# A method for rapid assessment of oil and protein contents in whole rapeseed kernels

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## Introduction

Rapeseed (*Brassica napus* L.) is appreciated for its high oil and protein contents and its applications for food, feed or industrial uses. Rapeseed oil can also be used for biodiesel production, which is the use that has seen the most dramatic expansion over the last ten years. Ethiopian mustard (*Brassica carinata* A. Braun) is another *Brassica* species of agronomic importance that has been included in several plant breeding programmes.[1]

Near infrared (NIR) spectroscopy is widely used to determine quality components in cereal grains, and also lately for the analysis of quality parameters in rapeseed. Most authors using NIR spectroscopy for analysing the seed composition of *Brassica* have focused their efforts on the species of *B. napus* and its double zero lines, because of the commercial interest of this species.[2,3] In contrast, those studies using NIR spectroscopy for determining the quality parameters of other *Brassica* species are scarce and have been focused on *B. campestris*, *B. juncea* and *B. carinata*.[1,4,5]

The aim of this study was the development of NIR calibrations for oil and protein contents in intact seeds of three cultivars of rapeseed (*Brassica napus* L.) and one cultivar of Ethiopian mustard (*Brassica carinata* A. Braun) collected in two different harvest years.

## Materials and methods

A field experiment including four *Brassica* varieties was carried out under non-irrigated conditions in the semiarid area of the province of Salamanca (Western Spain). The sowing season was in the fall of two consecutive years (2005 and 2006); a random design with four replications and several fertilising treatments was used.[6]

Prior to both chemical and NIR analysis, the total of 189 seed samples were cleaned and oven-dried at 60° C. For chemical analysis samples were taken immediately after grinding, and analysed by means of conventional chemical methods. Nitrogen content was determined by Kjeldahl analysis,[7] and a factor of 6.25 was used to estimate the protein content. Oil content

**Figure 1.** NIR measurement of *Brassica napus* and *B. carinata* intact seeds.

was determined by using nuclear magnetic resonance (NMR), according to the protocol of the AOACS.[8] Whole seed samples were scanned in a spectrophotometer NIRSystems 6500 (Silver Spring, MD, USA) over a wavelength of 400–2498 nm at 2 nm intervals. Analysis was performed using a transport module and samples were scanned on standard ring cells (Figure 1).

Mathematical procedures on the spectral information were carried out with WinISI v. IV (Infrasoft International, USA). In order to define the population and to select samples for equation calibration development the CENTER and SELECT algorithms were used.[9] Calibration equations were developed by means of modified partial least-squares regression (MPLSR) using different data pre-processing treatments: 0,0,1,1 (raw data); 1,4,4,1 (first derivative) and 2,5,5,2 (second derivative).

## Results and discussion

The range, mean and standard deviation of the parameters analysed by reference methods and the statistical results of the calibration development are summarised in Table 1.

Oil and protein content of seeds had a wide range of concentration as a consequence of including the different sources of heterogeneity considered (seeds from two species of *Brassica* including four varieties, different fertilization treatments and sampling years). The results of the calibrations were acceptable for the three mathematical treatments. Nevertheless, for both parameters the second derivative pre-processing showed the best results in the external validation (Table 1).

The external validation statistics obtained in the calibration for oil content displayed a greater predictive capacity than those reported by Font *et al.*[1] and Tkachuk[5] for *Brassica* species; the inclusion of several species in the studies of these workers could explain the differences observed in their standard errors of prediction (*SEP* = 0.83% and 1.49%, respectively). Other publications reported poor validation statistics with coefficient of determination ($r^2$) and *SEP* values which varied, respectively, from 0.71 to 0.85 and from 0.80% to 1.87%.[10,11] This could have been due to the use of a narrower wavelength range, only including the NIR region, or an interval within that region. Nevertheless, an identical $r^2$ value to our and similar *SEP* and ratio performance devia-

**Table 1.** Calibration and validation statistics obtained by MPLSR for the oil and protein contents in rapeseeds.

| Attribute | *n* | Range | Mean | *SD* | Treatment | Calibration | | Validation | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $R^2$ | *SEC* | $r^2$ | *SEP* | *RPD* |
| Oil (%) | 133 | 34.1–48.4 | 42.3 | 3.82 | Log 1/*R* | 0.96 | 0.79 | 0.93 | 0.92 | 3.81 |
| | | | | | 1D | 0.98 | 0.63 | 0.97 | 0.64 | 5.48 |
| | | | | | **2D** | **0.98** | **0.51** | **0.98** | **0.54** | **6.50** |
| Protein (%) | 189 | 14.8–31.2 | 20.1 | 3.61 | Log 1/*R* | 0.96 | 0.80 | 0.92 | 0.80 | 3.52 |
| | | | | | 1D | 0.97 | 0.63 | 0.95 | 0.65 | 4.34 |
| | | | | | **2D** | **0.98** | **0.52** | **0.96** | **0.57** | **4.95** |

*n*: number of samples, *SD*: standard deviation, $R^2$: coefficient of multiple determination, *SEC*: standard error of calibration, $r^2$: coefficient of determination, *SEP*: standard error of prediction, *RPD*: ratio performance deviation.

tion (*RPD*) values, were found in a study based on databases incorporating spectra from different instruments.[12]

The results of the external validation for protein content were more accurate than other results reported previously in samples of *B. napus* and *B. campestris* varieties.[5,10,13] Our prediction error (2.9 about the mean) was similar to those found by Font *et al*.,[1] Tillmann *et al*.[11] and Salgó *et al*.,[14] which varied from 2% to 3% about the mean. However, Daun *et al*.[12] achieved a *SEP* value lower than ours in a study with samples of two species of *Brassica*.

## Conclusion

The accurate predictions provided by the NIR equations confirm that NIR technology could be very useful for the rapid quality evaluation of oilseed species in intact seed samples. The calibration equations developed are robust enough, due to the variability of the sample set which includes several varieties, fertilisation treatments and sampling years.

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## References

1. R. Font, M. Del Río-Celestino and A. De Haro-Bailón, *Ind. Crop Prod.* **24,** 307 (2006).
2. N.H. Hom, H.C. Becker and C. Möllers, *Euphytica* **153,** 27 (2007).

3. T. Sato, *Plant Prod. Sci.* **11,** 146 (2008).
4. R. Font, M. Del Río, J.M. Fernández and A. De Haro, *J. Agric. Food Chem.* **52,** 3563 (2004).
5. R. Tkachuk, *JAOCS* **58,** 819 (1981).
6. A. García-Ciudad, C. Petisco, B.R. Vázquez de Aldana, L. García-Criado, S. Vicente Tavera and B. García-Criado, *Tierras de Castilla y León: Agricultura* **148,** 14 (2008).
7. AOAC *Official Methods of Analysis.* Association of Official Analytical Chemists, Arlington, USA (1990).
8. AOACS *Official Methods and Recommended Practices.* American Oil Chemist´s Society. Champaign, IL, USA (1980).
9. J.S. Shenk and M.O. Westerhaus, *Crop Sci.* **31,** 469 (1991).
10. V. Míka, P. Tillmann, R. Koprna, P. Nerusil and V. Kucera, *Plant Soil Environ.* **49,** 141 (2003).
11. P. Tillmann, T.C. Reinhardt and C. Paul, *J. Near Infrared Spectrosc.* **8,** 101 (2000).
12. J.K. Daun, K.M. Clear and P. Williams, *JAOACS* **71,** 1063 (1994).
13. L. Velasco and C. Möllers, *Euphytica* **123,** 89 (2002).
14. A. Salgó, Z. Fábián, E. Ungár and Z.S. WeinbrennerVarga, in *Making light work: advances in near infrared spectroscopy*, Ed by I. Murray and I. Cowe. Ian Michael Publications, Chichester, p. 336 (1992).