.5 ± 3.5	40.0 ± 2.0	38.8 ± 2.7	**	***	NS
	SFA	59.0 ± 4.5	57.1 ± 1.9	59.9 ± 4.1	54.8 ± 3.6	56.9 ± 1.7	57.5 ± 2.2	NS	*	NS

t-test: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; NS $p > 0.1$.

Table 3
Integral of each resonance area in ^{1}H and ^{2}H NMR spectra

	δ (ppm)	Assignment	Pasture			Maize silage		
			Le Pin	Mirecourt	Theix	Le Pin	Mirecourt	Theix
^{1}H	5.4	CH=	3.8 ± 0.3	4.0 ± 0.1	4.1 ± 0.3	3.8 ± 0.2	3.6 ± 0.3	3.8 ± 0.2
	4.25	Glycerol	3.6 ± 0.1	3.7 ± 0.1	3.7 ± 0.2	3.7 ± 0.1	3.5 ± 0.3	3.6 ± 0.1
	2.3	$CH_{2\alpha}CO$	5.8 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.8 ± 0.2	5.7 ± 0.3	5.8 ± 0.1
	2	$CH_{2\alpha}C=$	6.0 ± 0.6	6.4 ± 0.2	6.2 ± 0.6	5.9 ± 0.5	5.9 ± 0.7	6.6 ± 0.7
	1.6	$CH_{2\beta}C=$	6.2 ± 0.2	6.4 ± 0.2	6.0 ± 0.1	5.9 ± 0.2	6.0 ± 0.5	6.1 ± 0.2
	1.3	$(CH_2)_n$	65.3 ± 1.0	64.6 ± 0.4	64.7 ± 1.1	65.5 ± 0.7	65.9 ± 1.0	64.9 ± 0.5
	0.9	CH_3	9.1 ± 0.1	9.0 ± 0.2	9.5 ± 0.1	9.4 ± 0.4	9.5 ± 0.7	9.3 ± 0.2
^{2}H	5.4	CD=	1.7 ± 0.2	2.0 ± 0.3	2.0 ± 0.3	1.8 ± 0.2	1.8 ± 0.3	2.1 ± 0.6
	4.25	Glycerol	2.4 ± 0.6	2.8 ± 0.8	3.0 ± 0.9	2.5 ± 0.5	2.7 ± 0.4	3.0 ± 0.6
	2.3	$CD_{2\alpha}CO$	3.9 ± 1.2	4.1 ± 1.4	3.9 ± 0.5	4.1 ± 1.0	3.7 ± 1.2	36.6 ± 0.2
	2	$CD_{2\alpha}C=$	3.6 ± 0.7	4.7 ± 0.6	4.5 ± 1.0	4.5 ± 1.3	4.0 ± 0.2	5.0 ± 1.1
	1.6	$CD_{2\beta}C=$	6.6 ± 2.2	5.7 ± 2.0	4.9 ± 1.7	6.9 ± 2.0	6.0 ± 3.7	9.5 ± 3.8
	1.3	$(CD_2)_n$	72.7 ± 2.6	72.2 ± 2.3	72.9 ± 2.2	72.1 ± 2.7	72.2 ± 3.1	68.3 ± 2.8
	0.9	CD_3	9.0 ± 1.0	8.6 ± 1.5	8.8 ± 1.0	8.3 ± 0.8	9.6 ± 1.2	8.4 ± 1

Table 4
[D/H] ratios in meat fat according to the site and diet

δ (ppm)	Pasture			Maize silage			Effect		
	Le Pin	Mirecourt	Theix	Le Pin	Mirecourt	Theix	Site	Diet	Site × Diet
5.4	0.45 ± 0.05	0.50 ± 0.08	0.51 ± 0.10	0.47 ± 0.04	0.51 ± 0.10	0.55 ± 0.14	**NS**	**NS**	**NS**
4.25	0.66 ± 0.18	0.75 ± 0.23	0.81 ± 0.25	0.67 ± 0.13	0.78 ± 0.08	0.84 ± 0.16	**NS**	**NS**	**NS**
2.3	0.67 ± 0.20	0.68 ± 0.25	0.67 ± 0.08	0.70 ± 0.17	0.65 ± 0.19	0.63 ± 0.02	**NS**	**NS**	**NS**
2	0.60 ± 0.10	0.74 ± 0.11	0.73 ± 0.13	0.75 ± 0.22	0.68 ± 0.10	0.77 ± 0.23	**NS**	**NS**	**NS**
1.6	1.06 ± 0.38	0.89 ± 0.29	0.81 ± 0.28	1.16 ± 0.34	1.01 ± 0.61	1.56 ± 0.60	**NS**	*	**NS**
1.3	1.11 ± 0.03	1.12 ± 0.03	1.13 ± 0.04	1.10 ± 0.04	1.10 ± 0.03	1.05 ± 0.05	**NS**	*	**NS**
0.9	0.99 ± 0.11	0.93 ± 0.12	0.93 ± 0.12	0.87 ± 0.10	1.01 ± 0.13	0.91 ± 0.11	**NS**	**NS**	**NS**

t-test: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; NS $p > 0.1$.

reveal no significant differences according to diet or production site. However, the [D/H] ratio varied with the resonance (Table 4) and the D enrichment was not constant along the fatty acid chains. The ratios $[D/H]_{\delta:1.6}$ and $[D/H]_{\delta:1.3}$ differed significantly ($p < 0.05$) according to the diet (Table 4).

3.3. Characterization of the diet

In this study, the animals were fed on maize silage or pasture grass in each geographic region. A statistical analysis of the NMR and IRMS data showed that a single measured parameter could not discriminate the diet but that the NMR parameters were more pertinent. The degree of saturation of fatty acids and the specific D enrichment at positions 1.6 and 1.3 on the fatty acid chains were selected for a discriminant analysis. Using these three parameters, 89% of the animals were well classified and all animals were correctly assigned at the mountain site (Theix).

3.4. Characterization of the site

The geographic origin was determined for each diet (pasture or maize). All grazing steers were well classified, as were 16 of the 18 animals fed on maize ensilage (94%). The parameters identified as discriminant were derived from NMR but differed with the diet: MUFA, PUFA, $[D/H]_{\delta:2}$ and CH_3 for pasture and SFA, CD_2, CD= and $[D/H]_{\delta:0.9}$ for maize silage. The ^{2}H NMR results displayed significant differences. In the case of steers fed on maize silage, characterization of the geographic site was dependent on both the maize production site and the breeding site.

4. Conclusion

The $\delta^{18}O$ values in meat water reflect those of the oxygen entries, which depend on climatic conditions. A single ^{1}H, ^{2}H or ^{13}C NMR parameter was not sufficient to identify the production site and diet of the steers. The differences between groups were often not significant, due to the poor accuracy of the NMR measurements and the variability between animals. However, a combination of selected NMR parameters was adequate to characterize the meat production. We may conclude from this and other studies of the characterization of animal products that the analytical methods, NMR and IRMS, allow the authentication of production type. ^{1}H, ^{2}H and ^{13}C NMR and ^{18}O IRMS results are less dependent on husbandry (fertilizer application) than those of ^{15}N IRMS. These first encouraging results will now have to be validated at other sites in Europe.

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