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Development of near-infrared spectroscopy calibrations to measure quality characteristics in intact Brassicaceae germplasm☆

Emily A. Oblath[a,*], Terry A. Isbell[a], Mark A. Berhow[a], Brett Allen[b], David Archer[c], Jack Brown[d], Russell W. Gesch[e], Jerry L. Hatfield[f], Jalal D. Jabro[b], James R. Kiniry[g], Daniel S. Long[h]

[a] USDA-ARS-NCAUR, Peoria, IL 61604, USA
[b] USDA-ARS-NPARL, Sidney, MT 59270, USA
[c] USDA-ARS-NGPRL, Mandan, ND 58554, USA
[d] Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, ID 83844, USA
[e] USDA-ARS-NCSCRL, Morris, MN 56267, USA
[f] USDA-ARS-NLAE, Ames, IA 50011, USA
[g] USDA-ARS-GSWRL, Temple, TX 76502, USA
[h] USDA-ARS-CPCRC, Pendleton, OR 97801, USA

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ABSTRACT

Determining seed quality parameters is an integral part of cultivar improvement and germplasm screening. However, quality tests are often time consuming, seed destructive, and can require large seed samples. This study describes the development of near-infrared spectroscopy (NIRS) calibrations to measure moisture, oil, fatty acid profile, nitrogen, glucosinolate, and chlorophyll content in six species from the Brassicaceae family. Rapeseed and similar oilseeds are potential feedstocks for producing hydrotreated renewable jet fuel. Screening samples with NIRS would allow cultivars with desirable characteristics to be quickly identified. A total of 367 samples of six species (*Brassica napus*, *Brassica carinata*, *Brassica juncea*, *Brassica rapa*, *Sinapis alba*, and *Camelina sativa*) were scanned with NIRS. Global calibrations for all six species were developed using modified partial least squares regression with reference values obtained through wet chemistry techniques. Comparing predicted values to reference data, the coefficients of determination (r^2) and ratios of performance to deviation (RPD) varied, with some calibrations performing better than others. The calibration equations for seed oil content (r^2 = 0.98, RPD = 7.3) and nitrogen (r^2 = 0.98, RPD = 5.3) performed very well while the equations for seed moisture (r^2 = 0.93, RPD = 3.8) and total glucosinolate content (r^2 = 0.92, RPD = 2.3) were more qualitative. Large variation was observed for chlorophyll content (0–390 mg/kg) so two calibration equations were developed, one for the higher and one for the lower range of values. When combined, these calibrations also showed very good performance (r^2 = 0.99, RPD = 14). The performance of the calibrations for the fatty acids was more varied, with some performing very well, such as the calibration for $C_{18:3}$ (r^2 = 0.99, RPD = 9.9), and others, such as $C_{22:0}$ (r^2 = 0.69, RPD = 1.9), showing poor correlation.

1. Introduction

Jet fuel is primarily refined from crude petroleum feedstocks, but recent concerns over supply, cost, and sustainability have led to interest in the development of alternative feedstocks. Hydrotreated renewable jet (HRJ) fuels from plant oils including *Camelina, Jatropha,* and algae have been commercially demonstrated and cer-

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* Corresponding author at: USDA-ARS-NCAUR, 1815 N. University St Peoria, IL 61604, USA.
E-mail addresses: emily.oblath@ars.usda.gov (E.A. Oblath), terry.isbell@ars.usda.gov (T.A. Isbell), mark.berhow@ars.usda.gov (M.A. Berhow), brett.allen@ars.usda.gov (B. Allen), david.archer@ars.usda.gov (D. Archer), jbrown@uidaho.edu (J. Brown), russ.gesch@ars.usda.gov (R.W. Gesch), jerry.hatfield@ars.usda.gov (J.L. Hatfield), jay.jabro@ars.usda.gov (J.D. Jabro), jim.kiniry@ars.usda.gov (J.R. Kiniry), dan.long@ars.usda.gov (D.S. Long).

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tified for aviation use, but full scale production has not occurred because of higher costs and concerns over competition with food production (Liu et al., 2013; Rye et al., 2010). Rapeseed, the industrial oil, non-food form of *Brassica napus*, is another potential source for producing HRJ fuel that has the potential to be a beneficial rotation crop in wheat-growing regions (Gan et al., 2003; Kirkegaard et al., 2008; Ryan et al., 2006). Work on processing *B. napus* has been ongoing since the 1940s and the process of extracting oil from rapeseed is well understood (Hopkins, 1977; Ward, 1984). *B. napus* and potential competitors *Brassica carinata, Brassica juncea, Brassica rapa, Camelina sativa,* and *Sinapis alba* have high potential seed oil contents, some with greater than 50% total oil reported (Blackshaw et al., 2011; Taylor et al., 2010; Wang et al., 2010).

One challenge to using *B. napus* and other Brassicaceae oilseeds as feedstocks for HRJ fuel is minor seed components including glucosinolates and chlorophyll that can cause poor oil quality and additional refining costs. Glucosinolates are small organic molecules derived from glucose and amino acids containing sulfur and nitrogen. High levels of seed glucosinolates can cause higher sulfur content in biofuels (Soriano and Narani, 2012) but using lower sulfur jet fuel can improve air quality (Unger, 2011). Levels of total glucosinolates in *Brassica* seeds have been reported from 6 to 193 μmol/g (Velasco et al., 2008; Velasco and Becker, 1998a). Under stress conditions such as drought, these levels can nearly double (Bouchereau et al., 1996; Jensen et al., 1996). Chlorophyll presents a problem for oil quality because it is capable of poisoning the hydrotreating catalysts (Irandoust and Edvardsson, 1993). Levels of chlorophyll in *Brassica* seeds have been reported from 3 to 64 mg/kg with higher values in immature green seeds (Daun et al., 1994; Khattab et al., 2010).

It is desirable to rapidly screen seed samples for glucosinolate and chlorophyll content in addition to other quality characteristics including moisture, total oil, and nitrogen content, and fatty acid profile. Wet chemical methods can be time-consuming, destructive, and expensive. Analysis by near-infrared spectroscopy (NIRS) is both fast and non-destructive. NIRS calibrations for whole seeds of various *Brassica* species have been developed for oil content (Velasco et al., 1999a), moisture content (Hom et al., 2007), protein content (Petisco et al., 2010), fatty acid profile (Velasco and Becker, 1998b), chlorophyll content (Daun et al., 1994), glucosinolate content (Font et al., 2006; Velasco and Becker, 1998a), and other seed characteristics. NIRS calibrations have also been developed for analyzing characteristics of *C. sativa* seeds (Vollmann et al., 1997) and *S. alba* seeds (Jiang et al., 2013). Since several *Brassica* species as well as *C. sativa* and *S. alba* oilseeds are potential feedstocks for HRJ fuel, it is desirable to have a global calibrations covering all of the seed characteristics for all of the species of interest. In this study, we have developed calibrations for moisture, total oil, fatty acid profile, nitrogen, glucosinolate, and chlorophyll content of whole *B. napus*, *B. carinata*, *B. juncea*, *B. rapa*, *C. sativa*, and *S. alba* seeds with the same calibrations used for all six species.

2. Materials and methods

2.1. Samples

Seeds were obtained from an oilseed trial that was conducted during the 2013 growing season at seven sites across the United States: Ames, Iowa; Echo, Oregon; Mandan, North Dakota; Morris, Minnesota; Moscow, Idaho; Sidney, Montana; and Temple, Texas (Gesch et al., 2015). A total of 367 seed samples from 12 spring and 6 winter cultivars were used in this study: *B. carinata* spring ('080814EM' and 'AAC A110'), *B. juncea* spring ('Oasis' and 'Pacific Gold'), *B. napus* winter ('Amanda', 'Durola', 'Dwarf Essex', and 'Wichita') and spring ('DK3042RR', 'Gem', 'Invigor L130', and 'SC28'), *B. rapa* winter ('Largo') and spring ('Eclipse'), *C. sativa* winter ('Joelle') and spring ('CO46'), and *S. alba* spring ('Idagold' and 'Tilney').

2.2. NIR scanning

All 367 samples were scanned using a Foss XDS near-infrared Rapid Content™ Analyzer (Eden Prairie, MN) spectrophotometer with an iris adapter insert. Ring cup sample cells with a quartz window were used with a micro sample insert with a 1.8 cm inner diameter. Approximately 1 g of whole seed was placed in the ring cup sample cell and the cell was closed with a disposable foam board back. Scans were collected with a 14.5 mm spot size over a wavelength range of 400–2500 nm with 0.5 nm resolution and 32 scans were averaged for each sample.

2.3. Moisture

Moisture content was determined according to AOCS official method Ca 2c-25. Approximately 1 g of seed was weighed into a foil pan and dried for 3 h at 130 °C. The samples were then cooled in a desiccator for 1 h and weighed again. The difference in weight was used to calculate the percent moisture.

2.4. Total oil

Seed oil content was determined by pulsed Nuclear Magnetic Resonance (pNMR) with a Bruker Minispec mq-CU 20-series with firmware version 2.50Rev.00 using factory instrument setting 909_18A with a 0.47 T magnet maintained at 40 °C (Billerica, MA). The instrument settings were: NMR frequency 19.98 MHz, digital bandwidth 20,000 kHz, gain 63 dB, 16 scans with a 2 s recycle delay, pulse separation 3.5 ms, and a sampling window of 6.958–7.058. The instrument was calibrated using *B. juncea* oil on Kimwipes. The standard curve had a correlation coefficient of 0.999. Seed samples of 2.5–3.0 g were weighed into 18 mm × 150 mm test tubes (Pyrex, Corning, NY) and heated to 40 °C for at least 30 min before analysis with the pNMR. Oil content was determined on a dry weight basis, calculated using the moisture content.

2.5. Fatty acid profile

Fatty acid profile was determined with a fatty acid methyl ester extraction analyzed with gas chromatography (GC) similar to the procedure described in Alves et al. (2014). Fatty acid methyl esters were made by placing approximately 0.375 g of seed into a 20 mL scintillation vial with 5 mL of 0.25 M sodium methoxide in methanol. The seeds and solvent were mixed with a CAT Scientific X120 homogenizer (Paso Robles, CA) and incubated at 60 °C for 30 min. After cooling to room temperature, 5 mL of hexane followed by 5 mL of a saturated sodium chloride in water solution was added to the vial. Approximately 0.25 mL of the hexane layer was added to a 2 mL GC autosampler vial (Thermo Scientific, Rockwood, TN) and diluted to 2 mL with hexane.

A Hewlett-Packard 6890 gas chromatograph (GC) (Palo Alto, CA) equipped with a flame ionization detector (FID) and an Agilent 7683 autosampler/injector (Santa Clara, CA) was used to analyze the extract. Analyses were conducted on an SP-2380 30 m × 0.25 mm i.d. column (Supelco, Bellefonte, PA). GC conditions were as follows: programmed temperature ramp from 180 to 210 °C at 7 °C/min, then a ramp from 210 to 265 °C at 30 °C/min, and then a hold at 265 °C for 3 min. The injector temperature was 265 °C and the detector temperature was 250 °C with a helium flow rate through the column of 1 mL/min, a split ratio of 100:1, and a 4 mL/min septum purge. A standard mixture of C8-C30 saturated fatty methyl

esters from Nu-Check Prep (Elysian, MN) was used to identify retention times.

2.6. Nitrogen

Nitrogen content was determined by the Dumas combustion method with a LECO CHN628 instrument (St. Joseph, MI). Seeds were finely ground in a blade grinder (Mr. Coffee, Boca Raton, FL) and three replicates of approximately 150 mg of ground seed were analyzed for each sample. The calibration was made with EDTA standards from LECO over a range of 4.8–19.1 mg nitrogen.

2.7. Glucosinolates

Glucosinolate content was determined with high performance liquid chromatography (HPLC) similar to the procedure described in Berhow et al. (2013). Seeds were ground in a blade grinder, approximately 1.75 g of ground seed was weighed, placed into a filter paper packet, and defatted with hexane in a Foss Soxtec 2043 extraction unit (Eden Prairie, MN). After drying overnight, the percent hexane extractables was determined by the difference in weight. Then 0.25 g of defatted seed meal was placed in a vial with 4 mL of methanol, sonicated for 15 min, and left overnight at room temperature. After another brief sonication, the solution was filtered through a Millipore Millex-HV 0.45 μm PVDF filter (Billerica, MA) into a 2 mL autosampler vial.

For glucosinolate quantitation, samples were analyzed on an Thermo SpectraSystem HPLC system (SpectraSystem SCM1000 vacuum membrane degasser, P2000 binary gradient pump, and AS3000 autosampler) with a Spectra-Physics Spectra 100 variable wavelength UV/Vis detector (San Jose, CA) set to monitor at 237 nm. An Intersil reverse phase column (4.6 mm × 250 mm, ODS-3, 5 μm; GL Sciences, Inc., Torrance, CA) was used with a flow rate of 1 mL/min. The initial mobile phase conditions were 12% methanol/88% aqueous 0.005 M tetrabutylammonium bisulfate (TBAS). After injecting 20 μL of sample, the initial conditions were held for 2 min, then the methanol was increased to 35% over 20 min, then to 50% methanol over another 20 min, then to 100% methanol over another 10 min, and then held at 100% for 3 min. Freshly prepared sinigrin (Sigma-Aldrich, St. Louis, MO) standards diluted in water and sinalbin (Chromadex, Irvine, CA) standards diluted in methanol were used to make calibration curves. The sinigrin standard curve was used to calculate the concentrations of the aliphatic glucosinolates and the sinalbin standard curve was used to calculate the concentrations of the aromatic glucosinolates. Progoitrion and gluconapin (Chromadex) standards diluted in methanol were also analyzed to determine retention times.

To identify the glucosinolates found in the seed extracts, samples were also analyzed on a Thermo Electron LTQ Orbitrap Discovery Mass Spectrometer [a linear ion trap (LTQ XL) MS coupled to a high precision electrostatic ion trap (Orbitrap) MS with a higher energy C-trap dissociation (HCD) cell attached] with an Ion Max electrospray ionization (ESI) source and a Thermo Scientific ACCELA series HPLC system (ACCELA 1250 UHPLC pump, ACCELA1HTC cool stack autoinjector, and ACCELA 80HZ PDA detector) all running under Thermo Scientific Xcalibur 2.1.0.1140 LC–MS software. An Intersil reverse phase column (3 mm × 150 mm, ODS-3, 3 μm; GL Sciences, Inc.) was used with a flow rate of 0.25 mL min. The initial mobile phase conditions were 20% methanol with 0.1% formic acid/80% water with 0.1% formic acid. After injecting 1 μL of sample, the initial conditions were held for 2 min and then the methanol with 0.1% formic acid was increased to 100% over another 50 min. The MS was run with the ESI probe in the negative mode. The source inlet temperature was set to 300 °C, the sheath gas rate was set to 50 units, the auxiliary gas rate was set to 5 units, and the sweep gas rate was set to 2 units. The maximal mass resolution was set at 30,000, the spray voltage was 3.0 kV, and the tube lens was set at −100 V. The MS was calibrated with a standard calibration mixture recommended by Thermo Scientific. The software package was set to collect mass data between 100 and 2000 AMU. Generally, the most significant sample ions generated under these conditions were $[M-1]^-$ and $[M+HCOO]^-$. Six events were programmed to run in sequence in the MS detection scheme: (1) LTQ(IT)-MS full scan for *m*/*z* 150–2000; (2) LTQ(IT)-MS set to trap the most abundant ion above *m*/*z* 500 with CID at 35% energy, yielding a characteristic daughter ion for glucosinolates; (3) FT-MS(Orbitrap) full scan for *m*/*z* 150–2000, providing the accurate mass $[M-1]^-$ ion; (4) Mass-dependent MS/MS on the most abundant ion trapped by the IT-MS in event 1 with HCD performed at 25% energy and the resulting fragmentation ions detected by the FT-MS, providing the characteristic SO_4^- ion generated by HCD of any glucosinolate; (5) Mass dependent MS3 on the most abundant ion generated from event 2 with HCD performed at 25% energy with the resulting fragmentation ions detected by FT-MS; (6) Mass-dependent MS3 on the most abundant fragmentation ion generated from event 2 with CID at 35% energy and resulting ions detected by IT-MS. Glucosinolates were determined by the presence of the sulfate ion in event 4 and identified by the $[M-1]^-$ ion in event 3.

2.8. Chlorophyll

Chlorophyll content was determined though UV–vis spectrometry with a method adapted from AOCS official method Ak 2-92. Seeds were finely ground in a blade grinder, 1 g of ground seed was weighed into a 20 mL scintillation vial with 15 mL of extraction solvent (25% ethanol/75% isooctane), and the mixture was shaken for 1 h at 260 rpm. The solution was then filtered through Whatman #52 filter paper (Piscataway, NJ). The absorbance of the solutions was measured at 665, 705, and 625 nm with a Molecular Devices SpectraMax M5 UV–vis spectrophotometer (Sunnyvale, CA). The chlorophyll content was calculated using A_{corr} as described in the AOCS method. Calibration curves were made using crystalline chlorophyll A (Sigma-Aldrich) reconstituted in diethyl ether and diluted with the extraction solvent.

2.9. NIR calibrations

The NIR spectra from the samples were imported into WinISI version 4.6.8.14739 to develop calibration equations. Principle Component Analysis (PCA) was used to identify spectrally similar samples and select a subset of samples to analyze with the reference methods. All 367 samples were analyzed to determine moisture, total oil, and fatty acid profile (4 samples could not be analyzed for moisture content due to the small amount of sample available). A subset of 130 samples was analyzed for nitrogen content, 124 samples for glucosinolate content, and 123 samples for chlorophyll content. Those values were imported into WinISI and used to make the calibration. Three math treatments were tested for each calibration: 0,0,1,1 (raw data), 1,4,4,1 (first derivative), 2,4,4,1 (second derivative) each with and without SNV and detrend (scatter correction). Modified partial least squares (PLS) regression with cross-validation (4 groups) was used to develop the equations. Calibration models were compared using the coefficient of determination (r^2) and the standard error of calibration (SEC). For evaluating the calibrations, the ratio of performance to deviation (RPD), which is the ratio of the standard deviation of the reference data to the standard error of cross validation (SECV), was calculated (Williams, 2001).

Table 1
Calibration statistics for the equations developed for different characteristics of intact Brassicaceae seeds.

Characteristic	n[a]	Range[b]	Mean[b]	SD[b]	r^2[c]	SEC[c]	RPD[c]
Moisture (%)	363	3.1–13.8	5.9	1.7	0.93	0.39	3.8
Total oil (%)	363	17.6–51.0	37.5	7.3	0.98	0.90	7.3
$C_{16:0}$ (palmitic acid, %)[d]	367	3.0–7.5	4.4	0.9	0.91	0.25	2.8
$C_{18:0}$ (stearic acid, %)[d]	367	0.1–4.2	1.7	0.8	0.90	0.21	3.2
$C_{18:1}$ (oleic acid, %)[d]	367	7.1–65.5	34.0	20.8	0.98	3.12	5.1
$C_{18:2}$ (linoleic acid, %)[d]	367	10.6–27.1	17.9	3.6	0.89	1.19	2.6
$C_{18:3}$ (linolenic acid, %)[d]	367	4.7–38.6	13.1	8.7	1.00	0.59	9.9
$C_{20:0}$ (arachidic acid, %)[d]	367	0.0–2.8	1.3	0.4	0.89	0.12	3.1
$C_{20:1}$ (%)[d]	367	0.0–14.3	6.6	4.3	0.95	0.97	3.4
$C_{20:2}$ (%)[d]	367	0.0–1.7	0.6	0.6	0.95	0.13	4
$C_{22:0}$ (behenic acid, %)[d]	367	0.0–1.6	0.3	0.3	0.69	0.15	1.9
$C_{22:1}$ (erucic acid, %)[d]	367	0.0–47.9	15.7	16.2	0.97	2.83	5.3
$C_{22:2}$ (%)[d]	367	0.0–1.8	0.2	0.4	0.87	0.13	2.5
$C_{24:1}$ (nervonic acid, %)[d]	367	0.0–2.4	0.9	0.8	0.98	0.12	5.3
Nitrogen (%)	130	2.9–6.3	4.5	0.74	0.98	0.09	5.3
Glucosinolates (μmol/g)	124	5.5–117.4	44.2	29.2	0.92	8.19	2.3
Chlorophyll, low range (mg/kg)	86	0.0–17.6	4.1	4.8	0.93	1.16	2.2
Chlorophyll, high range (mg/kg)	51	10.8–389.8	107.9	105.6	0.99	9.68	3.8
Chlorophyll, combined (mg/kg)	123	0.0–389.8	46.0	85.5	0.99	6.1	14.0[e]

[a] Number of samples.
[b] Range, mean, and standard deviation are of reference data.
[c] r^2, SEC, and RPD are of the calibration model.
[d] Fatty acids are percentages of the total oil content.
[e] RPD for chlorophyll, combined was calculated as SD/SEC.

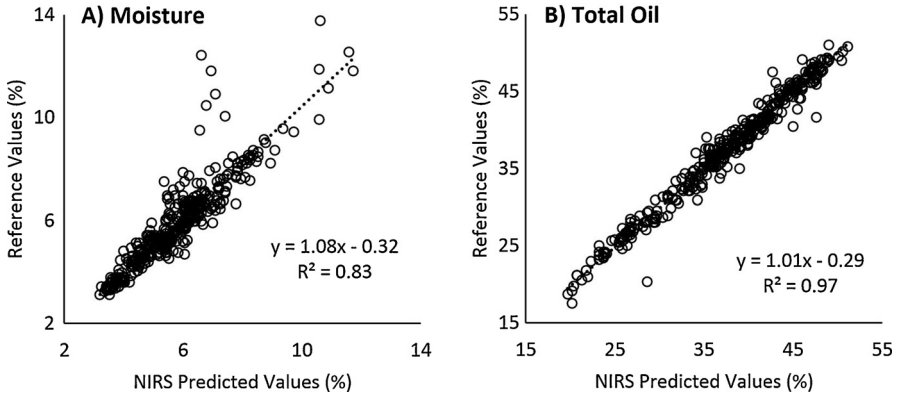

Fig. 1. Scatter plots of reference and NIRS predicted values for seed moisture (A) and oil content (B) calibrations.

3. Results and discussion

3.1. Moisture calibration

The samples used for the moisture calibration ranged from 3.1–13.8% moisture with a mean of 5.9% and a standard deviation of 1.7% (Table 1). The calibration settings were: math treatment 1,4,4,1, no scatter correction, 3 passes for outliers, and outliers downweighted. The calibration had an r^2 of 0.93, an SEC of 0.39%, and the RPD was 3.8. Reference values were highly correlated with the NIRS predicted values (Fig. 1a). Some outliers with predicted values much less than their reference values were observed, possibly due to a delay between when the moisture analysis was performed and when the samples were scanned with NIRS and varying humidity levels. Predicted outliers at the higher end of the calibration range suggest that it would be useful to dry the samples to a low moisture content before analysis, particularly since many of the other characteristics are calculated on a dry weight basis and may be affected by inaccurate moisture values.

3.2. Total oil calibration

The samples used in the total oil calibration ranged from 17.6–51.0% oil with a mean of 37.5% and a standard deviation of 7.3% (Table 1). The calibration settings were the same as those used for the moisture calibration. The calibration had an r^2 of 0.98, an SEC of 0.90%, and an RPD of 7.3. Similar to the moisture calibration, reference values were highly correlated with predicted values (Fig. 1b). The high RPD indicates a high prediction ability by NIRS for total oil content while covering a wide range of oil contents. Our calibration compares favorably with that developed by Petisco et al. (2010) for *B. napus* and *B. carinata* and the global calibration developed by Velasco et al. (1999b) for the Brassicaceae family.

3.3. Fatty acid calibrations

The performance of the calibrations developed for fatty acid composition was variable, with wide ranges in values and varying calibration statistics (Table 1). The calibrations for all 12 fatty acids used the following calibration settings: SNV/detrend scatter correction, 3 passes for outliers, and outliers downweighted. For $C_{16:0}$, $C_{18:0}$, $C_{18:2}$, $C_{20:0}$, $C_{20:2}$, $C_{22:0}$, $C_{22:1}$, $C_{22:2}$, and $C_{24:1}$, math treatment 1,4,4,1 was used, while for $C_{18:1}$, $C_{18:3}$, and $C_{20:1}$ the math treatment used was 2,4,4,1. Reference values showed different degrees of correlation with predicted values for $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ (Fig. 2). Several of the fatty acids had calibrations that performed very well, including those for $C_{18:1}$, $C_{18:3}$, and $C_{22:1}$ which agrees with that found by Velasco et al. (1999b). This is expected since

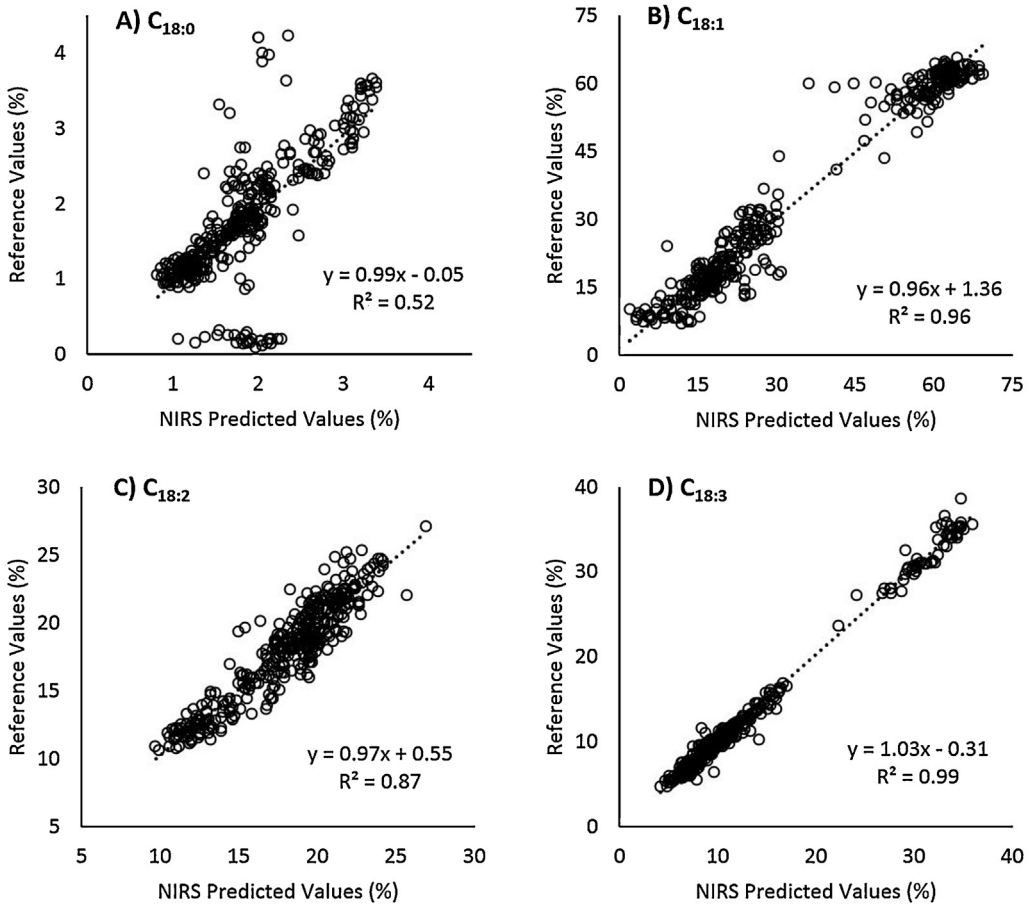

Fig. 2. Scatter plots of reference and NIRS predicted values for $C_{18:0}$ (A), $C_{18:1}$ (B), $C_{18:2}$ (C), and $C_{18:3}$ (D) fatty acid profile calibrations.

those three fatty acids are among the most abundant, with the largest range of values. Calibrations for a few of the less abundant fatty acids, including $C_{18:0}$, $C_{22:0}$, and $C_{22:2}$, were not suitable even for qualitative use.

3.4. Nitrogen calibrations

The samples used for the nitrogen calibration had reference values ranging from 2.9–6.3% nitrogen with a mean of 4.5% and a standard deviation of 0.7% (Table 1). The calibration settings were: math treatment 1,4,4,1, SNV/detrend scatter correction, 1 pass for outliers, and outliers were not downweighted. The calibration had an r^2 of 0.98, an SEC of 0.09%, and the RPD was 5.3. Predicted values were highly correlated with reference values (Fig. 3a). The high RPD indicates a high prediction ability for NIRS for nitrogen content. This calibration performs as well as those previously described by Daun et al. (1994) and Petisco et al. (2010) for protein, which is simply nitrogen content multiplied by the conversion factor of 6.25 (Mariotti et al., 2008).

3.5. Glucosinolates calibration

The samples used for the glucosinolates calibration had reference values ranging from 5.5–117.4 μmol/g with a mean of 44.2 μmol/g and a standard deviation of 29.2 μmol/g (Table 1). The calibration settings were the same as those used for the nitrogen calibration. The calibration had an r^2 of 0.92, an SEC of 8.19 μmol/g, and the RPD was 2.3. Similar to above, reference values were well correlated with predicted values (Fig. 3b). The low RPD indicates that the calibration is more qualitative than quantitative, although it would be useful for quickly screening samples to determine high or low glucosinolate content. The calibration likely suffers from the number of species used to build it. *C. sativa, S. alba,* and the *Brassica* species have widely varying glucosinolate profiles, and better predictions might be possible if separate calibrations were developed for each genus. With this global calibration, the prediction is less certain, but it can be used with unknown samples or those that have been contaminated or mixed with seeds from another species. Our results were similar to those obtained by Daun et al. (1994) and Font et al. (2004) although others have developed calibrations with much higher correlations (Petisco et al., 2010; Velasco and Becker, 1998a).

3.6. Chlorophyll calibration

The samples used for the chlorophyll calibration had reference values ranging from 0.0–389.8 mg/kg with a mean of 46.0 mg/kg and a standard deviation of 85.5 mg/kg. A calibration covering the entire range was made with the following calibration settings: math treatment 1,4,4,1, no scatter correction, 1 pass for outliers, and outliers were not downweighted. The calibration had an r^2 of 0.98, an SEC of 10.4 mg/kg, and the RPD was 5.5. A plot of the reference vs. predicted values is shown in Fig. 4a. While these statistics indicate high prediction ability over a wide range of chlorophyll contents, the prediction is poor at the lower end of the range where the majority of the samples were found. To improve the performance, the calibration set was split into two groups, one for the lower end of the range and one for the higher end of the range, and separate calibrations were developed for each group.

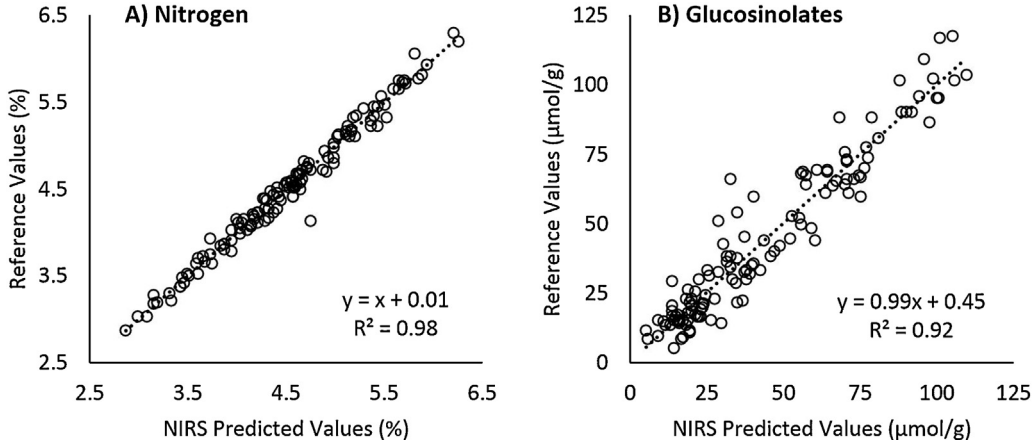

Fig. 3. Scatter plots of reference and NIRS predicted values for nitrogen (A) and glucosinolate (B) content calibrations.

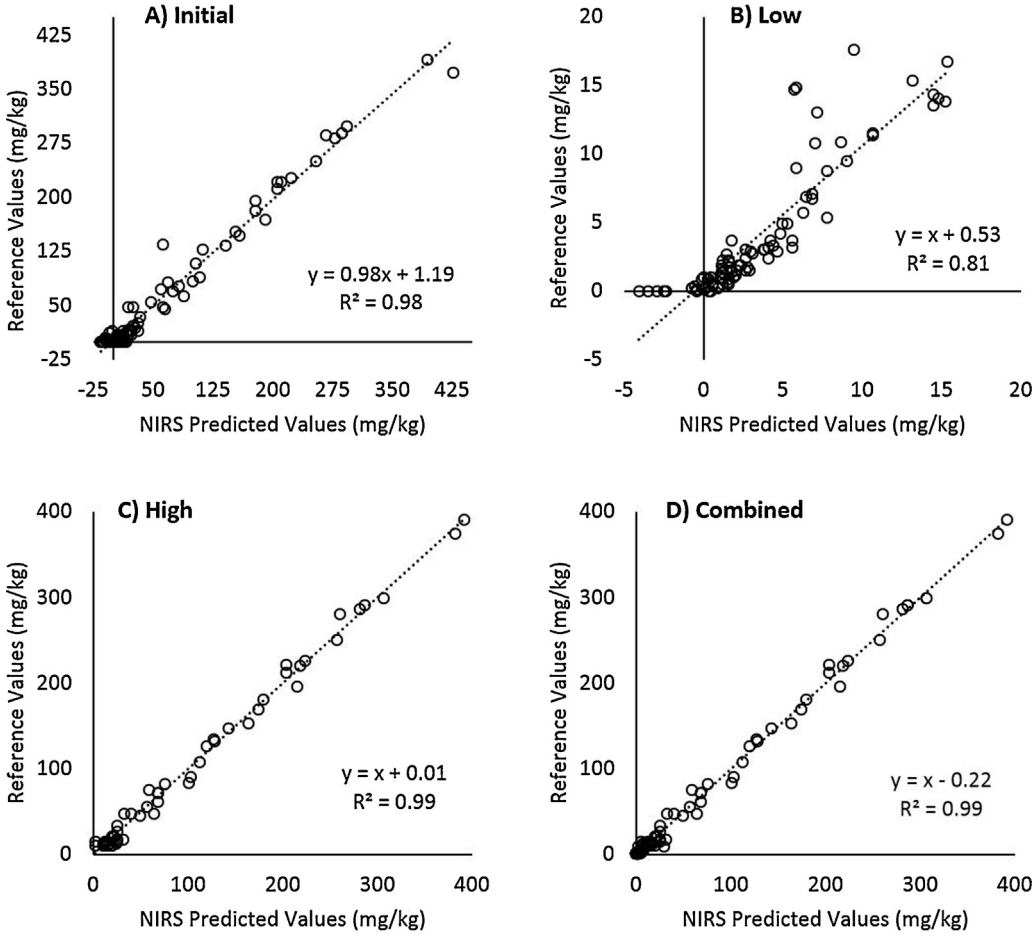

Fig. 4. Scatter plots of reference and NIRS predicted values for chlorophyll content for the initial calibration over the entire range (A), the calibration for the low end of the range (B), the calibration for the high end of the range (C), and the combined calibration (D).

The calibration for lower amounts of chlorophyll was developed with 86 samples over a range of 0.0–17.6 mg/kg with a mean of 4.1 mg/kg and a standard deviation of 4.8 mg/kg (Table 1). The calibration settings were: math treatment 2,4,4,1, SNV/detrend scatter correction, 1 pass for outliers, and outliers were not downweighted. The calibration had an r^2 of 0.93, an SEC of 1.2 mg/kg, and the RPD was 2.2. A plot of the reference vs. predicted values is shown in Fig. 4b. The calibration for higher amounts of chlorophyll was developed with 51 samples over a range of 10.8–389.8 mg/kg with a mean of 107.9 mg/kg and a standard deviation of 105.6 mg/kg (Table 1). The calibration settings were the same as those used for the lower range. The calibration had an r^2 of 0.99, an SEC of 9.7 mg/kg, and the RPD was 3.8. A plot of the reference vs. predicted values is shown in Fig. 4c.

The two calibrations were then combined by using the initial calibration that covered the entire range and the GH statistics from the low and high range calibrations. The GH statistic is reported in the WinISI software and is used to describe how similar a sample is to

the samples used to create the calibration (Shenk and Westerhaus, 1991). If the prediction from the full range calibration was less than 15 mg/kg, then the prediction from the low range calibration was used. If the prediction from the full range calibration was greater than 20 mg/kg, than the prediction from the high range calibration was used. For samples with full range predictions between 15–20 mg/kg, the calibration with the lowest GH was used. The predictions based on the three chlorophyll calibrations had an r^2 of 0.99, an SEC of 6.1 mg/kg, and an RPD of 14.0. Predicted values were highly correlated with reference values over the entire range (Fig. 4d). The high RPD indictates a high prediction ability for chlorophyll content by NIRS and the calibration performs even better than that described by Daun et al. (1994).

4. Conclusion

Global NIRS calibrations for moisture, oil, nitrogen, glucocosinolate, and chlorophyll content, and seed oil fatty acid profile were developed for six Brassicaceae species. The calibrations for oil, nitrogen, chlorophyll, and a few of the fatty acids showed good performance, while those for moisture and glucosinolate content were more qualitative. Calibrations for some of the fatty acids were not useful, with little correlation between predicted and reference values. These calibrations will be useful when analysis with reference methods is impractical due to the time required or a desire for non-destructive testing. Screenings in plant breeding programs and genetic studies often involve large numbers of samples with relatively small amounts of seed available for testing. In these cases, a high-throughput screening method such as NIRS is highly appropriate. In the future, updating the calibrations with more samples from additional growing years or sites will make them more robust, and may result in better moisture and glucosinolate calibrations or useable calibrations for some of the other fatty acids.

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