Nuclear Magnetic Resonance Spectrometry as a Quick Method of Determination of Oil Content in Rapeseed

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ABSTRACT

A comparison of results obtained by the traditional extraction method for determination of oil content in rapeseed and those achieved by a commercial nuclear magnetic resonance (NMR) spectrometer revealed a very high correlation (r = 0.987) between the results. The advantage of the NMR method, in relation to the extraction method, is the shorter time needed for a test: the result will be available in the course of a few minutes. Moreover, no inflammable chemicals are needed for the test, and the apparatus does not take up much room. On the other hand the NMR spectrometer must be placed in a room with a constant temperature, the seed samples must have the same temperature as the adjustment samples, and the oil content must be determined on dry matter basis.

INTRODUCTION

The determination of the oil content in seeds by an extraction method is a slow and time-consuming process. The apparatus requires much room when many samples are to be tested within a short time, and the inflammable solutions used at the determination require special arrangement of the laboratory. These serious drawbacks resulted in the preparation of new methods, one of which is nuclear magnetic resonance (NMR) spectrometry. By this method, it is possible to perform the test in a much shorter time and without the use of chemicals. The method has been used for determination of oil content in seeds and for selection of oil plants for breeding (1-5). Another method, the pulsed NMR method, seems to be well suited for a quick determination of the oil content in seeds (6).

NMR spectrometry has been used for many years for chemical research within the basic sciences, but the method was not profitable for commercial use until a special apparatus was designed for determination of the oil content in seeds. The method is based on the magnetic qualities of the atom nuclei. Some atom nuclei—in this case the hydrogen atoms—are able to absorb radio frequency energy of a certain frequency when placed in a magnetic field (7-10).

For the last six seasons, the Danish State Seed Testing Station has been using two apparatuses for routine determinations of oil content in rapeseed. The duration of the season has been 2-3 months, and ca. 50,000 samples have been tested.

Before the method was finally taken into use, various examinations of the apparatus and a series of comparative determinations with the traditional extraction method and the NMR spectrometry method were undertaken.

The NMR spectrometer is applicable only for oil determination in dried seeds or seeds with very low moisture content. Consequently, it was necessary to establish whether the extraction method would show different results of the oil content in seeds determined on the basis telle quelle and the oil content determined on dry matter basis and corrected to basis telle quelle, because oilseeds traded commercially are sold on basis telle quelle.

MATERIALS AND METHODS

Seed samples of spring and winter rape (*Brassica napus* L. var. *oleifera*) with different moisture content were selected for the examinations.

Three methods were used for comparison. For method I (standard method) seeds dried at 105 C for 16-18 hr (dry matter basis) were used. The examination was performed according to the following procedure: 11 g dried rapeseed, ground in a hand mill, weighed into two portions of 5 g each. The samples were placed in Soxhlet tubes and extracted for 24 hr with petroleum ether (bp under 50 C). Afterwards, the samples were ground in a mortar and extracted for 24 hr. The petroleum ether was evaporated, and the oil was dried to a constant weight, normally 2 x 2 hr in an incubator at 100 C; afterwards, the oil percentage was calculated.

In method II, the oil content was determined in the seeds as they were on receipt without drying (basis telle quelle); otherwise as in method I.

In method III, the oil content was determined in seeds on dry matter basis with a NMR spectrometer, type Quantity Analyser, Mk I (Newport Instruments, Ltd., Newport Pagnell, Buckinghamshire, England). The hydrogen atoms in the liquid stage of oil and water will absorb radio frequency energy in the same field (2.7 MHz), and it is therefore necessary to dry the seeds to make a measurement of the oil content in the sample possible.

For each measurement of oil content by the NMR method, 25 g of dried seeds were used. The samples were weighed with an accuracy of 0.001 g. The adjustment sample, sealed in a Nessler glass tube, contained 44.8% oil determined by method I.

Determination of moisture content and drying of the seeds were performed in an oven at 105 C for 16-18 hr.

RESULTS

Adjustment of the Apparatus

A certain current intensity is needed for the NMR spectrometer to obtain the greatest sensitivity and consequently the most reliable measurement. The intensity of the current depends on the species to be measured. Experiments showed that 275 μA provided the greatest sensitivity when rapeseed was to be measured. At this adjustment a deviation of 1 μA would result in a variation of 0.04% oil.

The influence of the room temperature on the NMR spectrometer was checked, and the results showed a deviation of 0.07% oil for each 1 C the temperature of the apparatus deviated from the adjustment temperature. Although varying temperatures may cause minor deviations only, the apparatus should nevertheless be placed in a room with a constant temperature.

The temperature of the seeds appeared to have a great influence on the result of determination. A difference of 1 C between the sample examined and the adjustment sample resulted in a deviation in the oil percentage of 0.2. Identity in temperature between the sample under examination and the adjustment sample is therefore necessary.

The reproducibility of the instrument was examined in 10 samples of 25 g each from the same lot in which the oil content had been determined 6 times in the course of one month. Between the determinations, the samples were kept in a desiccator with silica gel. Table I shows the results obtained in the single measurements as well as the average and standard deviation for each subsample and for each day

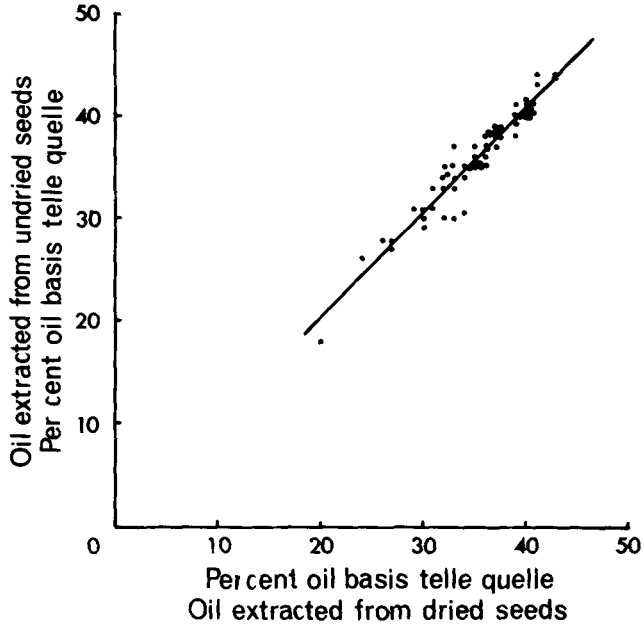

FIG. 1. Comparison of the oil content (basis telle quelle) in 64 samples of rapeseed determined by extraction of dried seeds and undried seeds. Correlation coefficient (r) = 0.959; regression line is determined by y = a + bx, where a = -0.117 and b = 1.023.

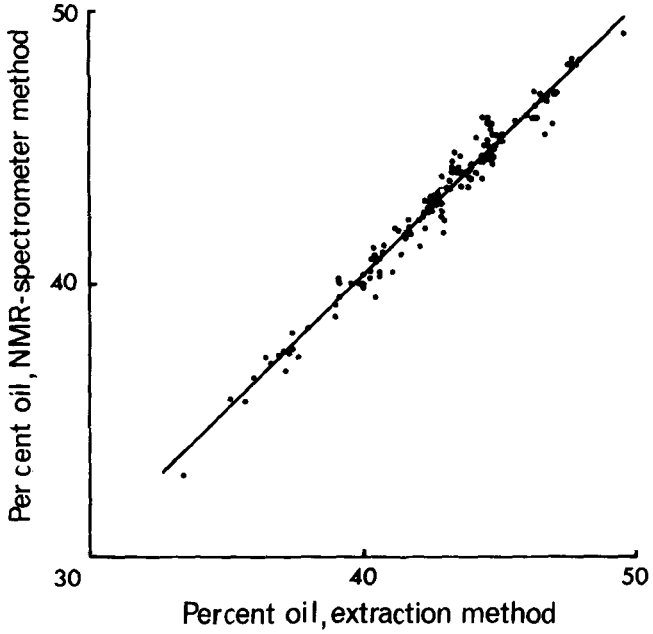

FIG. 2. Comparison of the oil content in 135 samples of rapeseed, determined by extraction of dried seeds and by a nuclear magnetic resonance spectrometer. Correlation coefficient (r) = 0.987; regression line is determined by y = a + bx, where a = 1.258 and b = 0.977.

of measurement. The average and standard deviation for all measurements constituted 42.5 ± 0.21%. A series of corresponding measurements performed by method I showed an average of 42.4 ± 0.22%.

An examination revealed that the measurements on the NMR spectrometer were independent of moisture contents of < 4% in the seeds, but for a correct measurement of the oil content there must be the same moisture content in the adjustment sample as in the seed sample to be examined.

Oil Content Measured by NMR Spectrometer and Ether Extraction

Because of the hydrogen atoms in the water, the oil content can be determined only in dried seeds by the NMR method. A comparison was made between method I and method II in order to find out whether the moisture content of the seeds did exert any influence on the oil content measured. The results, based on 64 samples with moisture content varying from 4 to 31%, are depicted in Figure 1. The correlation coefficient was (r) = 0.959, the regression coefficient (b) = 1.023, and the intersection point of ordinate (a) = 0.117. A t-test showed no significant difference in the oil content found by the two methods when the seeds had a moisture content up to 26%. Samples with a higher moisture content displayed a significant difference (P = 96.9); the results obtained with method II thus showed a higher oil content than those found by method I. For the 55 samples with a moisture content below 26%, the correlation coefficient was (r) = 0.971.

The results of a comparison of the oil content in 135 samples determined by methods I and III are given in Figure 2. The correlation coefficient was (r) = 0.987, the regression coefficient (b) = 0.977, and the intersection point of ordinate (a) = 1.258. A t-test revealed no significant difference in the results found by the two methods. As adjustment sample for method III, a sample with an oil content of 44.8% was used.

DISCUSSION

The reproducibility of results can be considered to be at least at the level of the traditional extraction method (Table I). A similar examination of the oil content in rapeseed (5) showed a standard deviation in results achieved by the NMR spectrometer of ± 0.23% for winter rape and ± 0.26% for spring rape. The corresponding standard devia-

TABLE I

Reproducibility of the Nuclear Magnetic Resonance Spectrometer[a]

Subsample number	Number of days from the first measurement						Mean
	0	13	27	27	28	30	
1	42.4	42.3	42.3	42.3	42.5	42.2	42.4 ± 0.10
2	42.3	42.2	42.3	42.2	42.4	42.0	42.2 ± 0.14
3	42.4	42.5	42.5	42.3	42.6	42.3	42.4 ± 0.12
4	42.3	42.5	42.5	42.3	42.6	42.5	42.5 ± 0.12
5	42.4	42.4	42.5	42.3	42.6	42.5	42.5 ± 0.10
6	42.4	42.5	42.3	42.3	42.5	42.4	42.4 ± 0.09
7	42.5	42.5	43.2	42.5	43.4	42.5	42.8 ± 0.42
8	42.6	42.6	42.7	42.5	42.6	42.6	42.6 ± 0.06
9	42.5	42.7	42.7	42.6	42.7	42.6	42.6 ± 0.08
10	42.7	42.6	42.4	42.4	42.5	42.5	42.5 ± 0.12
Mean	42.5 ± 0.13	42.5 ± 0.15	42.6 ± 0.27	42.4 ± 0.12	42.6 ± 0.22	42.4 ± 0.19	

[a]Tested by means of 10 rape samples, measured 6 times in the course of 30 days.

tions in results obtained by extraction were ± 0.23% and ± 0.36%, respectively.

There was good agreement between results obtained of seeds with no more than 26% moisture content and of dry seeds corrected to basis telle quelle (Fig. 1). A higher moisture content is of rare occurrence, even in seeds delivered from the farmer to the seed firm. This must also be a condition for using the NMR method for determination of oil content in commercial trade with oilseeds.

The very high correlation between results found by extraction and those measured by the NMR spectrometer (Fig. 2) was in agreement with the results of a previous examination (11) of 164 samples of spring rape ($r = 0.978$) and 42 samples of winter rape ($r = 0.987$).

An advantage of the NMR spectrometry method is the short time needed for the examination, namely, 5 min per sample (single determination on each of two parallel samples), whereas up to 2 hours' work is spent for the corresponding determinations by the extraction method.

Moreover, the NMR method does not require inflammable chemicals with consequent requirements for the premises. Less space is necessary when a large number of samples are to be tested, and the result is available in a few minutes instead of 48 hr.

On the other hand, the NMR spectrometer requires a laboratory with a constant temperature. Before measurement, the seed samples must have the same temperature as the adjustment sample, and measurements can be made only in seeds on dry matter basis.

On the basis of the results obtained, it may be concluded that the NMR spectrometer method is well suited for commercial use for determination of oil content in rapeseed.

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