Seed Proton NMR Spin-Grouping

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Proton spin grouping in the rotating frame allows for a distinction between oil, water and starch protons with a resolution which exceeds the one with the standard proton T_1 or T_2 oil and water separation experiments. Hence, it has clear advantages in plant breeding programs in those situations where, because of the relatively high oil and water content, the standard NMR technique fails unless the seeds are dried artificially. This technique is just as fast as the standard technique.

Nuclear magnetic resonance is a quick, nondestructive technique for determining the oil and moisture content of seeds and, as such, has become a valuable tool in plant breeding programs and in the determination of seed viability (1-5). Although some NMR studies have used spin-lattice relaxation (5,6), the majority have focused on the spin-spin relaxation characterization of the seeds. Recently, a two-dimensional time evolution method capable of improving the resolution of a heterogeneous NMR free induction decay (FID) has been introduced (7). This technique, called spin-grouping, resolves the FID by correlating the spin-lattice relaxation of the magnetization (characterized by the times T_1 or $T_{1\varrho}$) to the NMR FID shape characterized by the spin-spin relaxation times T_2. In this note, the first application of spin-grouping to sunflower and canola seeds is reported.

The NMR method for determining the oil and moisture content of seeds traditionally has been based (1-5) on the differences in the protonic spin-spin (T_2) relaxation times of the various hydrogen-containing constituents due to the different degrees of mobility. Typically:

$$(T_2)_{solid} \ll (T_2)_{moisture} \leqslant (T_2)_{oil}$$

where $(T_2)_{solid}$ is in the microsecond and $(T_2)_{moisture}$ and $(T_2)_{oil}$ in the millisecond range. The FID signal, G(t), following a 90° pulse, thus typically consists of a rapidly decaying "solid" component (A), and a much more slowly decaying "liquid" component (8). The A signal is proportional to the total number of hydrogen nuclei in the sample, i.e. solid plus liquid, whereas the B signal is proportional to the "liquid" content only. Under higher resolution the "liquid" signal itself can be decomposed into two components, a water and an oil one. In view of the heterogeneity of the sample, even this is only a rough approximation because not all oil and water environments are identical.

In order to improve the resolution of the NMR experiment for heterogeneous samples the spin grouping (lineshape-relaxation correlation) technique has been developed (7,8) in which each group of spins is assignated a unique set of spin-spin (T_2) and spin-lattice (T_1) relaxation times. The technique is based on the observation of the magnetization recovery at a number of different time windows along the axis of the sample's FID. The heterogeneous FID is thus T_1 resolved into several FID components. Each of these FIDs may then be resolved according to its T_2's into magnetization components $M_{o,i}$. These are measures of the relative percentages of protons (characterized with time constants $T_{1,i}$ and $T_{2,i}$) which form a particular "i" group. It should be noted that if the T_1's of protons in two different environments are the same they may be further T_2 resolved if their T_2's differ substitutionally. If their T_2's and their T_1's are the same, then they belong to the same spin group.

Instead of the T_1-T_2 spin grouping the $T_{1\varrho}$-T_2 spin grouping can be used in some situations. In this kind of spin grouping of protons their different relaxation times in the rotating frame are being used for resolution, in complete analogy to the T_1 spin grouping (8).

EXPERIMENTAL

The seeds were maintained at room temperature in a desiccator under a 100% relative humidity atmosphere for one month. The seeds were then flame sealed in 7 mm o.d. glass tubes. The NMR experiments were performed on a Bruker SXP spectrometer which was interfaced to a Hewlett-Packard 9845A computer via a Biomation model 805 sample and hold device. All data analysis was done on the HP 9845A computer. The experiments were performed at 38 MHz. The T_1's spin-grouping utilized the $\pi/2$-τ-$\pi/2$ pulse sequence; the $T_{1\varrho}$'s were measured with the spin-locking pulse sequence (9) ($\pi/2$-field pulse of duration τ) with a 10 G field pulse. In a T_1 experiment each magnetization evolution was recorded at 33 time windows along the FID for 33 values of the delay τ between the $\pi/2$ pulses. The first window was set at 13 μs to avoid the deadtime of the receiver circuit. The FID's of each sample were also recorded subsequent to the first $\pi/2$ pulse and characterized in terms of their T_2's. Due to the inhomogeneity of the external magnetic field, components of the FID's with T_2's greater than $\sim$ 2 ms were not resolved. The symbol $T_2^{\dagger}$ is used in such cases to indicate that macroscopic field inhomogeneity is causing the loss of spin coherence.

RESULTS AND DISCUSSION

The results of the spin-grouping and FID characterization of the proton magnetization of sunflower and canola seeds are presented in Table 1. The results of the T_1 spin-grouping of the sunflowerseed are illustrated in Figure 1. Two components of the seed magnetization are resolved by the FID analysis; one which decays with T_2 characteristic of a rigid solid ($T_2 \sim 14\mu s$)

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TABLE 1

The Results of the Proton FID Analysis and Proton Spin-Grouping of Sunflower and Canola Seeds

Experiment	Sunflower			Canola		
	T_1 or $T_{1\varrho}$ (ms)	T_2 (μs)	M_{oi}[b] (%)	T_1 or $T_{1\varrho}$ (ms)	T_2 (μs)	M_{oi}[b] (%)
FID		≥ 2000[a]	56±4		≥ 2000[a]	59±4
		13+2	44±3		14±2	41±3
T_1	317+20	≥ 2000[a]	28±2	285±20	≥ 2000[a]	59±4
		19+2	15±2		15+2	20±2
Spin-Grouping	97+7	≥ 2000[a]	31±2	88+6	≥ 2000[a]	35±1
		13+2	27±3		16+2	19±2
$T_{1\varrho}$	198+4	≥ 2000[a]	27±3	181+4	≥ 2000[a]	29±2
Spin-Grouping	50+3	$\geq 2000a$	24±2	56+2	≥ 2000[a]	22±2
	4+1	590+50	3±1	7+1	390+40	5±1
		16+2	64±4		13+2	42±2

[a] T_2^+ of 2 ms is the effective coherence loss time due to the macroscopic magnetic field inhomogeneity across the sample. It varies from one experiment to the next and if either the sample size or its position in the magnet are varied.

[b] The magnetization fraction M_{oi} represents the relative number of protons which constitute a particular proton spin group "i".

and one which decays with T_2 characteristic of a liquid, T_2^+. The large range of values quoted for the solid T_2 reflects the uncertainty resulting from the relatively long receiver deadtime of 9 μs. At 38 MHz the spin-lattice relaxation of both the sunflower and canola seeds is characterized by two T_1's of about 300 and 90 ms. Since the two T_1's differ by less than a factor of four, these times are to be considered a measure of the relaxation distribution rather than identifiable T_1's. The magnetization components corresponding to each T_1 are characterized by both solid-like and liquid-like FID's (Table 1). The spin-lattice relaxation in the rotating frame of both seeds is characterized by three $T_{1\varrho}$'s of about 190 and 50 ms have liquid-like FID's characterized with T_2^+. The solid-like magnetization of the sunflower and canola seeds have T_1's of 2 and 7 ms, respectively. Here it should be noted that a small component with T_2 of 590 and 390 μs, respectively, for sunflower and canola is also observed. Although this magnetization component is only 3% and 5%, respectively, of the total magnetization, it is a clear indication that water protons are exchanging with the solid protons. In this process the two reservoirs in contact have their relaxation rates as well as their relative magnetizations altered. Thus care has to be taken not to make conclusions on the basis of these apparent parameters.

In all experiments, the solid component of the seed's proton magnetization contributes 44±2% and 41±2% of the total magnetization of the sunflower and canola seeds, respectively. Therefore, less than half of the protons are in a truly rigid lattice (primarily starch) spin group. However, since the spin density of the primarily starch group is about half that of oil or water ($\sim$ 0.06 ^{1}H/unit molecular weight versus $\sim$ 0.1 ^{1}H/unit molecular weight, respectively), the primarily starch group contributes the largest weight fraction of the seeds. The remainder of the protons in seeds are on water or the oil molecules which can undergo reorientation and tumbling motions and, thus, are characterized by liquid-like or semiliquid-like T_2's. In our experimental set-up, these T_2's are not resolved because the magnetic field is not very homogeneous. Its field gradient determines T_2^+ to be in the 2 ms range.

The distribution of the magnetization components which appears to be represented by two groups, characterized with T_1's of 300 and 90 ms each, is attributed to water and oil. The water and oil contribute to both components. Thus, at 38 MHz with T_1 experiment the resolution is not satisfactory.

The $T_{1\varrho}$ spin-grouping, however, does resolve the FID's of the seeds into three components. The magnetization characterized by the short T_2 decays quickly in the rotating frame with $T_{1\varrho}$ of 4 and 7 ms for the sunflower and canola seeds, respectively, confirming that the protons in the primarily starch groups are in a rigid environment. The short $T_{1\varrho}$ also indicates, as does the T_2, that the thermally activated stocastic motion is slow. In the solid network the molecular correlation frequency ω_c is smaller than the dipolar width ω (dipolar) at room temperature. The magnetization components which decay with the $T_{1\varrho}$'s of about 190 and 50 ms correspond to protons in nonrigid environments. The magnetization decaying with the shorter $T_{1\varrho}$ is identified with the water protons and the magnetization component having $T_{1\varrho}$ of $\sim$ 190 ms with the oil spin group. This resolution of the liquid-like magnetization

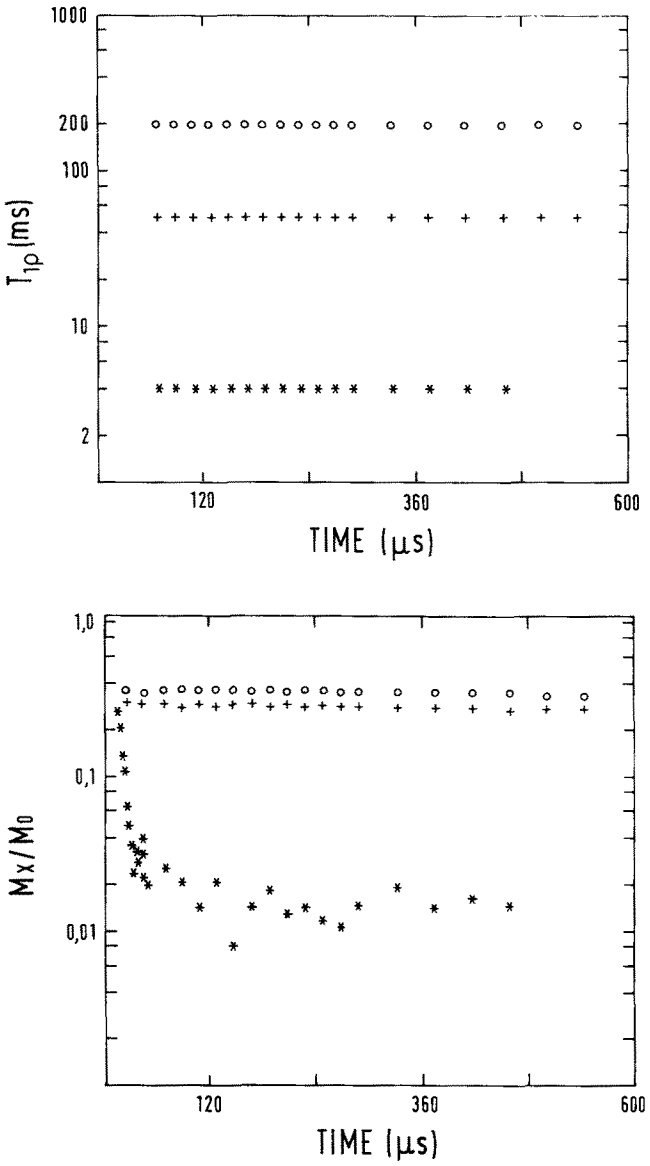

FIG. 1. The results of the rotating frame spin-grouping of sunflower seeds: upper part, A, the $T_{1\varrho}$'s averaged over all time windows on the free induction decay. Three $T_{1\varrho}$'s (198±14, 50±3 and 4±1 ms) characterize the recovery of proton magnetization in the rotating frame. Lower part, B, the FID's corresponding to the three $T_{1\varrho}$'s shown in Fig. 1A. The FID's corresponding to the $T_{1\varrho}$'s of 198 and 50 ms are characterized by $T_2 = T_2^+$, the FID of the $T_{1\varrho}$ = 590 and 14 μs. Here Mx stands for the instantaneous value of the transverse proton magnetization at time t and Mo for the relative number of protons in a given spin group. Mx/Mo thus represents the FID signal.

into two components is not possible with the FID analysis alone, nor with the T_1 experiment.

The results at high fields, with FID's corresponding to the two T_1's components having both solid-like and liquid-like T_2's, indicate that some of the protons on the starch are coupled strongly to the water spin-group while the rest may be strongly coupled with the oil protons. That is, there is a magnetization transfer among the various spin groups constituting the seeds.

NMR spin grouping experiments on other seeds have been completed, also. It should be stressed that the NMR spin-grouping method gives results identical to the standard proton T_1 or T_2 oil and water separation technique wherever the standard technique can be applied.

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