Calibration of NMR spectroscopy for accurate estimation of oil content in sunflower, safflower and castor seeds

Praduman Yadav[1,*] and I. Y. L. N. Murthy[2]

[1]Biochemistry Unit, and
[2]Agricultural Chemistry Unit, Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad 500 030, India

Oil content analysis in oilseed crops requires methods that are non-destructive, accurate, fast, eco-friendly (without the use of solvent), inexpensive in terms of consumables, and easy to use. Pulsed low-resolution nuclear magnetic resonance (NMR) satisfies all these conditions. The objective of the present study was to develop an oil measurement method for seeds using NMR spectrometry. A bench-top pulsed NMR analyser was calibrated with respect to temperature. Six genotypes each of sunflower, safflower and castor were used for the analysis. Changes in sample conditioning temperature can lead to significant changes in the calibration graph. Based on the statistical parameters (correlation coefficient, variance and standard deviation) obtained, the best calibration was found for sunflower and safflower at 40°C and castor at 44°C among the temperature ranges tested.

Keywords: Calibration, nuclear magnetic resonance spectrophotometer, oil seed crops, oil content analysis.

IN crop improvement programmes, screening of a large number of seed samples is required and precise estimation of oil content is needed, especially for developing superior cultivars with increased seed oil content. Oil content can be measured by direct methods (grinding the seed and extracting the oil using a solvent or by supercritical fluid extraction using CO_2), or indirect methods nuclear magnetic resonance (NMR) spectroscopy or near-infrared (NIR) spectroscopy). Direct methods are slow, laborious and expensive. In addition, many hazardous chemicals are used in such estimations. Supercritical fluid extraction is reasonably fast but requires high maintenance and the cost of compressed CO_2 used to extract the oil is also significant. When protons are placed in a magnetic field, they acquire the ability to resonate when irradiated with radio waves of the correct frequency. The NMR technique measures the resonance energy absorbed by protons, while NIR utilizes the absorption of near-infrared energy (1100–2500 nm). NIR spectroscopy is commonly used to estimate oil content, but it is difficult to apply on opaque samples as it can only scan the surface. It is also complex and time-consuming to calibrate (requires a large number of samples), and therefore it is difficult to maintain accurate results on a large variety of product types. Hence, NIR has limited applicability for the estimation of oil content in oilseeds. In contrast, NMR provides a simple, accurate and inexpensive measurement of oil content in oilseeds as it requires small sample size to calibrate. Proton NMR methods give accurate, precise and reproducible results in comparison to the conventional method of extraction1. Seed asymmetry, hydrogen content and moisture associated with the seeds have been shown to affect seed oil estimation by NMR2. In some cases, it is necessary to preheat samples to liquefy fat, e.g. cocoa beans which contain a high percentage of saturated fat that is semi-solid at room temperature, or when the laboratory temperatures vary from 1°C to 2°C (ref. 3). Hence, temperature is also an important factor that affects the reproducibility of quantitative results. It should be kept constant throughout the study, even during the calibration of the external reference used for quantitation. Temperature variation also affects the relaxation properties of the molecules4. Data on the effect of sample conditioning temperature on oil content are meagre.

Whenever rapid and accurate estimation of oil content is the primary concern, simple calibration maintenance is desired. Keeping all this in view, the present study was undertaken to determine the effect of conditioning temperature variation on the oil content data of NMR estimations.

Six genotypes each of sunflower, safflower and castor seeds having a wide range with respect to oil content were used for the analysis (Table 1). Unless stated otherwise, all experiments were replicated six times.

Oil from seeds was extracted in hexane on soxhlet apparatus (Extraction unit, E-816, Buchi). Seeds were oven-dried at 80°C for 8 h. Five grams of dry seeds was ground using mortar and pestle and taken into a cellulose thimble of 25 mm × 80 mm size (Whatman, England) suspended onto the thimble holder. The pre-weighed extraction beaker was then kept on the hot plate of the equipment. For complete extraction of oil, the extraction, rinsing and drying process was carried out for at least 80 (30 cycles), 60 and 30 min respectively. Level of the extraction solvent was maintained by putting extra solvent from the top of the collecting vessel. Then the extraction beaker containing oil and hexane was removed from the hot plate and transferred into an oven maintained at 55°C for evaporating traces of the solvent. The beaker containing oil was weighed. The amount of oil was calculated by taking the difference between the weight of the beaker before and after extraction. Results are expressed as percentage of seeds.

A bench-top pulsed NMR-MQC-5 analyser (Oxford, London) supplied with preloaded 'easy cal' software was calibrated at different conditioning temperatures in 2013–14. Before making the calibration curve, seeds were dried by keeping them in a hot-air oven at 80°C for 8 h. The calibration was performed with 40 mm diameter sample

*For correspondence. (e-mail: praduman1311@gmail.com)

Table 1. Oil content of sunflower, safflower and castor genotypes by soxhlet method

Sunflower genotype	Oil%	Safflower genotype	Oil%	Castor genotype	Oil%
NSFH-1	33.85	NARI-57	37.80	GCH-2	41.40
KBSH-1	35.80	NARI-52	32.51	DCH-177	42.10
KBSH-44	38.73	A-1	25.18	GCH-6	47.24
KBSH-53	38.95	NARI-38	25.83	GCH-4	45.70
DRSH-1	41.4	Bhima	31.94	DCH-519	50.44
DRSF-108	36.51	NARI-NH-1	35.74	48-1	52.21

Table 2. Statistical parameters of developed calibration process

Crop	Conditioning temperature (°C)	Fitted equation	Correlation coefficient (R^2)	Standard deviation (SD)	Variance
Sunflower	36	$y = 106.55x - 1061$	0.99	0.38	0.14
	38	$y = 106.87x - 1055.52$	1.00	0.17	0.03
	40	$y = 106.37x - 1031.90$	1.00	0.09	0.01
	42	$y = 113.71x - 1302.68$	1.00	0.23	0.05
	44	$y = 126.91x - 1821.87$	0.98	0.51	0.26
	46	$y = 140.40x - 2267.26$	0.95	0.78	0.61
Safflower	36	$y = 81.20x - 176.12$	0.99	0.53	0.28
	38	$y = 77.59x - 65.79$	1.00	0.30	0.09
	40	$y = 79.06x - 101.73$	1.00	0.22	0.05
	42	$y = 87.28x - 348.90$	1.00	0.43	0.18
	44	$y = 87.02x - 359.30$	0.99	0.61	0.37
	46	$y = 84.87x - 306.28$	0.98	0.84	0.70
Castor	36	$y = 12.35x + 940.06$	0.90	1.97	3.89
	38	$y = 14.68x + 832.52$	0.96	1.17	1.36
	40	$y = 12.11x + 670.82$	0.99	0.67	0.45
	42	$y = 13.42x + 891.11$	0.99	0.65	0.42
	44	$y = 14.02x + 863.09$	1.00	0.12	0.01
	46	$y = 15.96x + 775.64$	0.99	0.53	0.29

y, NMR data; *x*, Concentration.

probe, 5 MHz operating frequency, four scans, 1 sec recycle delay and 40°C magnetic box temperature. NMR room temperature was maintained at 23 ± 2°C. The calibration was made by conditioning the samples in a heating block for 90 min at different temperatures from 36°C to 46°C at 2°C interval using reference values obtained from the soxhlet method.

All statistical analyses were performed on replicated data and the results were expressed as mean. The data were compared based on statistical parameters (correlation coefficient (*R*2), standard deviation and variance) with fitted line obtained using 'easy cal' software of NMR.

Oven-dried seeds of sunflower; safflower and castor were used for the analysis. The seeds were maintained at different temperatures (36–46°C) for conditioning before the analysis. According to the manufacturer's directions, the optimum temperature for analysing samples by NMR is 40°C. A linear calibration curve was constructed for oil content by measuring the NMR resonance signal, normalizing by sample mass and plotting against the sample of known oil content. Study results indicate that small difference in conditioning temperature can have a substantial difference in NMR data. In case of sunflower and safflower at 40°C, sample conditioning resulted in obtaining a good calibration curve, but in case of castor it showed variation. In order to determine the ideal sample temperature conditions, a study was done with different degrees (36–46°C) of sample temperature conditions. It indicates that keeping castor sample conditioning temperature at 44°C gave a correlation coefficient of $R2 = 1$, with minimum standard deviation (0.12) and variance (0.01) compared at 40°C. It also indicates the optimum temperature for sample conditioning is 44°C, which resulted in accurate estimation of oil content in castor (Table 2).

In an oilseed with less than 8% moisture, the signal intensity in the spin echo arises solely from the oil because it is the only component in the liquid phase. No other component has to be accounted during the calibration. Therefore, calibration is a simple linear equation of increasing signal proportional to increasing oil content. NMR response is received from the whole seed sample,

Table 3. Comparison of reference oil content (soxhlet values) of six castor genotypes by NMR calibrations at 40°C and 44°C conditioning temperature

Genotype	Oil% (reference)	Oil% (40°C)	Oil% (44°C)	Difference columns 2,3	Difference columns 2,4
GCH-2	41.4	40.77	41.52	0.63	0.12
DCH-177	42.1	43.38	41.94	1.28	0.16
GCH-6	47.24	48.31	47.39	1.07	0.15
GCH-4	45.7	45.32	45.81	0.38	0.11
DCH-519	50.44	49.29	50.56	1.15	0.12
48-1	52.21	51.27	51.98	0.94	0.23
Mean	46.52	46.39	46.53	0.91	0.15

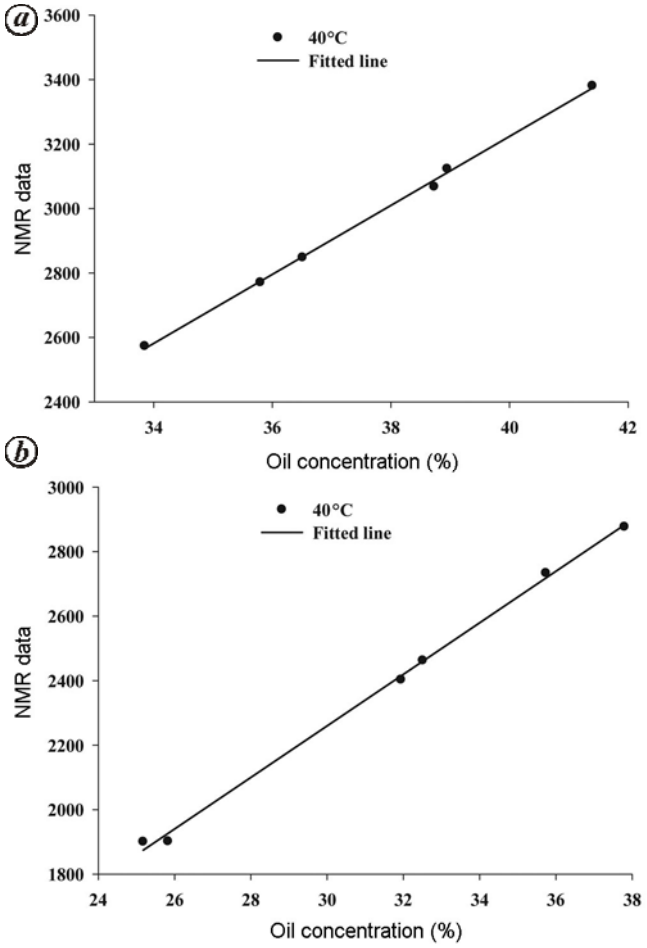

Figure 1. Calibration curve for (***a***) sunflower oil concentration and (***b***) safflower oil concentration at 40°C along with the fitted line ($R^2 = 1$).

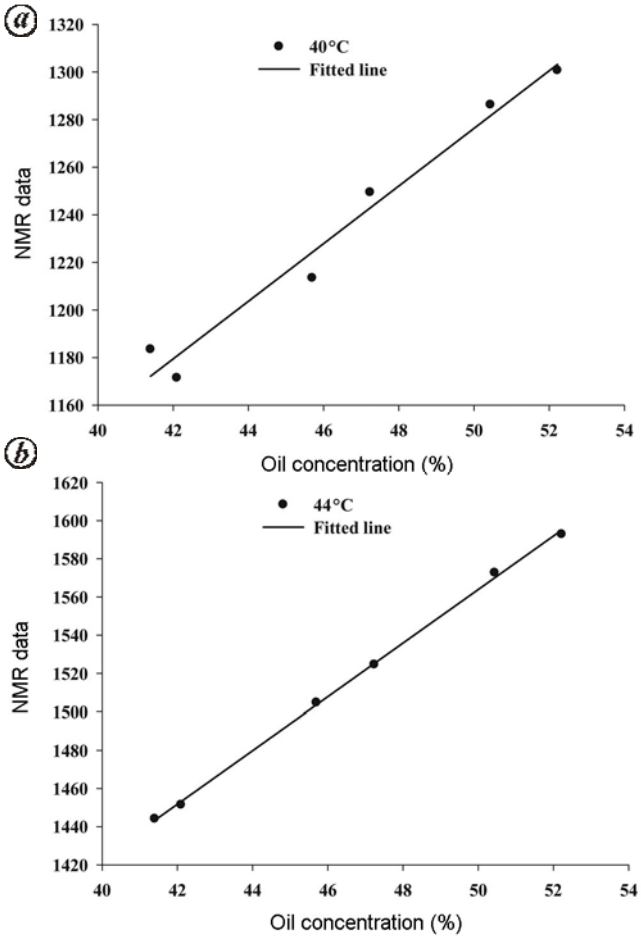

Figure 2. Calibration curve for castor oil concentration at (***a***) 40°C ($R^2 = 0.99$) and (***b***) 44°C along with the fitted line ($R^2 = 1$).

since in NMR duration of re-emitted resonance signal depends on the physical phase of the proton-containing material. Signals from H^+ in solids tend to have very short durations, whereas signals from H^+ in liquid tend to last much longer. NMR uses this to distinguish between different phases in samples in order to eliminate a solid background signal[5]. Hence, viscosity of the oil plays a crucial role in the determination of oil content. The fatty acid profile is another factor to be considered in NMR calibration[6]. Fatty acids present in the oilseeds differ significantly in the percentage of hydrogen and iodine value. It is well known that chain length, saturation and unsaturation, and hydroxyl group have a direct influence on the viscosity of the oil[7]. Castor has a unique fatty acid called ricinoleic acid (12-hydroxy-9-*cis*-octadecenoic acid) with hydroxyl group. NMR analyser maintains 40°C magnet temperatures during sample analysis and normalizes (conditioning before analysis) the sample temperature.

Below this temperature, NMR signals may vary from the actual. For accurate analysis seed temperature should be 40°C. Since room temperature is around 25°C during the analysis, it is obvious that while transferring the sample tube from conditioning block to NMR, the temperature will go below 40°C. Castor seed has a hard outer layer (seed coat) compared to other oilseeds crops and its oil is positioned in the endosperm rather than kernel of the seed. At 40°C, viscosity of castor oil is $2.7135 \times 10–1$ Pa,S (ref. 8) which is high compared to sunflower oil $0.3627 \times 10–1$ Pa,S (ref. 7) and safflower oil $0.2999 \times 10–1$ Pa,S (ref. 9) at the same temperature. In case of sunflower and safflower, NMR magnet conditioning before the actual analysis is adequate to bring the desired temperature (Figure 1 *a* and *b*), but in case of castor results show that by conditioning the castor seeds at 44°C, temperature loss can be compensated (Figure 2 *a* and *b*). To study the actual variation in oil content, six reference castor seed samples were analysed by making a calibration curve at 40°C and 44°C respectively (Table 3). It was found that calibration made at 40°C gave 0.91% variation in oil content, while that made at 44°C gave only 0.15%. Hence, by keeping sample conditioning temperature at 44°C, a good calibration curve and precise oil content of castor seeds can be determined.

NMR spectroscopy is one of the most important and widely used methods for oil content determination in the oilseed crops. In this study, the effect of change in sample conditioning temperature on calibration statistics and calibration linearity was described, and NMR spectrometry has been calibrated with respect to conditioning temperature for castor seed oil measurements. The study concludes that optimum sample conditioning temperature for sunflower and safflower seeds is 40°C, while for castor seeds it is 44°C.

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Arsenic and other metals in the groundwater samples of Ranchi city, Jharkhand, India

Poonam Tirkey, Tanushree Bhattacharya* and Sukalyan Chakraborty

Department of Civil and Environmental Engineering,
Birla Institute of Technology, Mesra, Ranchi 835 215, India

This study was aimed to monitor and quantify the metals present in the groundwater of Ranchi city, state capital of Jharkhand, India. Samples were collected from 44 locations during three seasons. The results show that arsenic concentration ranged from 0 to 0.2 and 0 to 0.015 mg/l in monsoon and pre-monsoon seasons respectively, but was below detection limit in the post-monsoon season. Manganese varied from 0 to 4.199, Nickel from 0 to 0.077, Selenium from 0 to 0.14, and Fe varied from 0 to 0.047 mg/l. Presence of toxic metals, especially arsenic, above acceptable limits is alarming and needs immediate attention.

Keywords: Arsenic, groundwater, land use, metal contamination.

DEGRADATION of freshwater resources in terms of quality and quantity is posing a great threat to human civilization. Moreover, metal contamination in freshwater resources can lead to serious public health concern. Metals in groundwater generally originate from weathering of minerals and rocks which can cause contamination of water resources. However, anthropogenic activities like disposal of industrial effluents from metal processing, storage, battery, chemicals and glass industries, release of

*For correspondence. (e-mail: tbhattacharya@bitmesra.ac.in)