both surface and subsurface Sr^{90} in the Atlantic contrasted with data from other oceans (*13*).

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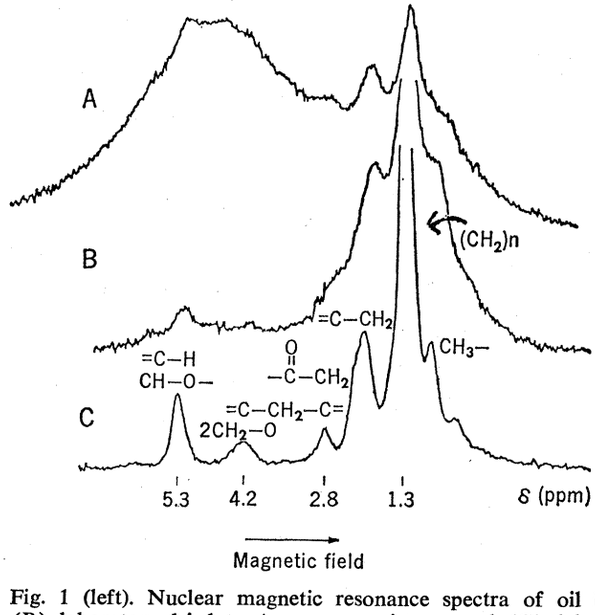
Nuclear Magnetic Resonance Measurement of Oil "Unsaturation" in Single Viable Corn Kernels

Abstract. *High-resolution nuclear magnetic resonance spectroscopy has been used to demonstrate the feasibility of determining iodine value and average molecular weight of oil in individual corn kernels. The procedure is rapid and nondestructive. Depending on heritability of individual fatty acids, this technique may greatly increase selection efficiency in breeding programs to alter the fatty acid composition of corn oil.*

Traditionally, selection for chemical composition of plants has been accomplished by methods that destroy the sample. Consequently, in plant breeding programs, selections normally are made on the basis of population averages. Nondestructive methods to identify variant individuals permit specific choices in breeding for a desired trait. One such method is wide-line nuclear magnetic resonance (NMR) spectroscopy adapted to the analysis of single seeds for total oil content (*1*, *2*). This technique has been applied advantageously in selecting for higher oil content in corn. Bauman *et al.* (*3*) and Dumanović and Trifunović (*4*) showed that differences in oil content of kernels from the same ear were heritable. Silvela (*5*), using NMR selection techniques, was able to increase the average oil content of corn 2.25 times faster than was possible with traditional selection methods. Of practical importance, the number of plants grown each year was reduced significantly while a germ plasm base at least five times greater than that retained by the classical selection method was maintained. Applying somewhat similar NMR techniques at higher selection pressures and using conventional methodology Zupančič *et al.* (*2*) increased the oil content of corn in four to five generations by an amount that normally would have been obtained only in 20 to 30 generations.

A negative correlation between the total amount of oil in corn and the iodine value of oil exists (*6*). However, since the correlation is not so high as to preclude the development of high-oil lines with oil of medium or high iodine values, there is a need for a rapid, nondestructive method to measure the iodine value of oil *in situ* in single corn kernels. In a study of a set of diallel crosses of nine inbred lines of corn, Poneleit (*7*) found additive gene action to predominate in control of total oil and fatty acid composition. Therefore,



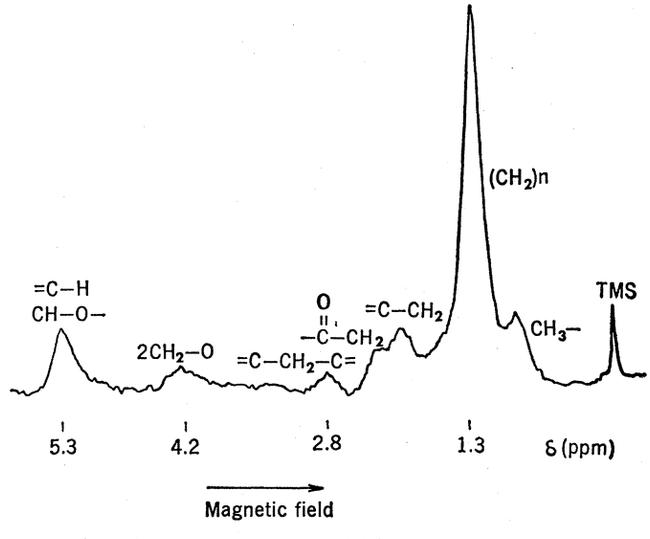


Fig. 1 (left). Nuclear magnetic resonance spectra of oil in single corn kernels. (A) Commercial-dried to 15 percent moisture; (B) laboratory-dried to 4 percent moisture; and (C) laboratory-dried to 4 percent moisture and soaked in Freon-113 for 6 days; (100 Mhz). Fig. 2 (right). Fourier transform spectrum of oil in single intact corn germ soaked in Freon-113; (100 Mhz).

it is possible to predict from this study that single-kernel differences would be heritable and that breeding programs, such as mass selection and single recurrent selection which make use of high heritability, would probably be effective for modification of corn oil quality. Consequently, a high-resolution NMR selection technique for unsaturation may prove as advantageous as the wide-line NMR single-kernel technique in speeding the development of new corn strains.

In 1962 Johnson and Shoolery (*8*) described a high-resolution NMR method for determining the average molecular weight and iodine value of natural fats dissolved in carbon tetrachloride. With this method the NMR integral signal from C(1) and C(3) glyceride protons provides an internal standard from which the olefinic and total number of hydrogen atoms are measured. From these, an average molecular weight and iodine value are calculated. Agreement between NMR and Wijs iodine values is remarkably good.

To demonstrate viability under the conditions of our method, we dried whole kernels of 25 commercial corn hybrids to 4 percent moisture and subjected them to 95°C for periods up to 90 minutes. Others were soaked in Freon-113 for periods up to 6 days. Subsequent germination tests showed a high degree of tolerance to low moisture heating (94 percent survival) and to the solvent treatment (98 percent survival).

For NMR experiments proposed earlier (*9*), individual corn kernels or excised germ were mounted on a Teflon-glass pedestal, transferred to 12-mm tubes and covered with Freon-113 to minimize the large discontinuities in magnetic susceptibilities within the sample cell. Samples were examined with a Varian model HA-100-15 high-resolution spectrometer equipped with a wide gap magnet. Typical spectra from kernels of commercial-dried (15 percent corn moisture), laboratory-dried (4 percent corn moisture), and laboratory-dried corn soaked in Freon-113 for 6 days are shown in Fig. 1 with NMR chemical shift assignments. The spectrum from the commercial-dried kernel (Fig. 1A) was not usable because of the broad absorption signal. The laboratory-dried kernel (Fig. 1B) exhibited a poor NMR spectrum; resolution and signal-to-noise ratio were less than satisfactory. Surprisingly, heating the dry sample up to 95°C to alter the mobility of the fat did not improve resolution. By contrast, solvent treatment of dried seeds altered the mobility of the oil sufficiently to provide very good signal-to-noise ratios and adequate resolution to separate the olefinic and glyceride methylene protons (Fig. 1C). Proper integration of spectra from single seeds permits determination of iodine value and average molecular weight by means of the Johnson-Shoolery (*8*) calculation. These data indicate that sample drying and solvent treatment are necessary to obtain usable spectra. Variations in NMR spectra resolution have been observed between seeds which are attributed to differences in magnetic susceptibilities between the oil and the corn germ matrix as well as between the seed and surrounding solvent. Minimizing these should improve spectra resolution.

The spectra shown in Fig. 1 were obtained with 250-second sweep time. Through use of NMR radio-frequency pulse techniques and Fourier transformation of the data (*10*), it is possible to greatly reduce instrument time. Studies with the Varian FT-100 Fourier transform accessory (Fig. 2) show a 10-second examination of an excised corn germ which was fitted into a 5-mm tube and surrounded by Freon-113. These results suggest that unsaturation in the whole kernel can be measured in times as short as 10 seconds.

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Simultaneously Recorded Trains of Action Potentials: Analysis and Functional Interpretation

Abstract. *A new kind of statistical display, the joint peri-stimulus-time scatter diagram, facilitates the analysis and interpretation of two or more simultaneously recorded trains of action potentials. The display is a generalization of the cross correlation and the peri-stimulus-time histogram, and it reflects specific underlying neuronal interactions. The technique yields quantitative measures of interaction in terms of effectiveness of synaptic connections.*

Much of the known picture of the nervous system has been obtained from single neurons individually studied for an extended period of time. On the other hand, anatomical, physiological, and behavioral evidence overwhelmingly suggest that integrative functions in the nervous system are performed by simultaneous activity in groups of neurons. It has become possible to record several trains of action potentials (spike trains) simultaneously and to analyze their temporal relationships. The results of these analyses may, in turn, be interpreted in terms of connections which underlie the integrative process in the group of neurons under observation (*1*, *2*). We describe here a method of performing such analyses for experiments in which a repeated stimulus is presented to a system of neurons and in which two or more spike trains are simultaneously monitored. This method is more powerful and sensitive than that suggested by Perkel *et al.* (*2*, p. 429) and permits a relatively direct functional interpretation.

Our basic data consist of the times of occurrence of three sets of events: a train of periodic (identical) stimuli, and two trains of action potentials (A and B). Let the time of occurrence of the ith stimulus be S_i; that of the jth spike of train A be A_j; and that of the kth spike of train B be B_k. We con-