Diffusion Properties of Lipids — Pure and in Seeds

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ABSTRACT AND SUMMARY

Diffusion constants of some fatty acids and triglycerides in pure form and also in seeds have been measured at different temperatures in order to elucidate the motion and organization of triglyceride molecules in oils. The size of the oil storage cavity (droplet) in seeds has been estimated to be 0.8 μ. Temperature dependences of the proton T_1 relaxation time in some samples have been measured by pulsed nuclear magnetic resonance (NMR). From these measurements it is concluded that since activation energies for the relaxation mechanism, diffusion, is the same in seeds as in pure oils, 0.22 eV, the nature of the motion is similar.

INTRODUCTION

Recent nuclear magnetic resonance (NMR) studies (S. Ratkovic and L. Ehrenberg, private communications) of lipids (oil) in samples of plant origin, such as seeds, have shown that in these systems the relaxation times T_1 and T_2 are nonexponential. They usually consist of two, or maybe more, components. Reasons for this are not completely understood. Usually it is argued that nonexponential T_2 relaxation times appear because part of the oil molecules interact with the solid matrix of the seed, and therefore their T_2 relaxation time is shortened due to exchange. On the other hand, measurements on sunflower oil suggest that nonexponential behavior of T_1 relaxation time in oils may be due to different intramolecular and intermolecular contributions to the relaxation. Intermolecular contribution should be highly dependent on the motion and organization of the triglyceride molecules in oil. These would also be reflected in diffusion measurements. We have undertaken this NMR study of diffusion properties of oils and fatty acids with the hope of:

1. determiming the basic diffusion properties;
2. correlating this information, and the activation energy extracted from the diffusion constant D vs. temperature dependence, with the proton relaxation behavior, and the activation energy extracted from the T_1 vs. temperature dependence;
3. using the decrease of D in the sequence: pure fatty acids - pure triglycerides - oils in seeds, to study the nature of storage of oils in seeds;
4. estimating the size of the oil droplet (storage cavity) in seeds from the conditions of restricted diffusion;
5. determining whether the dependence of D on the saturation factor (f = unsaturated fats/saturated) is sufficiently strong to be used as a nondestructive method for oil "quality" determination in single seeds.

EXPERIMENTAL PROCEDURES

The diffusion measurements were made using the attenuation effect of a pulsed magnetic field gradient on the proton NMR spin-echo (1). In case of constant field gradient being negligible compared to the pulsed gradient, the ratio of the spin echo amplitudes, with and without the field gradient present, is given by

$$\frac{A}{A_0} = \exp[-\gamma^2 g^2 D^2 (\Delta - \delta/3)]$$

where g = field gradient in Gauss/cm (190 Gauss/cm); δ = gradient pulse width; Δ = delay between gradient pulses; γ = proton gyromagnetic ratio; and D = diffusion constant in cm^2/sec.

The onset of restricted diffusion (2) at longer diffusion times is observed as an initially linear decrease in $\ln A/A_0$ (the slope of which gives the diffusion constant) followed by an asymptotic approach to a constant attenuation effect; for $\delta << \Delta$ and free diffusion within a cavity of

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TABLE I

Diffusion Constants of Lipids, Pure and in Single Seeds, at Various Temperatures

Sample	D $[10^{-8}\ cm^2/sec]$ T = 24 C	45 C	60 C	75 C
Octanol (standard)	140			
Linoleic acid	36			
Oleic acid	26			
Trilinolein	15		38	
Triolein	9		29	
89% Trilinolein + 11% tripal			34	
1–Oleodipalmitiol			11.2	
2–Oleodipalmitiol			8.6	14
Groundnut seed # 1				12.6
Groundnut seed # 2	4.8	7		
Groundnut seed # 3	5.0	7		
Single corn seed	3.5			
Four corn embryos	2.6			
Sunflower seed # 1	6.0			
Sunflower seed # 2	6.2			
Sunflower seed # 3	6.0			
Sunflower seed # 4	6.0			
Sunflower seed # 5	4.4			
Sunflower seed # 6	5.0			

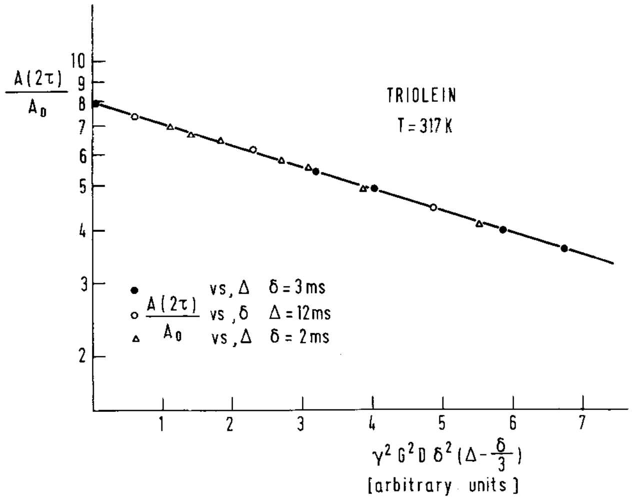

FIG. 1. Dependence of the spin-echo signal amplitude ratio $A(2\tau)/A_0$ on $\delta^2(\Delta - \delta/3)$ at constant δ (full circles and triangles) and constant Δ (open circles), in triolein at 317 K.

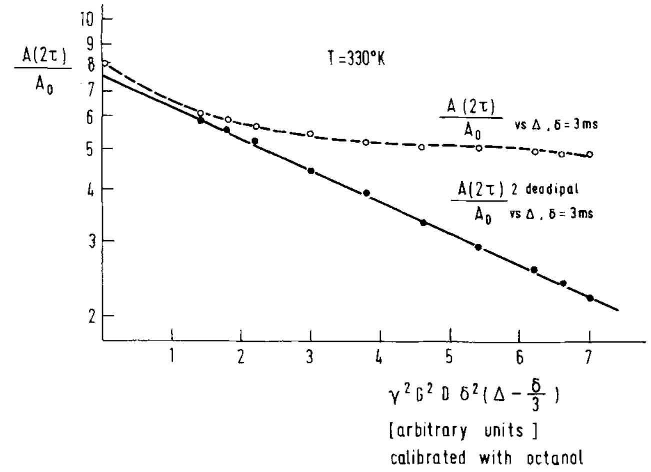

FIG. 2. Restricted diffusion of oil in groundnut (open circles) at 330 K, compared to diffusion in 2-oleodipalmitiol (full circles).

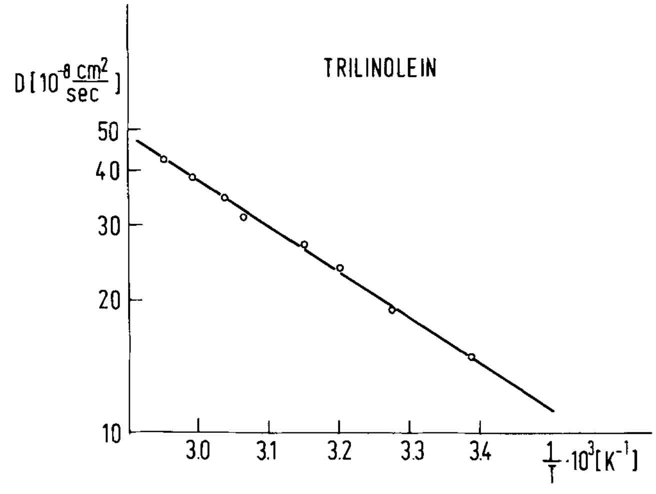

FIG. 3. Temperature dependence of the diffusion constant of trilinoleic oil. E_a = 0.20 eV.

TABLE II

Proton T_1 Relaxation Times and Activation Energies of Oil in Intact Seeds of Sunflower, Corn, Groundnut, as Well as Triolein, and Linoleic Acid, at 21 C and NMR Frequencies 32 MHz and 16 MHz

	T = 21 C		
Sample	T_1 [msec] ν = 32 MHz	T_1 [msec] ν = 16 MHz	E_a [eV]
Sunflower	110	90	0.22
Corn	150	150	0.20
Groundnut	100	80	0.22
Triolein	190	130	0.25
Linoleic acid	150	140	0.25

radius ρ, the asymptotic value is given by

$$\lim \ln A/A_0 = -\frac{1}{5}(\gamma\delta g\rho)^2$$

In the limiting case, ρ is the mean free path traveled by molecules during the diffusion time Δ, and is given by

$$\rho = \sqrt{2D\Delta}.$$

RESULTS AND DISCUSSION

The results for the determination of the free diffusion constants for fatty acids, triglycerides, and oils in seeds are shown in Table I. Note that the important saturated fatty acids, whose relative presence in oils is wished to be measured, are absent as these are solids in the temperature range investigated. The results presented in Table I have an estimated error of 10%, and are based on repeated measurements, holding either δ or Δ constant. The attenuation of spin-echo amplitudes vs. δ^2 $(\Delta - \delta/3)$ follows a single curve in both types of experiments, as shown on Figure 1. Typically, δ = 1-5 ms; Δ = 6-40 ms; and τ = 10-25 ms.

The well-known increase in viscosity of lipids with increased saturation and chain length (3) is mirrored in their decreased diffusion constant. An example is the decrease from $D = 36\cdot10^{-8}$ cm²/s to $D = 26\cdot10^{-8}$ cm²/s for linoleic acid C(18:2) and oleic acid C(18:1), respectively, or the corresponding decrease in their respective triglycerides, trilinoleic $D = 15\cdot10^{-8}$ cm²/s and trioleic $D = 9\cdot10^{-8}$ cm²/s at 24 C. In each case the attachment to glycerol reduces the diffusion constant of free fatty acid by a factor of two to three.

It is interesting to compare the diffusion constants of two synthetic triglycerides, 1-oleodipalmitiol and 2-oleodipalmitiol. They differ only in the position of the oleic acid on the glycerol, but their diffusion constants differ markedly, $11.2\cdot10^{-8}$ cm²/s and $8.6\cdot10^{-8}$ cm²/s, respectively.

The diffusion constants of oils in samples of groundnut, sunflower, and corn seeds are also shown in Table I. Note that diffusion rates for individual sunflower seeds can vary by over 20%. Diffusion rates for all seeds tested, typically from 4 to $7\cdot10^{-8}$ cm²/s, are sufficiently low, when compared to those of pure oils, to consider the involvement of restricted diffusion. To check this, the spin-echo attenuation vs. diffusion time was measured. Due to the weak single seed signals, the relatively large oil cavity size, and slow diffusion rate, conclusive proof could not be observed at room temperature. However, at increased temperature, the increased diffusion and relaxation rates made measurements at longer diffusion times possible. The results for a single groundnut seed at 75 C are shown in Figure 2, together with 2-oleodipalmitiol, having a comparable free diffusion rate, for comparison. The onset of restricted diffusion in groundnut is clearly observed, although the asymptotic value is not quite reached. The oil cavity (droplet) radius ρ is calculated from these measurements to be 0.8 μ. This is in the upper range of values given by Ratković (S. Ratković and L. Ehrenberg, private communications), 1 mμ-1 μ. Essentially the same value of ρ has also been found in sunflower.

The temperature dependence of the diffusion constant was measured to determine the activation energy E_a for the diffusion process where,

$$D \propto \exp(-E_a/kT).$$

The results for trilinoleic oil over the temperature range of T = 24-67 C are shown in Figure 3. The value of E_a is 0.20 eV.

We have measured temperature dependences of the proton T_1 relaxation time of oil in intact seeds of sunflower, corn, and groundnut, as well as in triolein and linoleic acid. From these measurements activation energies E_a for the relaxation mechanism, i.e., diffusion, were calculated and are presented in Table II, together with T_1 relaxation times of oil protons in the above samples, at 21 C. The measurements were performed at two NMR frequencies, 32 MHz and 16 MHz, and though the relaxation times of a certain sample, measured at the two different frequencies, do differ somewhat, the activation energies are the same in both cases. This is not an unexpected result, assuming a diffusion correlation time τ_c which is short compared to the inverse NMR frequency,

$1/\nu_{NMR}$.

Virtually the same activation energies in all samples measured support the argument that the nature of diffusion is similar in all cases studies. This is further indicated by the value of the activation energy, $E_a = 0.20$ eV, in trilinoleic oil, as determined by the diffusion measurements.

By adopting a model of diffusion in liquids, described in detail in the book of Abragam (4), a diffusion correlation time τ_c can be extracted from our data, yielding a value of ca. $2 \cdot 10^{-9}$ sec. From the well-known relation between the length d of the "diffusion jump," the diffusion constant D, and the diffusion correlation time τ_c,

$$d = (6D\tau_c)^{1/2},$$

the value of d is calculated to be 5Å. It is gratifying to note that, despite the crudeness of the model and the somewhat arbitrary choice of the value of interproton distance in a lipid molecule, as required by the model, the value obtained for d is of the same order of magnitude as the diameter of the lipid molecule. Thus, consistency of our measurements of the temperature dependences of the proton T_1 relaxation times of oil in seeds and its diffusion constants is established.

Measurements extending the present study of diffusion properties of lipids to further samples are in progress. Also, attempts to increase the accuracy of the measurements are being made.

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