^{1}H & ^{13}C NMR STUDIES OF FAT IN MEAT PRODUCTS

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INTRODUCTION

As compared with the extensive use made of low resolution NMR for determining fat content in food science, high resolution NMR spectroscopy has been very little used so far. The ^{1}H high resolution NMR allows very fast determination of fat content. It has been demonstrated that ^{13}C NMR spectroscopy has a high potential because of its superior separation of resonances. The natural abundance level and the relatively low sensitivity of this nucleus ^{13}C restrict the number of compounds which can be detected without long acquisition times. Spectra from triglycerides of vegetable oils, have been obtained. The nature of the fatty acids (Ng & Ng, 1983; Ng, 1984) and the position where they are attached on the glycerol carbons were determined (Ng, 1985). This study deals with the NMR application to fatty acid composition in meat products.

MATERIALS AND METHODS

Different "Foie Gras" and animal fats were analyzed. For ^{13}C NMR analysis, the fat was extracted by melting at 60°C for 12h., filtered and dissolved in chloroform-*d* in the volume ratio 200mg/ml.
All CPG experiments were performed on Girdel DI 700 with carbowax 20M column (0.3mm x 25 m) at 200°C.
Lipid content was determined by ^{1}H spectroscopy with a magnet at 0.47T. and a 10 cm wide bore on samples of about 60ml.
The ^{13}C NMR spectra were recorded at 35°C on a Bruker AM400 spectrometer at ^{13}C frequency of 100.6 MHz. The total ^{13}C spectrum was recorded with a sweep width of 20.000Hz and a 90° pulse of 5µs. The selective spectrum was obtained with a sweep width of 200 Hz and a 90° pulse of 105 µs when the low transmitter power was used. The recycle time was 20 s. Each spectrum was a result of 3000 transients. The proton decoupling was achieved by "inverse gated decoupling" mode to suppress NOE (Breitmaier & Völter, 1987). A maximum field homogeneity was obtained by adjusting the shims to obtain a half-height line width of ≈ 0.5 Hz. An exponential line broadening of 0.05 Hz was applied before Fourier transformation.

RESULTS

Figure 1 shows the typical ^{1}H spectrum obtained from a single scan. Signal to noise ratio was excellent and resolution sufficient at low field to separate both lines : water and fat (Renou *et al.* 1987).

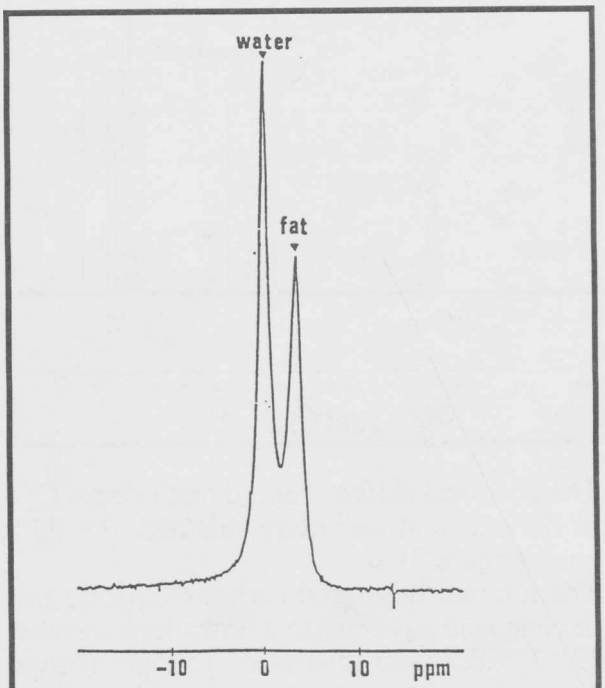

FIGURE 1

The ^{13}C resonances on figure 2 were assigned on the basis of chemical shifts previously reported from lipid compounds (Canioni *et al* 1983, Asworth *et al.*1985)
Peaks in the 14-40 ppm region arose from saturated carbons of all fatty acyl chains. Resonances around 130 ppm were characteristic of unsaturated carbons. The carbonyl groups (ester and fatty acids) appeared at high field 173 ppm. The glycerol

$C_{1',3'}$, $C_{2'}$ carbons gave rise to the two peaks at 62 and 69.5 ppm respectively.
All central methylenes of fatty acid chain were magnetically similar and appeared at ∂ value of 29.5 ± 0.5 ppm. The C_3 resonance was always observed at 24.8 ppm. The introduction of a double bond in fatty acyl chain induced the 2.5 ppm shielding of external carbon in α position ,(∂ = 27.3 ± 0.1 ppm).

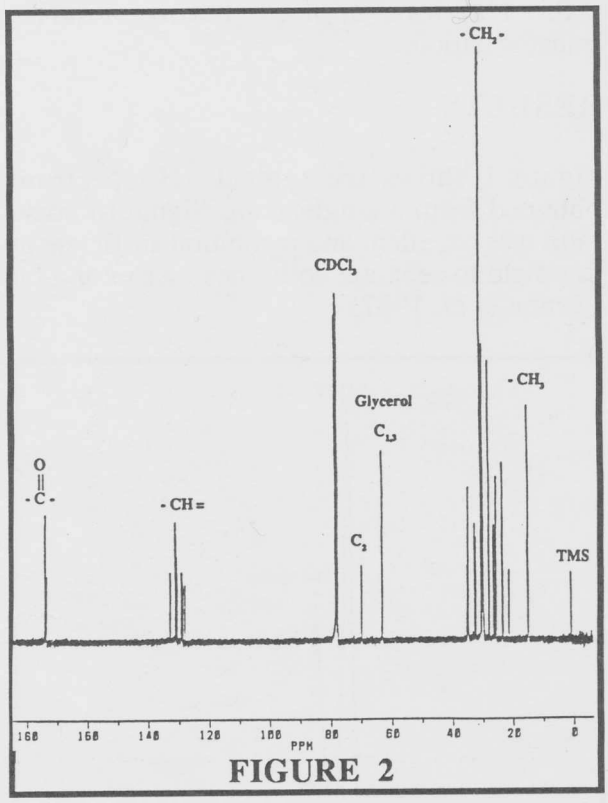

FIGURE 2

The chemical shift of central methylenes C_{11} of linoleic acid was more shielded :∂ = 25.5 ppm. (figure 3).
The internal unsatured carbons C_{10}, C_{12},of linoleic acid gave rise to a downfield signal at 128.1 ± 0.5 ppm instead of 130ppm (figure 4).

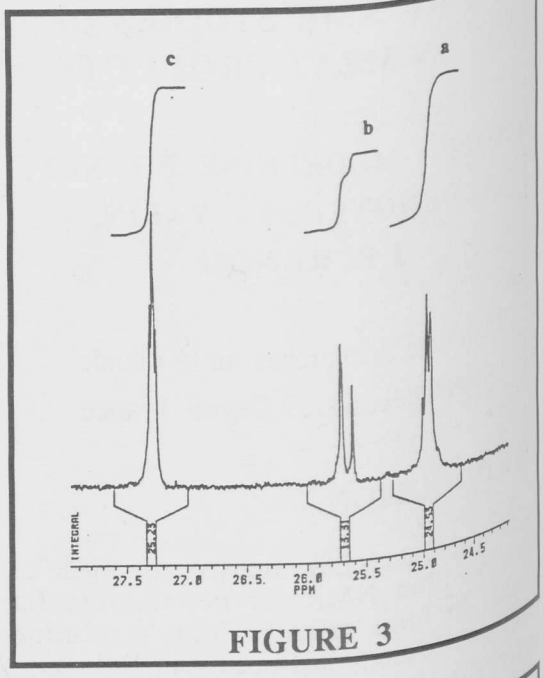

FIGURE 3

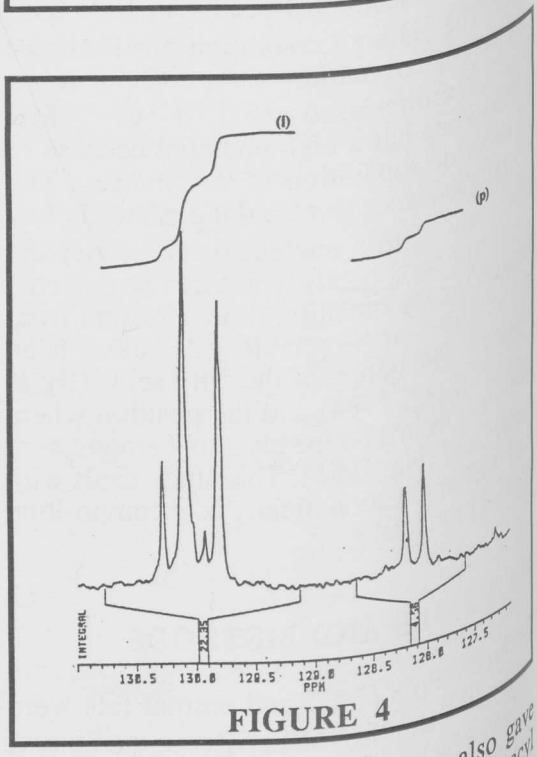

FIGURE 4

The study of carbonyl carbons also gave information on the saturation of fatty acyl chains and on its position on the glycerol carbons. The peaks in the 173.2-173.25 ppm region were assigned to carbonyl carbons in $C_{1',3'}$ position, while the peaks in the 172.8-172.85 ppm region were assigned to carbonyl carbons in $C_{2'}$ position of glycerol.

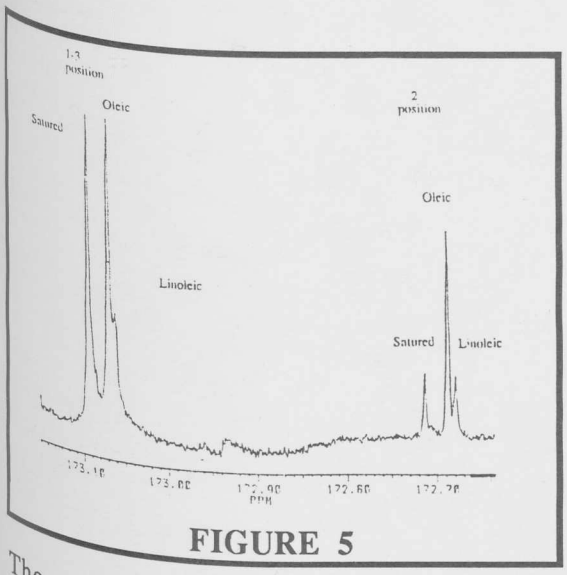

FIGURE 5

The resonance at high field 173.29 coresponded to saturated fatty acyl chains, the resonance at 173.25 and 173.24 ppm arose from mono- and poly-unsaturated carbons, respectively. The same order was obtained for internal position. The chemical shift differences were low but large enough for determination of the location of the saturated, mono- and polyunsaturated fatty acid chain on the glycerol backbone (figure 4).

The quantitative determination of the composition of animal fat in respect of the saturated, mono- and polyunsaturated fatty acid chain of the triglyceride molecules were performed by intensity measurements of characteristic carbons. The ***a*** intensity of 24.8 ppm resonance, which was assigned to C_3 of all fatty acyl chains was directly proportional to the total number of chains, the ***b*** intensity of 25.7 ppm resonance, which was assigned to C_{11} of polyunsatured fatty acyl chains corresponded to the number of polyunsatured fatty acyl chains while the ***c*** intensity of 27.3 ppm resonance which was assigned to C_8, and C_{14} of all unsatured fatty acyl chains gave two times the number of unsatured fatty acyl chains (figure 3). The ratio of the number of mono- and poly-unsatured fatty acid chains was also determined from the intensities of internal unsatured carbons of dienes at ≈128pm and external unsatured carbons at 130 ppm. The simple calculation from different integral values allowed the determination of the % of the saturated, mono- and polyunsaturated fatty acid chain.
The correlation between NMR results and CPG results was high (R=0.99) as seen in figure 6.

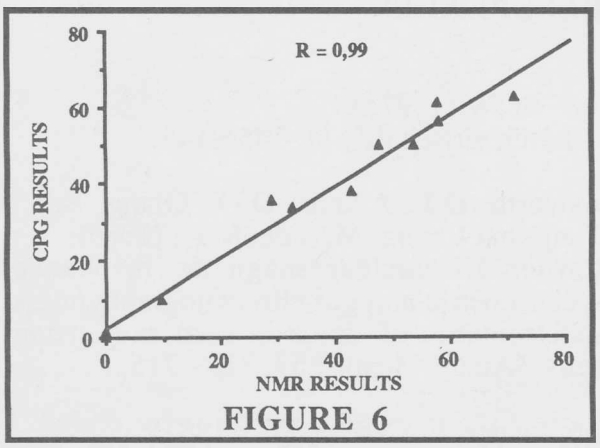

FIGURE 6

The histogram on figure 7 shows the repartition of satured mono and poly unsatured tissue on glycerol position for "foie gras" and subcutaneous fatty tissue of duck.

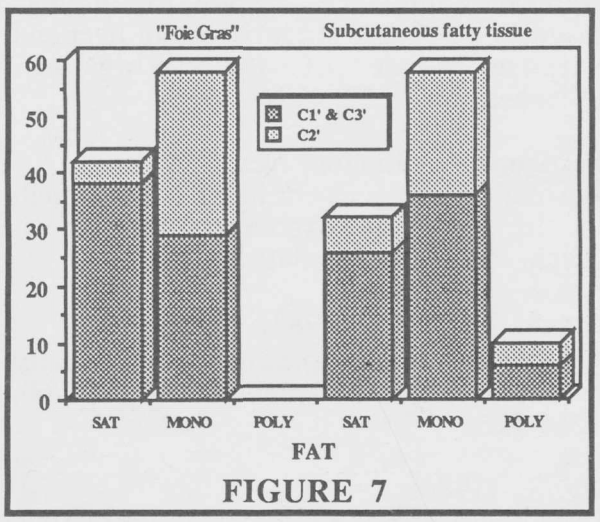

FIGURE 7

CONCLUSION

Analysis of the chemical shifts revealed no information about fatty acid chain length. The distribution of fatty acid chains on the glycerol required a very good resolution The gaussian multiplication improved resolution of overlapping resonances but the use of this function gave rise to spectral distorsion and so decreased the integral accuracy. Best results were obtained by reducing the spectral width by using the low transmitter power to obtain selective pulse. ^1H NMR method has the advantages of rapidity, sensitivity on Soxhlet method The integrals measured on ^{13}C NMR spectra agree with the CPG results. The sample preparation is less time-consuming. Moreover NMR spectroscopy gives information on glycerol substitution.

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