Sources of Error in the Determination of Oil Content of Rapeseed by Nuclear Magnetic Resonance (NMR)

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Introduction

The technique of Nuclear Magnetic Resonance (NMR) is widely used for the determination of oil content in rapeseed, for purposes of plant selection and breeding (1). In principle, when a dried seed sample is inserted into the NMR instrument, a digital "count" or number is produced, based on the electromagnetic properties of the spinning hydrogen nuclei of the liquid lipids contained in the seed. The count is proportional to the amount of oil (lipid) in the seed sample. Moisture in the seed will contribute to the produced count. In our laboratory, with this technique, large samples (25 g rapeseed) give results of high precision (reproducibility), similar to the conventional Soxhlet extraction procedure. Small samples (1 g), however, give considerably lower precision (0.8% relative standard deviation).

In an effort to increase the precision of analysis, particularly for the small samples, a study is being made of individual sources of error in the method. We wish to report on some observations made in the course of this study, concerning (a) optimal instrument settings of radiofrequency (RF) level and gain, (b) the effect of sample temperature on precision, (c) difficulties in equalising the sample and magnet temperatures, and (d) the occurrence of a gradual increase in the apparent oil content of dried rapeseed (as determined by NMR).

Experimental

The NMR analyses were performed with an MKII Newport Analyzer (Oxford Analytical Instruments Ltd., England).

Method. Samples of rapeseed (1 g), placed in NMR sample vials, are heated to dryness in an air oven, cooled to room temperature, weighed, and assayed for counts in the Analyzer. A "standard" rapeseed sample (dried) of known oil content, permanently kept in a stoppered or sealed NMR vial, is similarly assayed for counts at intervals during the period of analytical work. The oil content of the unknown sample is calculated by comparison with the standard, assuming a strict proportionality of the oil content and the counts per gram of sample.

Results and Discussion

All the factors discussed below are of potential significance in affecting the precision of analysis with the NMR method. The discussion is concerned largely with the small analytical samples (1 g); we have no problems with precision of analysis for the large samples (25 g).

Optimal RF Level and Gain. Contrary to recommendations in the Instrument User Handbook, we have not yet been able to establish an optimal value for the RF level. The precision for repeated counts for a one-gram seed sample remained approximately the same within the RF range of 100 to 250 μA, with a trend towards higher precision at 250 μA. A lower precision was obtained below 100 μA.

Similarly, we did not find any optimal value over a wide range of gain settings, for any given RF level.

Effect of Sample Temperature. With a constant magnet temperature, the count for a rapeseed sample (1 g) varied linearly with the sample temperature, at a rate of -1.2% (relative) per degree C. This rate was four times higher than specified in the User Handbook (-0.3%/°C). The Handbook specification is probably correct for large samples (25 g, not examined); a correction for the small samples has appeared in more recent instrument specifications (2). The corresponding rate of change reported by Madsen (1) for 25-gram samples, using an MKI Newport Analyzer, was -0.4% to -0.5% (relative)/°C, similar to the value specified by the instrument company.

Temperature Control. In view of the recently realized high effect of sample temperature on precision (vide supra), a temperature-controlled room or a temperature-controlled magnet (commercially available) and sample rack would be desirable. To circumvent the expense of temperature controls, we have been operating in a laboratory with fluctuating temperatures. This was expected to be satisfactory provided the standard samples were measured at sufficiently frequent intervals in between the unknown samples.

We found, however, that the temperature of the sample assembly (holder) in the magnet usually differs (by 0.2 to 1.0°C) from the temperature of the samples to be analysed (stacked in an adjacent rack on the table). This occurs because, with a drift in room temperature, upwards or downwards, the samples (with a relatively low mass) will change in temperature at a faster rate than the magnet and sample assembly (with a higher mass). This situation leads to reduced precision of repeated consecutive counts. A sample of a certain temperature, inserted into a sample assembly of a slightly different temperature, will undergo a gradual change in temperature until, after 1-2 minutes, it equilibrates with the assembly temperature. Consecutive counts registered for the sample during this equilibration time will therefore be unreliable and of poor reproducibility. We are endeavouring to overcome this problem by equalising the magnet and sample rack temperatures through some forced air circulation or heat sink arrangement. Also, we are now leaving each sample in the sample assembly for 32 seconds to equilibrate in temperature, before initiating the counting procedure.

Instability of Dried Rapeseed. Seed samples (1 g), after having been dried for analysis, appear to undergo some changes of an unknown nature which result in a gradual increase in the NMR counts (i.e., apparent oil content) with time. This increase may amount to 1% (relative) within the first hour after cooling the dried sample, and as much as 4% after four years. This instability has been evident with most, but not all the one-gram samples investigated; it has not been noticeable with the 25-gram samples. Also, it does not occur with undried samples (telle quelle).

This phenomenon might be partly explained if the drying process should be causing an increased proportion of the seed lipids to become bound to protein and thus to become less "liquid" and therefore less receptive to NMR resonance. Furthermore, if this additional amount of bound lipids should gradually revert to

the free state with time, one would expect the seed to first decrease in NMR counts by the drying process (disregarding here the influence of seed moisture on the NMR count), and subsequently to gradually increase in counts again. Experiments are underway to see if this theory can be supported.

Choice of Standard. The use of a permanent standard, if stable, should result in a higher precision of analysis than the use of a series of daily prepared standards from a bulk seed sample. The above discussion, however, has demonstrated that a dried one-gram sample of rapeseed usually is not stable but gradually increases in apparent oil content with time. To partly overcome this problem, we have been using dried seed samples as standards only after long-time storage, when the rate of change has diminished. Furthermore, the assumed oil content of the standards has been adjusted periodically, to compensate for any further change that may have occurred. In a similar manner, any other material (paraffin oil, rubber, etc.), if stable, could be used as standard and assigned an appropriately assumed oil content.

A different approach, which we expect to adopt, is to use an undried or partly dried rapeseed sample as standard. An undried sample should remain stable for many years (we have evidence for nine years). Such a standard may be assigned an assumed oil content or, alternatively, the contribution of its seed moisture to the NMR count may be taken into account in the calculations.

Choice of Sample Treatment. The observed gradual increase in the apparent oil content of dried rapeseed samples affects not only the rapeseed standards but also the samples to be analysed. If, for example, a group of identical samples were dried and cooled to room temperature, ready for NMR analysis, the first few samples analysed might not yet have undergone any change in apparent oil content. Later samples, however, might have changed, and different samples might have changed to a different degree. A series of different values would thus be obtained for the oil content of these identical samples.

To minimise this effect, we now store all dried samples for at least 24 hours (at room temperature) before starting the NMR analyses. Since the rate of change in the samples is lower after 24 hours than immediately after drying and cooling, the results

should thus be somewhat more reliable. This is not a complete solution to the problem, however, since all samples do not undergo this change at the same rate and to the same degree.

We expect in the future to fully overcome this problem by analysing all samples undried. Such samples should be stable with regard to the apparent oil content. After the NMR analysis, the samples will have to be dried for moisture determination, and the contribution of the seed moisture to the NMR count will have to be incorporated into the calculation of the oil contents.

Conclusions

In order to improve the precision of analysis in the NMR determination of oil content in rapeseed, we have modified our analytical procedure (a) by using dried rapeseed samples as standards only after prolonged storage, (b) by storing the dried, unknown rapeseed samples for at least 24 hours prior to NMR analysis, and (c) by leaving each sample in the magnet sample-assembly for 32 seconds before initiating the NMR counting.

We expect in the future to obtain further improvement in precision by cancelling the above modifications and (a) ensuring equal temperature of the sample assembly and the adjacent sample rack (even if the room temperature fluctuates), (b) using a permanent rapeseed standard of undried seed (telle quelle), and (c) performing the NMR analyses on undried seed samples, followed by a moisture determination.

Acknowledgement

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References

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