^{13}C NMR Spectroscopic Analysis of the Fatty Acid Composition of Palm Oil

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ABSTRACT

Gated decoupled ^{13}C NMR spectra of the saturated and olefinic carbons in palm oil can be used for direct determination of the composition of the fatty acids in mole fractions of the saturated, monoene and diene acid chains. The results are more informative than the conventional iodine value which is used as a measure of the total unsaturation in the fatty acids.

INTRODUCTION

The characteristics of oils and fats are expressed in terms of some chemical constants derived from specific reactions with the oil sample. These constants can be divided into three classes according to the nature of functional groups involved in the reactions (1): (a) acidimetric, which is based on the carboxyl or ester group; (b) enometric, which is based on the double bonds; and (c) oxidimetric, which is based on oxygen-containing groups other than the carboxyl.

In characterizing palm oil or its products, iodine value (IV), which is the number of grams of iodine that react with 100 g of oil, is the only enometric constant conventionally used. This parameter indicates the degree of unsaturation of the total amount of glycerides and free fatty acids present. Its determination using Wij's reagent (iodine monochloride in acetic acid) is carried out in a stepwise manner according to the official method (Cd 1-25) of the American Oil Chemists' Society (2). A certain amount of the sample is first allowed to react with an excess of the reagent in the dark for ca. 2 hr. The unreacted reagent is then determined by iodometric titration. This quantitative analysis is not only time-consuming but also involves a rather cumbersome preparation of the Wij's reagent which is both thermally and photolytically unstable. A method that has also been used is to calculate the iodine value (3) from the gas chromatogram of the fatty acid methyl esters obtained according to the official method (Ce 1-62) of AOCS (2). This method, however, is equally tedious in sample preparation as well as in obtaining the gas chromatogram needed for the calculation.

The increasing importance of using IV as a quality parameter in the palm oil industry has made it desirable to seek a simple physical method that can provide speedy, sensitive and reproducible evaluation of IV or its equivalent.

In this paper we discuss the measurement by the ^{13}C nuclear magnetic resonance (NMR) technique of the composition of palm oil in respect of the saturated, monoene and diene fatty acid chains of the triglyceride molecules. This spectroscopic technique offers a more direct and more informative measure of the olefinic content of palm oil in mole fractions of the fatty acids.

EXPERIMENTAL

The ^{13}C NMR spectra were recorded with a JEOL JMN FX100 high resolution Fourier transform spectrometer operating at 25.05 MHz. The gated decoupling pulse sequence (suppressed nuclear Overhauser effect) was used with the following parameters: data memory 8 k words, spectral width 600 Hz, acquisition time 1.066 sec, pulse delay 9.0 sec and pulse angle 45°. The spectrum in Figure 1 was obtained with 400 free-induction-decay (FID) accumulations for sample concentration of 1:3 v/v. It gives a precision of ±0.5% for the peak area analysis described in the text. However with a slightly more concentrated solution (e.g., 1:2 v/v) a usable spectrum can be obtained in one-fourth of the experimental time with a precision of less than ±1.0% for the peak area analysis.

For the spin-lattice relaxation time T_1 measurements the samples were sealed in 10 mm tubes with chloroform-*d* as solvent and were degassed through five freeze-pump-thaw cycles. The inversion-recovery method was used and all necessary precautions were taken (4).

The triglycerides (triolein and tripalmitin) were obtained from Sigma Chemical Co. (St. Louis, MO). The corn oil was a commercial sample. The palm oil was obtained from a palm oil mill and was homogenized before use.

RESULTS AND DISCUSSION

Assignment of the peaks in the ^{13}C NMR spectra of the glycerides of various fatty acids has been established by Shoolery (5). Figure 1 shows the spectrum of the saturated carbons of the fatty acid chains of a sample of crude palm oil. The peak at δ 24.8 ± 0.2 belongs to C_3 of all of the chains, whether fully saturated or containing one or two double bonds. The peak at δ 25.7 ± 0.2 belongs to C_{11} which is allylic to both double bonds of a *cis-cis* diene (linoleic) chain. The peak at δ 27.3 ± 0.2 belongs to the two carbons allylic to *cis* double bonds, i.e., $C_{8,11}$ of the monoene (oleic) chain and $C_{8,14}$ of the diene chain. As Figure 1 shows, natural palm oil is composed of the triglycerides of saturated (mainly palmitic (C16:0) and to a small extent stearic (C18:0), oleic (C18:1) and linoleic (C18:2) fatty acids, and the presence of the triene chain, if any, is not detected. Normally in crude palm oil, as a result of hydrolysis, small, but variable, quantities of the free fatty acids, mono- and diglycerides can be found. The corresponding carbons in the free fatty acids and the mono- and diglycerides are found to have the same δ values as in the triglycerides, so that the peak at δ 24.8 represents the total number of saturated, monoene and diene chains in the palm oil, that at δ 25.7 represents the total number of diene chains, while that at δ 27.3 represents twice the total number of monoene and diene chains. The areas of these three peaks therefore permit quantitative analysis of the proportion (mole fraction) of saturated, monoene and diene chains in a sample of palm oil. Electronic integration of these peaks is facilitated by the proximity of the peaks.

The ratio of the number of monoene to diene fatty acid chains can be determined from the spectrum of the olefinic carbons. In Figure 2 the pair of peaks at δ 129.9 and δ 129.6 belong to C_{10} and C_9 of the oleic chain, while the two smaller pairs at δ 130.1, 129.9, 128.1 and 127.9 belong to C_{13}, C_9, C_{10} and C_{12} of the linoleic chain. (Specific assignments of these peaks are attempted on the basis of spin-lattice relaxation times T_1 (see below).) In Figure 2 are shown the integration values t and ℓ and the ratio of monoene to diene chains is given by (t − ℓ):ℓ.

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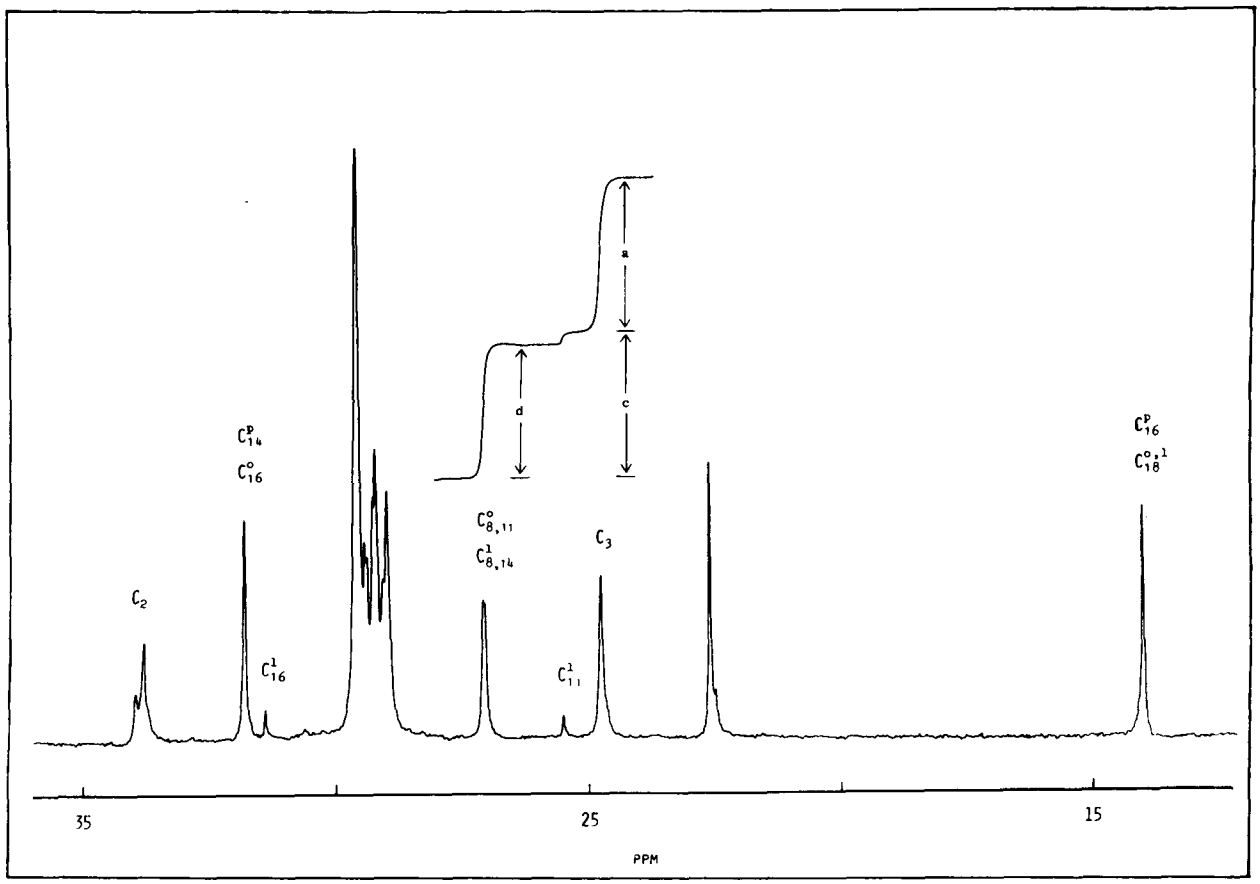

FIG. 1. Gated decoupled ^{13}C NMR spectrum of the saturated carbons of the fatty acid chains in palm oil with integration of three peaks. The integral value d is for peak at δ 27.3, the value c is for the sum of the two peaks at δ 27.3 and δ 25.7, and the value a is for the peak at δ 24.8. In the assignment of the peaks, the superscripts of symbol C are defined as follows: p for palmitic, o for oleic and l for linoleic; where no superscript appears the peak represents the specified carbon in all the fatty acid chains.

The calculation of the mole fractions of the fatty acid chains follows from the integration of the three peaks in Figure 1.

Total number of chains $\propto$ a

Number of olefinic chains $\propto \frac{1}{2}$d

Number of diene chains $\propto$ (c − d)

Mole fraction (mf) of saturated chains = $(a - \frac{1}{2}d)/a$

mf of diene chains = (c − d)/a

mf of monoene chains = $[\frac{1}{2}d - (c - d)]/a$

For the sample of palm oil shown in Figure 1, a = 44.5, c = 43.5 and d = 39.5, giving the following mole fractions of fatty acid chains: saturated = 0.560 ± 0.005, monoene = 0.350 ± 0.005, and diene = 0.090 ± 0.005. If the average molecular weight of all species present in the palm oil is known, the corresponding iodine value can be calculated from the above data. The data obtained for this one sample of crude palm oil by ^{13}C NMR analysis may be compared with the data obtained by gas chromatographic analysis of the fatty acid methyl esters for a large number of samples of crude palm oil (6): saturated fatty acids 47.1–54.7%, oleic acid 37.3–40.8% and linoleic acid 9.1–11.0%.

If the intensity of the δ 25.7 peak in Figure 1 is too low for a reliable integral value, the integrals in Figure 2 should preferably be used in the above calculation, as the higher field pair of peaks of the linoleic chain arises from two olefinic carbons. The calculation becomes:

mf diene chains = $(\frac{1}{2}d/a)(\ell/t)$

mf monoene chains = $(\frac{1}{2}d/a)[(t - \ell)/t]$

For quantitative analyses, the ^{13}C spectra are acquired using gated decoupling pulse sequence (suppressed nuclear Overhauser enhancement) in which the pulse-repetition time should be at least $5T_1$ and preferably $10T_1$ times the longest T_1 of the carbons concerned. Table I shows the T_1 data for various triglycerides which can serve as guide in setting the parameters for spectra accumulation. If a relaxation agent is used, the T_1 values would be reduced con-

TABLE I

Spin-Lattice Relaxation Times T_1[a] of Selected Carbons of Triglycerides at 28 C in $CdCl_3$ Solution

		δ/ppm								
					Oleic		Linoleic			
Triglyceride	Conc	24.8 C_3	25.7 C_{11}	27.3[b] $C_{8,11}$ $C_{8,14}$	129.9[c] C_{10}	129.6 C_9	130.1 C_{13}	129.9[c] C_9	128.1 C_{10}	127.9 C_{12}
Palm oil	1:3 v/v[d]	0.58		(1.2, 0.9)	(1.5)	1.4				
Triolein	5 mol %	0.55		(1.1, 0.9)	1.31	1.22				
Tripalmitin	5 mol %	0.61								
Corn oil	1.3 v/v	0.61	2.0	1.4	(1.7)	1.7	2.8	(1.8)	1.8	2.7

[a]Error estimated at ± 3%, except for values in parentheses for which error > ± 3%.

[b]Incompletely resolved pair, Δδ = 0.054 ppm.

[c]Peaks overlap.

[d]v/v refers to volume by volume, with solvent the larger component.

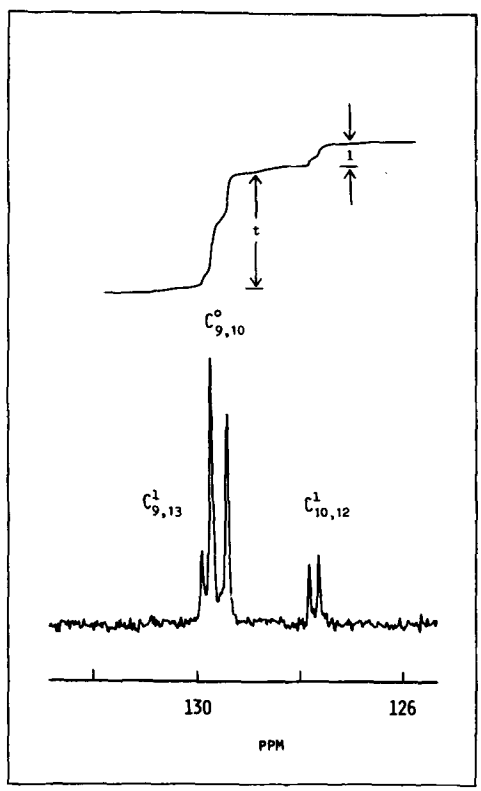

FIG. 2. Gated decoupled ^{13}C NMR spectrum of the olefinic carbons in palm oil with integration of the peaks. The integral value l is for the pair of peaks at δ 128.1 and δ 127.9, and the value t is for the sum of the pair of (oleic) peaks at δ 129.9 and δ 129.6 and the pair of (linoleic) peaks at δ 130.1 and δ 129.9. For definition of superscripts of symbol C for peak assignment see Fig. 1. For specific assignment of the peaks see Table I.

siderably (7). Indeed with Cr (III) acetylacetonate (0.04 M) as the relaxation reagent the T_1 value for C_{11} of the linoleic acid chain is reduced by one-half to below 1.0 sec in $CdCl_3$ solution (sample concentration 1:3 v/v) at 28 C, and the T_1 values of the other carbons concerned are considerably smaller. Consequently, the experimental time for spectrum acquisition is reduced considerably when the relaxation reagent is used.

The olefinic carbons are found to have slightly different but distinguishable T_1 values. The olefinic carbon which has the more mobile portion of the chain attached to it is assigned the longer T_1; hence the assignment shown in Table I.

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