

SHORT COMMUNICATION

Determination of Oil, Starch, and Protein Content of Viable Intact Seeds by Carbon-13 Nuclear Magnetic Resonance

ABSTRACT

Dipolar-decoupled natural abundance ^{13}C NMR spectra of some intact seeds have been obtained at 22.6 MHz. The spectra display reasonably well resolved resonance lines for rigid materials, such as the starch and protein components of the seeds, in addition to well resolved lines for the mobile oil component, and, hence, should be useful in rapid nondestructive analyses of total composition.

INTRODUCTION

We recently have shown the practicality of the determination of the oil composition of intact oilseeds by ^{13}C NMR (1). Essentially, high-resolution spectra of the mobile oil fraction are obtained by standard proton-decoupled

Fourier transform NMR techniques. Under these standard conditions, the resonances of the immobilized, or rigid, carbohydrate and protein components of the seeds are not observed. This is due to the substantial dipolar broadening of the ^{13}C lines of the rigid components by directly bonded or nearby protons. The resonances of the rigid components in the solid state are simply too broad to be observed in a standard experiment.

METHOD

Static dipolar broadening can be removed by strong proton decoupling (2,3) which we refer to here as dipolar decoupling. Dipolar decoupling involves applying a decoupling field at the proton radio frequency (rf) comparable in strength to the ^1H linewidth. For the rigid protein and carbohydrate components of most seeds, dipolar decoupling requires a field strength of ca. 10 gauss. In practice, this means that dipolar decoupling requires ca. 100 times as much power as is used in standard scalar decoupling. The dipolar decoupling removes static dipolar interactions in the same way conventional weaker decoupling removes weaker scalar interactions by rapid rf stirring of the protons (2). The principle of the dipolar decoupling experiment is straightforward. The difficulty lies in the experimental problem of detecting the weak natural abundance ^{13}C NMR signal in the presence of the very large rf decoupling field. This difficulty has been overcome, however, by the proper transmitter and receiver coil design along with the use of the appropriate rf filters and traps (3).

RESULTS AND DISCUSSION

Examples of dipolar-decoupled spectra of some common seeds, along with some other materials of biological interest, are shown in Figure 1. The sharp lines in the spectra of the seeds, especially obvious in the spectrum of the soybean, are due to the oil fraction. (Actually, the resolution of the oil lines has been intentionally degraded somewhat for purposes of presentation relative to some of the broader lines.) The resonances due to the starch and proteins in the seeds can be identified by comparison with the spectra of various isolated materials. The relatively broad line in the center of the seed spectra is due to starch and other immobilized carbohydrates, while the presence of the protein in the seeds is evident in a broad resonance beneath the high-field oil line. (Compare, for example, the broad resonance in the high-field region of the spectrum of the relatively protein-rich, oil-deficient wheat compared to that of corn.)

Detection of the protein content of oil-rich seeds, such as soybean, under the conditions employed in these experiments, is made difficult by the intense oil resonances. We presently are working on mechanical spinning techniques to sharpen the carbohydrate and protein resonances in a dipolar decoupling experiment, so that oil, carbohydrate, and protein content of any kind of seed, regardless of

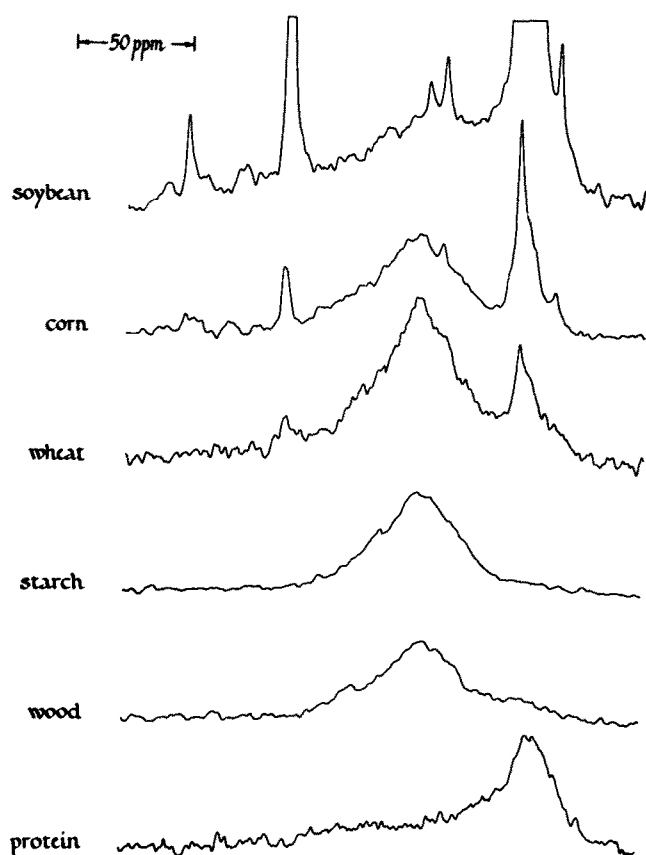


FIG. 1. Dipolar-decoupled, 22.6-MHz ^{13}C NMR spectra of some seeds, starch, wood, and a protein (human serum albumin). Resonances of those immobilized carbons having a substantial chemical shift anisotropy, such as olefinic and carbonyl carbons, are too broad to be observed, at least under the conditions of these experiments. Thus, the dipolar-decoupled spectra of many solid materials, for example, are similar and consist mainly of broad methylene- and methyl-carbon resonances. The ^{13}C pulse repetition period for all spectra was 4 sec. The magnetic field increases from left to right.

total composition, can be determined by a nondestructive ^{13}C NMR analysis. We feel this is a promising approach, since a major source of residual line broadening in the dipolar-decoupled seed spectra is due to what is called chemical shift anisotropy (3). This broadening is especially pronounced for asymmetrically substituted carbons but has been shown both theoretically and in practice (4) to be subject to removal by high speed, mechanical sample spinning.

Nevertheless, for a wide variety of seeds, including some oilseeds such as corn, it is possible by spectral decomposition (1) of dipolar-decoupled spectra, such as those shown in Figure 1, to obtain at least a semiquantitative, i.e. $\pm 10\%$, determination of the overall composition of the seed. Since the determination is nondestructive and since suitably chosen coil dimensions can be shown to provide sufficient sensitivity and accuracy to permit rapid analyses of single

seeds (1), this technique appears to be suitable for the selection of seeds for breeding programs.

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[Received August 9, 1974]