Wild Mustard (*Sinapis arvensis L.*) and the Analysis of Canola (*Brassica napus L.* and *Brassica rapa L.*) by NIR and NMR

James K. DAUN, Douglas DeCLERCQ, H. Kenneth HOWARD, Kathleen M. CLEAR AND C. Thomas THORSTEINSON

Canadian Grain Commission, Grain Research Laboratory,
1404-303 Main St., Winnipeg, MB R3C 3G8, CANADA

Abstract.

Wild mustard or charlock (*Sinapis arvensis* L.) is a common weed seed contaminant in rapeseed and canola. The similarity in shape and size between wild mustard and rapeseed and canola make separation and even identification of admixed seeds difficult. Besides lowering oil content, wild mustard contamination contributes significant amounts of erucic acid and glucosinolates to the seed with which it is mixed. In addition, the presence of wild mustard may cause difficulties in the instrumental analysis of components of canola including low results for oil content by NMR and NIR and low results for protein and glucosinolates by NIR. Low results in NMR analysis are at least partially due to differences in hydrogen density caused by wild mustard. NIR is a possible mechanism for accurate determination of wild mustard contamination in canola and rapeseed.

Introduction.

Sinapis arvensis L., a common weed seed contaminant in rapeseed and canola, is a cruciferous weed commonly known as wild mustard in North America or charlock in Europe . The similarity in colour, shape and size between wild mustard and rapeseed and canola make separation and even identification of admixed seeds difficult. Canadian grade specifications currently allow up to 5% contamination of wild mustard without penalty[1]. Wild mustard analysis requires microscopic examinaton of seeds in a sample in order to detect the wild mustard seeds which are similar in colour but are more round in appearence, have a stippled seed coat and lack the distinctive crease of the *Brassica napus* and *Brassica rapa*.

Wild mustard has less oil and more protein than canola[2] . It also has about 10 times as much glucosinolate although the predominant glucosinolate found in wild mustard is sinalbin (hydroxybenzylglucosinolate) which is not found in *B. napus* or *B. rapa*. The differences in composition mean that wild mustard contamination in canola has a serious effect on the quality of the product[3]. *S. arvensis* from North America has been shown to have a different fatty acid composition to S. arvensis from other parts of the world. The erucic acid content of North American S. arvensis is only 6-8% compared with 35% in that from other locations. Even with the reduced erucic acid content, Canadian *S. arvensis* has a significantly different fatty acid composition to canola and only small amounts of contamination can cause problems with oil specifications.

In addition to decreasing the quality of the seed, the presence of wild mustard may cause difficulties in the instrumental analysis of components of canola. In this work we show how wild mustard contamination leads to low results for oil content by NMR and NIR and low results for protein and glucosinolates by NIR.

Materials and Methods

The canola samples used in this study were derived from an agronomic study designed to evaluate the effect of a wild mustard controlling herbicide on the yield and quality of canola[3]. This study provided us a selection of samples of *B. napus* and *B. rapa* canola from two different years and with a range of admixture of wild mustard ranging from less than 1 to over 80%.

Oil contents were determined by extraction according to the FOSFA method[4] and also by NMR spectroscopy using a multipoint calibration procedure described by FOSFA[5]. Calibration standards were all canola seeds. Protein was determined as nitrogen x 6.25% on a LECO FP 428 Nitrogen Analyzer according to the AOCS method[6]. Glucosinolates were determined by HPLC according to the ISO method[7].

NIR analysis was carried out on a NIRSystems 6500 spectrometer which allows analysis of whole seeds and scans spectra from 400 nm to 2500 nm. For this study, the NIR instrument was calibrated for determination of oil, protein and glucosinolates using canola samples from harvest surveys. Whole seed samples were scanned in reflectance mode and the calibrations were prepared using partial least squares analysis of the log 1/R spectra[8].

Results and Discussion

The NMR instrument was calibrated with canola seed having oil contents ranging from 36% to 50%. Although most of the samples contaminated with wild mustard had oil contents within the calibration range, NMR results were progressively lower with increasing wild mustard contamination (Figure 1). It was possible to explain the NMR effect by the differences in hydrogen density between canola and wild mustard. Hydrogen density was calculated as the average number of hydrogen atoms per triglyceride molecule based on the fatty acid composition. Correction of results for hydrogen density removed the bias from the NMR determination.

A similar effect was noted for determination of oil content by NIR. Differences between true oil content and NIR determined oil content increased with increasing wild mustard with the NIR method finding more oil than was truly present (Figure 2). An effect inverse to the oil effect was noted for the NIR determination of protein. With increasing wild mustard contamination, the variance between true protein and NIR protein increased with the NIR finding less protein than was really present (Figure 3).

The largest difference in results was for glucosinolates where the difference between NIR glucosinolates and HPLC glucosinolates was approximately equivalent to the sinalbin content of the sample (Figure 4). This suggests that, glucosinolate wavelengths chosen are not universal since the NIR was calibrated with samples containing little or no sinalbin.

Differences in the spectra between wild mustard and canola seeds (Figure 5) suggested that it would be possible to use NIR as a tool to rapidly determine the extent of contamination. A calibration set was prepared using samples with a range of wild mustard from 0 to 100%. Whole seed samples were scanned between 400 and 2500 nm in the reflectance mode and a calibration for wild mustard content was determined using multiple linear regression of the 2nd derivatized spectra. The calibration showed good linearity with R^2 of 0.94 and a standard error of calibration of 0.5%. When the prediction set was analyzed however, results were not as favorable with an standard error of prediction of 9% and R^2 of 0.885 (Figure 6). One of the problems was that, although the calibration set included samples of both dark colored *B. napus* yellow-colored *B. rapa* varieties, the spetrometer reported that two samples of pure yellow seeded *B. rapa* were had 60-70% wild mustard. Further work with different coloured varieties will be required to resolve the problem of this type of outlier.

Based on the above results, it will be some time before we will be able to replace the human eye with an NIR instrument for the accurate detection of wild mustard contamination in canola and rapeseed.

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Figure 1 : Differences between NMR and Extraction values for oil content for samples with different levels of wild mustard contamination

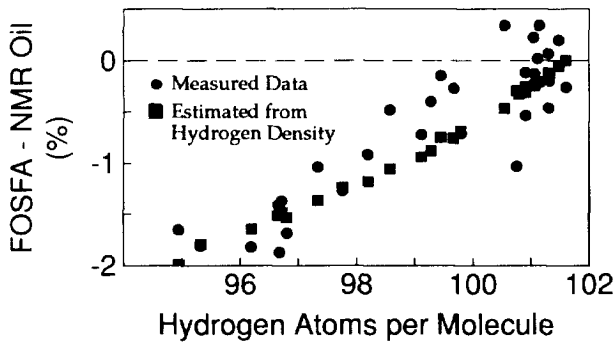


Figure 2 : Differences between oil content determined by NIR and extracted oil content with increasing wild mustard

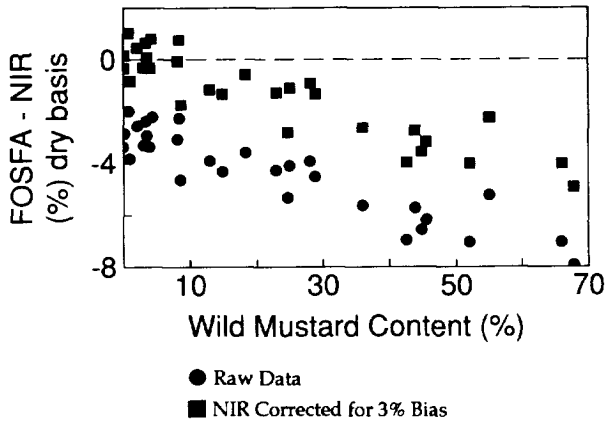


Figure 3 : Differences between protein content determined by NIR and protein content determined by combustion (% N x 6.25) with increasing wild mustard

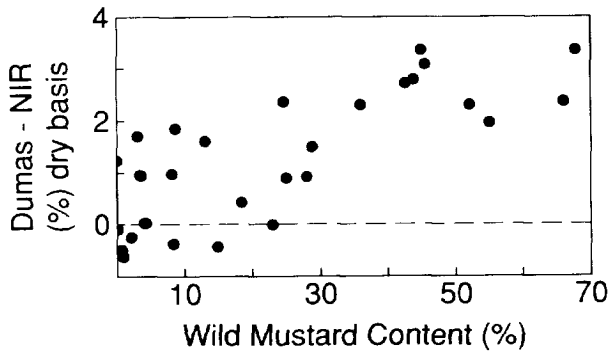


Figure 4 : Differences between glucosinolate content determined by NIR and glucosinolate content determined by HPLC with increasing wild mustard

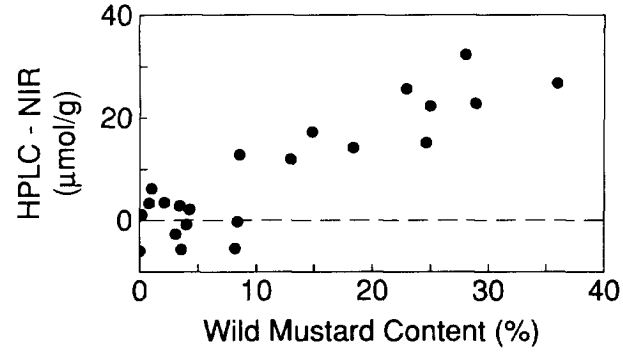


Figure 5 : Near Infrared spectra of whole seed samples of *S. arvensis* L. (solid line), *B. napus* L. (dotted line), and *B. rapa* L. (dashed line)

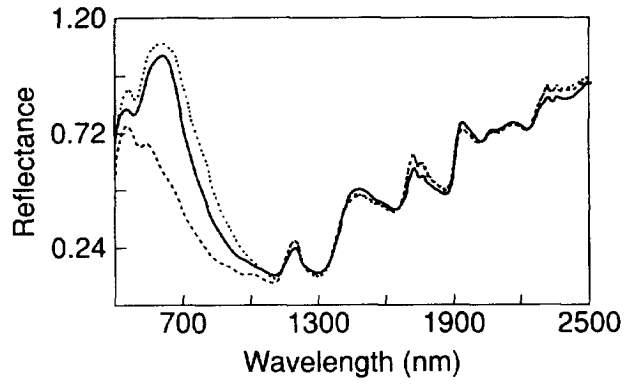
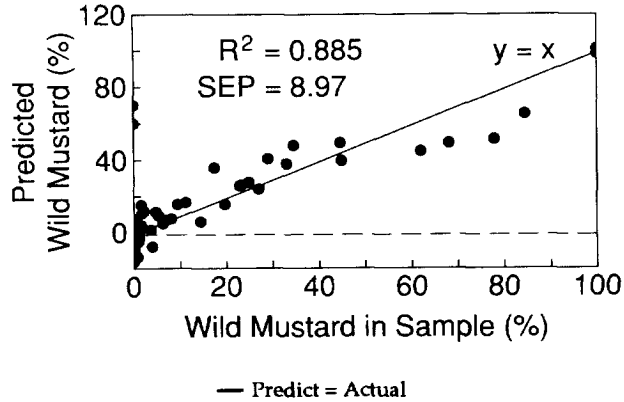


Figure 6 : Prediction plot for determination of wild mustard content by NIR spectroscopy



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