Seed Oil Determination by Pulsed Nuclear Magnetic Resonance without Weighing and Drying Seeds

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ABSTRACT

Pulsed nuclear magnetic resonance (NMR), which takes about 10 sec per analysis, has been used for rapid nondestructive determination of oil in oilseeds without weighing and oven drying the seeds. This has been done by measuring the free induction decay (FID) signal of solid and liquid in oilseeds. The oil values determined by this method for mustard, sunflower, and soybean seeds have been compared with the values determined by measuring the oil signal alone in the intact seeds, which takes about 2 min per analysis. Correlation for mustard is 0.988, for sunflower 0.945, and for soybean 0.931. The reasons for better agreement for mustard and the way of improving it for sunflower and soybean have been discussed.

INTRODUCTION

It is necessary to use a rapid and nondestructive method of determining oil in seeds for selecting seeds of higher oil content among thousands of progenies in plant breeding work. Methods based on wide-line and pulsed nuclear magnetic resonance (NMR) have been developed and used for this purpose (1,2). In these methods, seeds have to be oven dried and weighed. This paper deals with a pulsed NMR method in which it is not necessary to oven dry and weigh the seeds. It is based on the measurement of the free induction decay (FID) signal of the solid phase (protein and carbohydrate) and the liquid phase (oil) of oilseeds and is called the solid-liquid signal method of seed oil determination. The transverse relaxation time T_2 of the solid phase is much smaller than that of the liquid phase, which enables the separation of the two signals.

The percentage of solid or liquid in a mixture of solid-liquid fat has been determined by measuring the FID signal of the solid and liquid phases (3). There are some problems in using it for seed oil determination. Figure 1 shows the FID signal of the same mustard seeds dried in the sun and at 105 C. The signal measured at 10 μsec immediately after

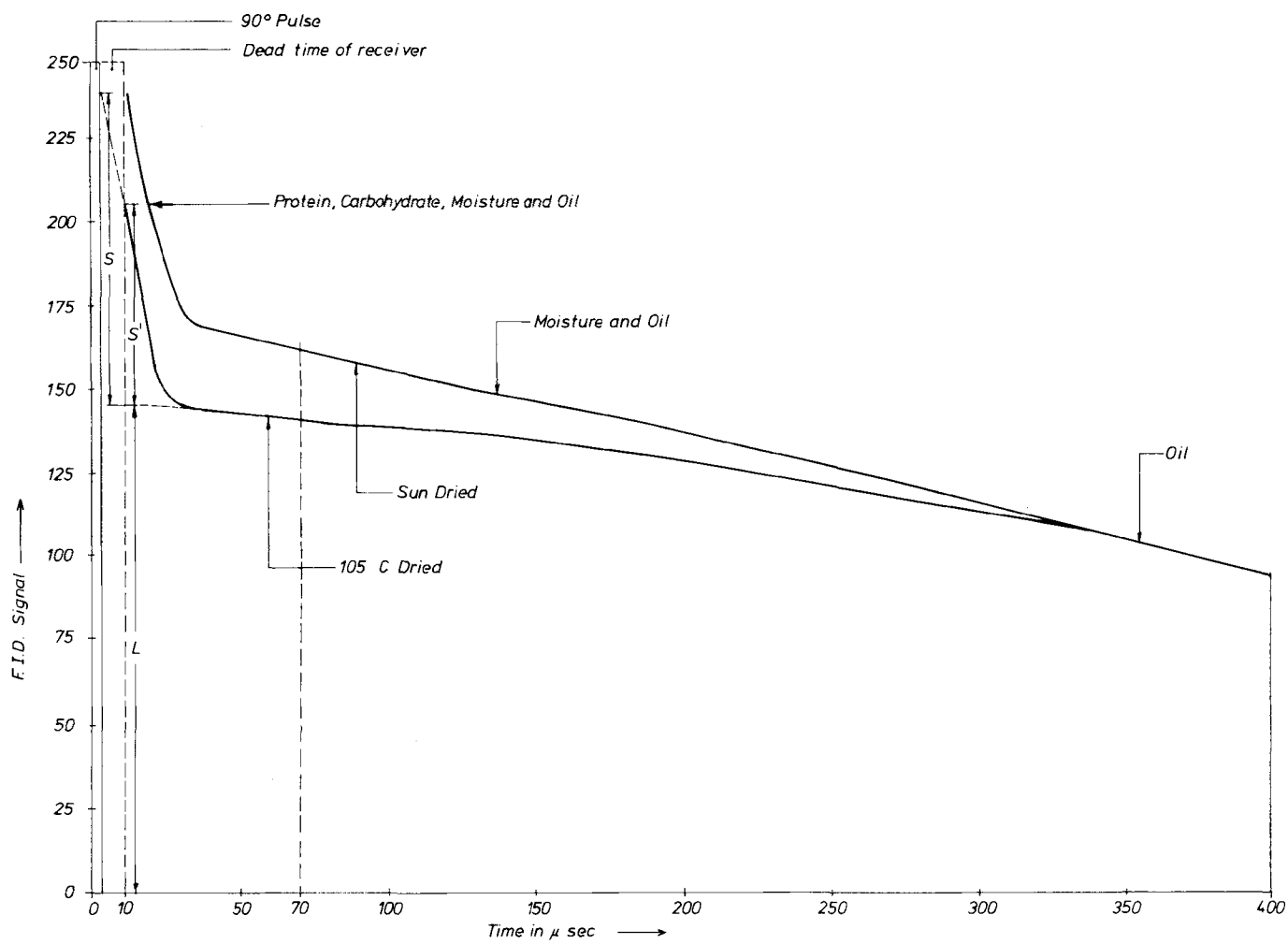

FIG. 1. FID signal of the same mustard seeds dried in the sun and at 105 C.

the dead time of the receiver gives a part of the solid signal S′ plus full oil signal L as shown on the lower curve of Figure 1. The solid signal decays to negligible value by about 70 μsec. The decay of the liquid signal at this point is not very significant. The signal measured at 70 μsec gives nearly the full oil signal. The full solid signal S can be obtained multiplying S′ by a correction factor f which depends on T_2 of solid and dead time of the receiver. If the proton density in the solid and liquid phase is the same, the oil percentage can be expressed as:

$$\text{oil \%} = \frac{L}{L + fS'} 100 \quad [1]$$

There are three main problems in using this expression for calculating oil percentage in sun-dried seeds (exposed to summer sun for several days after harvest). First, the seeds have about 6% moisture, which contributes to the oil signal at 70 μsec as shown by the upper curve of Figure 1. Second, the proton density in the solid phase (protein and carbohydrate) is not the same as in the oil phase of oilseeds. Third, there might be a considerable difference in solid phase T_2 of different samples of the same crop, making f different for different samples. If the moisture content, proton density difference, and solid phase T_2 do not change significantly from sample to sample, the oil percentage in seeds can be determined by measuring S′, L, and using another correction factor F. The oil percentage can be expressed as:

$$\text{Seed oil \%} = \frac{L}{L + FS'} 100 \quad [2]$$

EXPERIMENTAL PROCEDURES

The transverse relaxation time T_2 of the solid phase of several samples of mustard, sunflower, and soybean seeds was measured to study its variation. A Bruker Minispec p20i with two-coil arrangement, signal averager, and transient recorder was used. The FID signal of the solid phase was found to decay to an insignificant value after about 70 μsec for all the samples. The oil signal remains nearly constant during this period, which makes it convenient to measure the decay of the solid signal and determine T_2 by a semilog plot of the signal against time squared (t^2), as the solid signal is Guassian in shape. It was found that T_2 of the solid is nearly constant (about 12 μsec) for all the measured samples of sun-dried oilseeds.

The F-factor, which is automatically calculated and displayed on the Minispec p20i, was determined for different varieties of mustard, sunflower, and soybean to study its variation. For this, the oil content of these varieties was determined by the oil signal method (2), which is an accurate method of seed oil determination, but the seeds must be oven dried and weighed. The F-factor of sun-dried seed samples of each variety was measured by putting such samples in the probe and adjusting the F-factor as necessary to arrive at the oil percent of the variety, determined by the oil signal method. The F-factor of sun-dried seeds was found to be greater than that of oven-dried seeds because of the contribution of moisture to the liquid signal of the former. Some variation was found in the F-factor of different varieties of the same oilcrop. Therefore, the mean F-factor for each crop was calculated. The mean F-factor of a particular crop was used to measure the oil content of different samples of that crop by the solid-liquid signal method (3). The Bruker Minispec p20i with two-coil arrangement was used for the determination of oil content by both methods (2,3). Samples of mustard, sunflower and soybean having a maximum weight of ca. 2.5 g, 1.5 g, and 2.3 g, respectively, can be analyzed by this instrument. It has a sample volume of about 3.5 cc. After making all these measurements, the moisture content of the same samples was determined by drying them at 105 C to

TABLE I

Oil Percent, F-Factor and Moisture Percent of Mustard, Sunflower, and Soybean Seeds

Crop	Sample no.	Oil, % by oil signal method	F-Factor sun-dried seeds	Oil, % by solid-liquid signal method with mean F-factor	Moisture, %
Mustard	1	37.2	3.20	37.3	6.3
Mustard	2	37.9	3.24	37.9	6.2
Mustard	3	43.1	3.14	42.6	5.7
Mustard	4	40.5	3.18	40.3	6.1
Mustard	5	42.2	3.34	43.3	5.5
Mustard	6	43.7	3.28	44.1	6.4
Mustard	7	41.3	3.18	41.1	6.7
Mustard	8	41.3	3.22	41.3	6.8
Mustard	9	43.5	3.22	43.4	6.8
Mustard	10	46.5	3.22	46.4	6.2
Sunflower	1	35.2	2.84	35.0	6.0
Sunflower	2	38.4	2.92	39.0	5.1
Sunflower	3	42.7	2.64	40.6	5.9
Sunflower	4	37.1	2.84	37.3	6.1
Sunflower	5	44.2	2.94	44.6	5.7
Sunflower	6	39.4	2.90	39.8	6.1
Sunflower	7	42.9	3.00	44.6	7.9
Soybean	1	19.9	3.20	21.1	7.0
Soybean	2	23.7	2.84	22.9	6.5
Soybean	3	22.2	2.78	21.0	6.8
Soybean	4	19.6	2.94	19.8	7.5
Soybean	5	23.3	2.98	23.2	6.9
Soybean	6	22.0	2.88	21.7	6.9
Soybean	7	20.7	3.00	20.7	6.5
Soybean	8	18.0	3.20	19.2	7.2
Soybean	9	20.2	2.98	20.3	6.9

constant weight.

RESULTS AND DISCUSSION

The oil values determined by the oil signal and the solid-liquid signal methods, moisture content, and F-factor for each sun-dried sample of mustard, sunflower, and soybean have been given in Table I. The mean F-factors of these crops for their sun-dried seeds are 3.22, 2.87 and 2.98, respectively. The correlation in oil values determined by the two methods is 98.8% for mustard, 94.5% for sunflower, and 93.1% for soybean. It might be possible to improve the correlations for sunflower and soybean which have much larger seeds than mustard by analyzing larger samples of ca. 5 g in weight which would provide a more representative sample. This could be achieved by using an NMR probe head with a larger volume over which the RF and the magnetic fields are constant.

In the present experiment, the requirement of the representative sample has been fully met for mustard crop by using ca. 2.5 g seeds. The agreement between the oil values obtained by the two methods is very good for mustard, which means that the solid phase T_2, moisture content, and proton density difference between the two phases of the seeds do not change significantly from sample to sample. The good agreement between the methods shows that it is possible to determine oil in seeds by pulsed NMR without weighing sun-dried seeds in plant breeding.

REFERENCES

1. Conway, T.F., and F.R. Earle, JAOCS 40:265 (1963).
2. Tiwari, P.N., P.N. Gambhir, and T.S. Rajan, Ibid. 51:104 (1974).
3. Van Putte, K., and J. Van den Enden, Ibid. 51:316 (1974).

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Preparation of Heptadecenoic Acid from *Candida tropicallis* Yeast

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ABSTRACT

In this paper a method is described for preparing 10 g or more of heptadecenoic acid (C17:1ω8) of 99 p.100 purity from *Candida tripicallis* yeast. Three cycles of treatment, based on crystallization techniques, were used successively: (1) Crystallization of fatty acids (in free form) from acetone at -25 C induced the elimination of most of the saturated fatty acids, and at -60 C, of all of the polyunsaturated acids. (2) Inclusion formation of fatty acids (as methyl esters) with urea at hC induced the removal of all of the remaining saturated methyl esters and most of methyl oleate. (3) Crystallization of fatty acid methyl esters from acetone at -60 C removed almost all the remaining monounsaturated methyl esters (methyl palmitoleate and methyl oleate). Total efficiency of the preparation was about 17 p.100.

INTRODUCTION

Thorough studies of lipid metabolism involve qualitative and quantitative analyses of lipid classes and their fatty acids in biological samples. Among the different dosage methods, the addition of internal standards (free or esterified fatty acids) in the samples has the following advantages: sensitivity, specificity, and efficiency (simultaneous quantification of several kinds of lipids).

Among all the natural fatty acids readily available, heptadecenoic acid (C17:1ω8) is the one that best possesses all the features which an internal standard should have: scarcity in most animal tissues, length of the carbon chain close to that of the main fatty acids in the biological samples, and easy usage in organic synthesis of esters (triglycerides, phospholipids, and cholesterol esters). Grown on hydrocarbons, *Candida tropicallis* yeast are rich in this acid (27.9 p.100 of total fatty acids). They are therefore an excellent biological source for preparing highly pure heptadecenoic acid.

Fractional distillation, often used in the past, could alter the structure of the fatty acid. Zhukov and Vereshchagin (1) avoided this problem and used preparative gas chromatography. Nevertheless, the authors worked out only some hundred milligrams of pure heptadecenoic acid, while a specialized, expensive apparatus would have been required for the preparation of several grams of this acid needed for our studies.

It is possible to separate great amounts of fatty acids into classes in a single step according to the degree of unsaturation with a simple liquid chromatography device. Florisil columns or ion exchange resins columns impregnated with silver nitrate have been used respectively by Anderson and Hollenbach (2) and Willner (3), and Wuster et al. (4) and Emken et al. (5). This technique, whose various uses and limits have been described by Morris (6), has been applied particularly to purify cis-olefins from trans-isomers by De Vries (7) and Emken et al. (5). It is, however, expensive, and its use is especially valuable for the purification of the polyunsaturated fatty acids.

Fractional crystallization techniques, based on the differences in physical properties of fatty acids, allow a greater field of purification possibilities. They usually do not cause any damage to fatty acids, are cheap and allow great quantities of pure fatty acids to be extracted. Brown (8) and Brown and Kolb (9), on the one hand, described the potentially numerous applications of low temperature fractional crystallization from solvents, and Schlenk (10) and Iverson and Weik (11), on the other hand, showed the advantages of the formation of inclusion complexes with urea. In both cases, the purification of a given fatty acid can be obtained only after several successive steps in the same treatment. Similarly, several authors have worked out methods of preparing highly pure fatty acids (99 p.100 pure or more) based on the use of either one of these two techniques or a combination of both. The raw sources used have been from either vegetable or animal origin: purification of oleic acid from olive oil by Swern and Parker (12), Keppler et al. (13), Rubin and Paisley (14), Fremont and Gozzelino (15), purification of linoleic acid from safflower oil by Brown (8) and Keppler et al. (13), or from corn oil by Schlenk and Holman