NMR AS A TOOL FOR THE PLANT BREEDER

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N.M.R. AS A TOOL FOR THE PLANT BREEDER

N.M.R. is a phenomena first used in the mid 1940's, since which time it has become a standard analytical tool in the field of chemistry. Since 1968 N.I. have manufactured a broad line low resolution instrument called the Newport Analyser. This instrument is now used in 40 countries world wide as a quality control tool.

In the next ten minutes I will explain to you the ease of using this technique and instrument in the field of plant breeding.

SLIDE 1

This shows the Newport Analyser MK111A. The unit consists of two pieces of equipment:

1) the electronics console
2) Magnet and sample assembly.

The equipment is all solid state and requires only 30 minutes warm up.

SLIDE 2

This shows the sample assemblies, capable of taking samples of up to:

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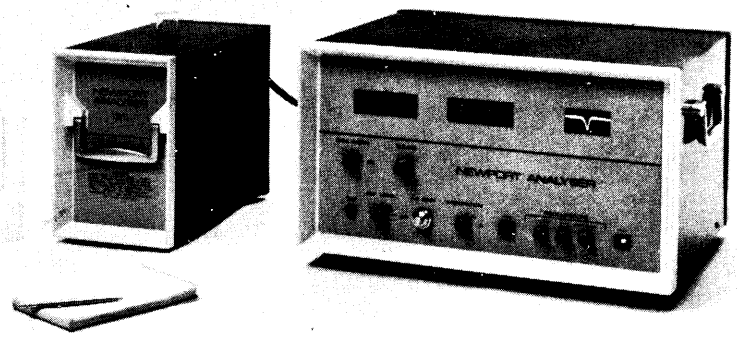
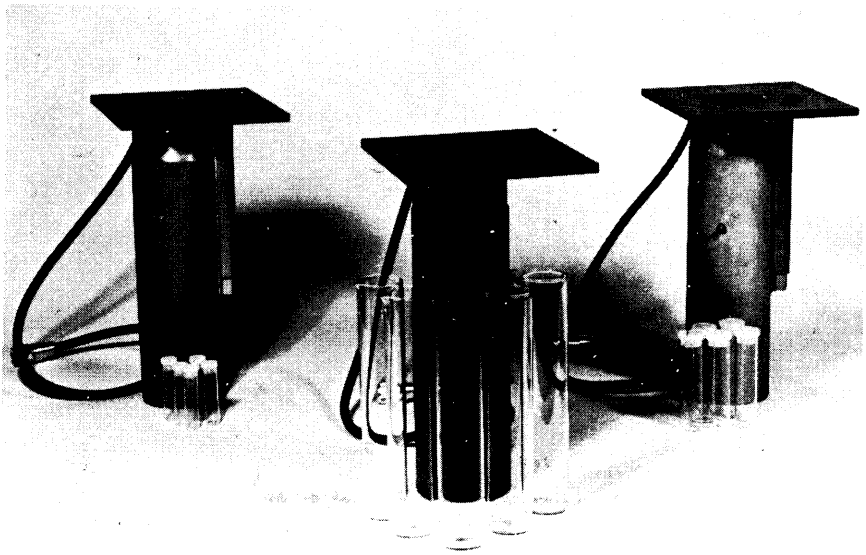
Blakelands North,
Milton Keynes,
MK14 5AW



SLIDE 1

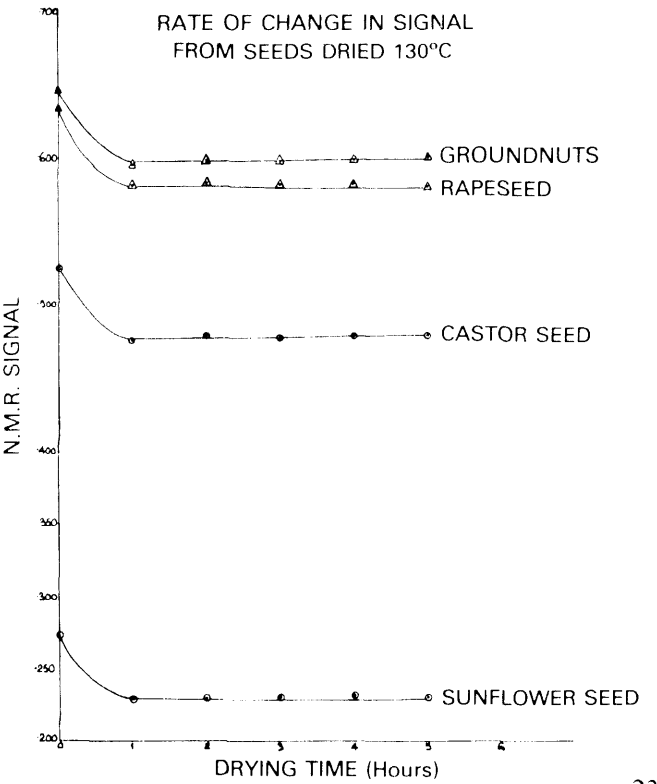


SLIDE 2

1) 2 ml maximun, this is used for single seed measurements
2) 6.5 ml maximum, this is used when measuring a small number of seeds
3) 40 ml maximum, this is the tube for general use and can hold up to 16 g of sunflower seed
4) 150 ml maximum, this is not illustrated but can be used to measure the oil content of all the seeds in one sunflower head. It will hold up to 62 g.

SLIDE 3

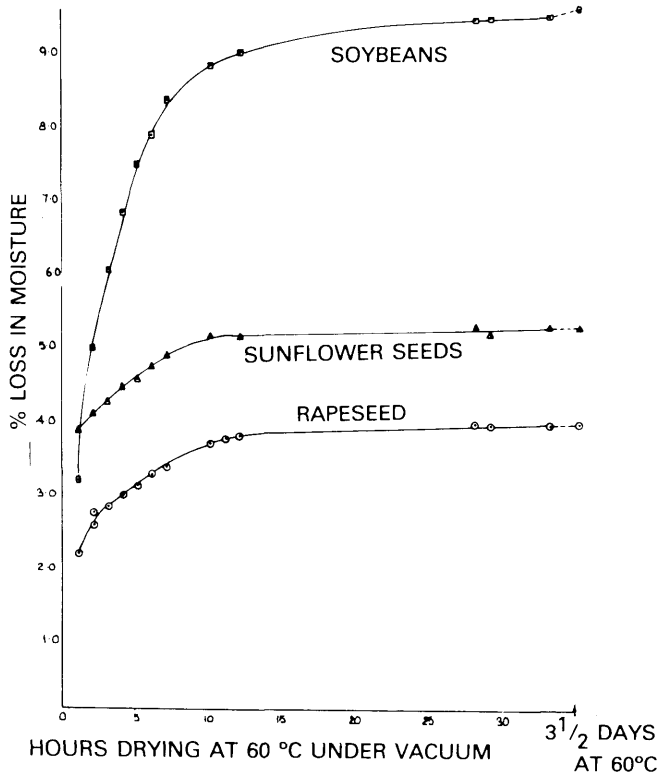
The Newport Analyser measures the total number of mobile hydrogen atoms (or protons) in a sample. In a seed this means in the oil and water phase. This slide shows the times typically needed to reduce the moisture content in a seed sample to a level where it will not affect the n.m.r. response. These times are given for drying at 130° C and an hour at this temperature will reduce the viability of the seed.



SLIDE 3

SLIDE 4

This shows the reduction of moisture in a sample using a vacuum oven at 60° C, drying at this temperature does not destroy the viability of the seed. A more popular method than drying is to equilibrate samples in a constant humidity environment for approximately two weeks prior to measuring the samples. As the Newport Analyser measures all the mobile protons it will measure both oil and moisture but all the seeds will have attained the same moisture content so any variation in signal will be due to change in the oil content. These results will only be relative to other seeds of the same moisture content and if absolute results are required it is necessary to determine the exact moisture content. However with the MK111A Analyser it is possible to differentiate between moisture and oil provided the moisture level is below a certain critical level. This level is typically the'safe storage'level.



SLIDE 4

The MK111A instrument has a gatewidth control switch which allows the electronic gate to be widened or narrowed from the standard 1 1/2 gauss gatewidth. By taking measurements at two different gatewidths it is possible once the original calibration charts have been prepared to measure the oil and moisture content on the same seed sample.

Briefly, if the gate is set to 1 1/2 gauss the resonance from the oil signal and much of the moisture is admitted to the measuring circuit. If the gatewidth is reduced below 1 1/2 gauss more and more of the resonance from the moisture is excluded, but the resonance from the oil (being much narrower) continues to pass through the gate and is measured. Thus by taking two measurements at different gatewidths it is possible to determine the moisture and oil content separately.

SLIDE 5

This slide depicts results obtained on a sample of maize.
The samples were split into four.
One sample was left (as received).
A second sample was semi dried.
A third sample was semi dried.
The fourth sample was dried for two hours at 130° C to reduce the moisture to zero.

Sample	Seed type	Measurement at 1/4 g.	Measurement at 1 g.	Moisture content as determined by drying
1	as received maize	13.9	19.2	13.2 %
2	semi-dried maize	8.2	12.3	7.8 %
3	semi-dried maize	5.1	8.4	4.1 %
4	fully-dried	3.8	4.5	0.0 %

The results depicted at 1/4 and 1 gauss are "apparent oil content" (oil equivalent) based on measurements on a wet weight basis.

Apparent oil content =

$$\frac{100 \times \text{reading from maixe sample} \times \text{weight of calibrant oil}}{\text{Reading from calibrant sample} \times \text{weight of maixe sample (wet weight)}}$$

SLIDE 6

These results are then used to plot a graph showing the relationship between the difference in apparent oil content at two different gatewidths and the moisture content by oven drying.

Sample	Difference between apparent 1.0 g - 0.25 g	% Moisture
1	5.3	13.2
2	4.1	7.8
3	3.3	4.1
4	0.7	0.0

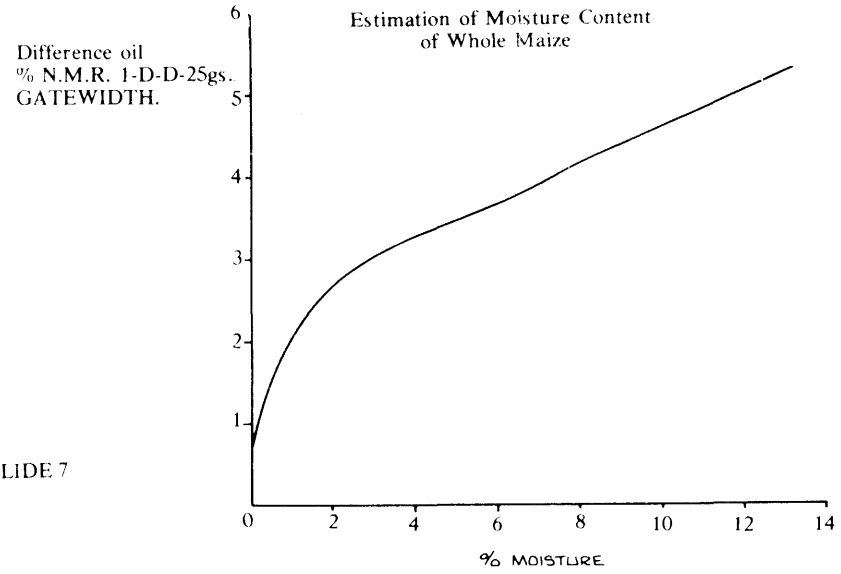
SLIDE 7

This shows the shape of the graph generally obtained from these measurements.

SLIDE 8

The difference between the apparent oil contents at different gatewidths is then plotted against the error in the oil content. For

this report the true oil content was taken as the oil content by n.m.r. on the fully dried samples using a gatewidth of 1 gauss but the oil content by solvent extraction can be used. The error was calculated



SLIDE 7

Sample	Difference between apparent oil content 1·0g – 0·25g	Error 1. NMR 1g – NMR 1g at 0% moisture
1	5·3	14·7
2	4·1	7·8
3	3·3	3·9
4	0·7	0·0

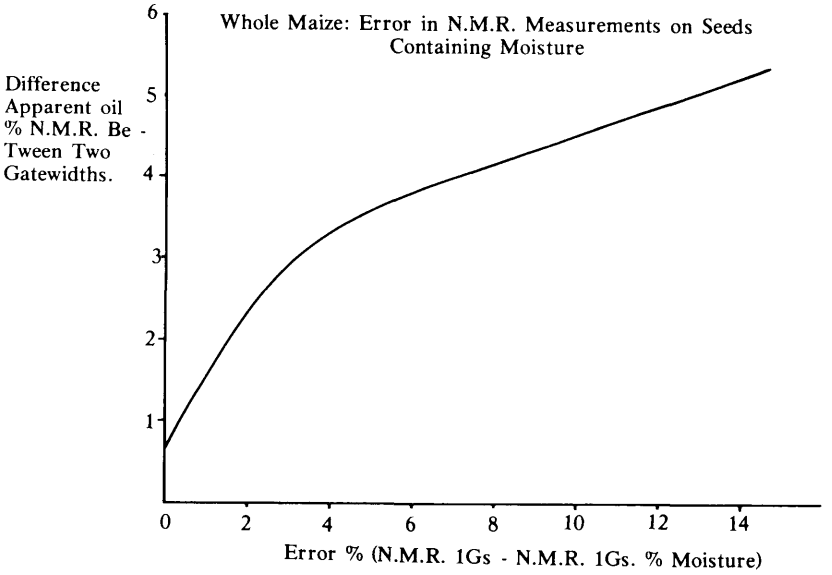
SLIDE 8

by subtracting this figure from the apparent oil content of the undried samples at 1 gauss.

SLIDE 9

This shows the second and final curve necessary for measuring oil and moisture in the same sample.

Taking now an unknown seed sample we measure it in the analyser at preselected gatewidths and instrument conditions. We calculate the difference in apparent oil contents and by referring to the appropriate graph we can obtain the % moisture and % errors. With this error figure and the apparent oil content at 1 gauss figure we can calculate the true oil content.



CONCLUSION

Although the results already achieved at Newport Instruments and elsewhere are very encouraging there have been some anomalies found when using seeds straight from the field but research is continuing into this area.

Overall however the use of broad line NMR can only be a great improvement in time, manpower and accuracy over the standard extraction techniques.