



Development of near infrared reflectance spectroscopy (NIRS) calibration model for estimation of oil content in a worldwide safflower germplasm collection

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Received ----- May 2010; Accepted after revision 3 July 2010; Published online -----

Abstract

The development of NIRS calibration model as a rapid, precise, robust, and cost-effective method to estimate oil content in ground seeds of worldwide safflower germplasm collection grown under different agro-climatic conditions was the key objective of this research project. The oil content was measured by accelerated solvent extraction method in a total of 328 samples collected across 2004 (165 samples) and 2005 (163) growing seasons and used as reference values. Two thirds of the measured samples were used for building the calibrations and one third for the validations. Combined and annual calibration and validation models were carried out by NIRCal 4.21 using the partial least squares (PLS) regression. Different data pretreatments such as full multiplicative scatter correction (MSC), first derivative or smoothing way of Savitzky-Golay with a gap of 9 data points were used to improve the calibration models. The optimum PLS factors for developing the best calibration were 12, 10, and 14 for combined model, annual model of 2004 and of 2005, respectively. In combined and annual models, the statistical parameters in calibration model were consistent with the respective parameters in validation model. Coefficient of variation (15.5 to 25.1) demonstrated high variability in calibration and validation models. The standard error of estimation (SEE) and standard error of prediction (SEP) for combined model were 1.40 and 1.43, respectively. Although the quality value (Q-value) of calibration was slightly higher in annual models (0.66 for both), the combined calibration model (0.64) precisely predicted oil content as indicated by higher coefficient of determination (0.90) and RPD (3.2%) compared to annual calibration. The accuracy and precision of the combined calibration model were sufficient to use NIRS as a tool for screening of oil content in a diverse safflower germplasm in the range obtained.

Keywords: Safflower; Oil content; Near-infrared spectroscopy; Calibration; Validation.

Introduction

Over the last 3 years the significant increase of commodity prices on all oilseeds was driven by demand from developing countries, biofuels initiatives, adverse weather patterns,

and tightening supply stocks (DeBonte, 2008). Safflower (*Carthamus tinctorius* L.) is placed eighth among the major oilseeds crop grown worldwide (Singh, 2006). It has a great potential which stems from the good agronomic characteristics, highly polyunsaturated fatty acids coupled with elevated levels of tocopherol and from the medicinal uses (Velasco et al., 2002). Depending on the hull-kernel ratio, the safflower oil content varies between 25 and 47% (Nagaraj, 1993). Safflower oil contains palmitic and stearic acids as saturated fatty acids and oleic and linoleic as unsaturated ones (Vasishtha et al., 1994). Germany ranks third (13317 tonnes) after United States and Japan in importing safflower oil (FAOSTAT, 2006). Since 2002, several projects were initiated in Germany to promote safflower as an alternative oil crop in organic farming system by screening a worldwide germplasm collection and ideotyping a high safflower oil (Reinbrecht et al., 2004; Khan et al., 2004; Elfadl et al., 2005; Wurl et al., 2007; Rudolphi, 2007).

One of the major concerns for both oil chemists and breeders is the time-consuming methods available for routine analysis of seeds (Panford and deMan, 1990). The Soxhlet is the standard method that provides reliable and robust oil extraction. However, it is impractical for analysis of large number of samples (Leon et al., 2004). Recently, several new extraction methods have been developed, one of which is the accelerated solvent extraction (ASE). The ASE employs a combination of increased temperature and pressure to reduce samples preparation time and amount of solvent required to complete the extraction and to maintain relatively constant extraction conditions (Tao et al., 2004). However, these extractive methods are laborious and require more than 3 g sample size to operate. Plant improvement relies on the ability to assess large numbers of individuals (Kohel, 1998). Usually such ability requires methods of analysis that are rapid, inexpensive and robust. For this aim it is a benefit to use near infrared reflectance spectroscopy (NIRS) after successful calibrations for estimation of oil content. The NIRS has become standard method for estimation of oil content in many oilseeds crops (Orman and Schumann, 1991; Daun et al., 1994; Perez-Vich et al., 1998; Hom et al., 2007). The NIRS is time-saving, cost-effective, repeatable, environmentally safe, and allows the simultaneous estimation of several traits in one measurement (Wu et al., 2002). These advantages make it an ideal technique for plant breeding researches.

Calibrations of NIRS have shown to be sensitive to year, time of sowing, crop location and variety (Dardenne, 1996). Samples evaluated over the course of several years can further enhance the robustness of the calibration because the samples best represent the seeds that would be grown under different conditions (Shenk and Westerhaus, 1991). To date, little has been reported regarding the development of NIRS calibrations for estimating oil content in a diverse safflower germplasm sown in many locations under different climatic and farming conditions. The current study investigated the potentiality of using NIRS to predict oil content using ground safflower achenes samples.

Materials and Methods

Materials

A worldwide safflower germplasm collection and 18 lines selected out of 65 accessions evaluated under similar conditions in Germany in 2003 growing season were used in this

study. The collection is representing 48 countries across Europe (152 accessions), Asia (147), America (87), Mediterranean (44), Africa (21), and Australia (2). Fifteen accessions of unknown origin were contained in this collection. The accessions and selected lines were sown in Germany, Switzerland and Austria under conditions of organic and low-input farming system during 2004 and 2005 growing seasons. Organic and inorganic fertilizers and plant protection measures were not applied under organic farming. However, nitrogen fertilizer in the form of calcium ammonium nitrate (KAS) was applied under low-input farming. A set of 326 accessions were selected and analyzed by ASE and NIRS for developing calibration model for oil estimation, as described below. To test calibration accuracy across years, annual calibration models were developed with samples collected in 2004 (163 samples) and 2005 (163 samples) growing seasons.

Oil content extraction

Prior to grinding, achenes were cleaned manually to remove dirt and other foreign materials. The achenes samples were ground (1 min, < 3 mm particle size) using an IKA-Universalmuehle M20 (20000 t/min) grinder (Janke and Kunkel, Staufen, Germany) which was equipped with a water cooling system to prevent heating of samples. The samples were scanned by the NIRVIS1038 instrument (INF2000 v3.00-07/98/ NVD-driver version 4.25 Build 335) immediately after grinding and the spectra recorded prior to chemical analysis. Around 3 g from each ground achene sample was dried in an oven (103 °C) for one hour. The sample was loaded into a cell of stainless steel, placed into the ASE 100 system (Dionex, Sunnyvale, CA, USA), rinsed with petroleum ether as extraction solvent (80%) and set under elevated temperatures (80 °C) and pressure (1015 mbar). Increased temperature accelerates the extraction, while the elevated pressure keeps the solvent below its boiling point (40-60 °C) for safe and rapid extractions. After a static phase (1 min.) the solvent in the cell was exchanged and eluted into a glass vial. The cycle was repeated 5 times to ensure complete extraction of the oil. To separate solvent from oil, the pressure was reduced to 600 mbar and the extract was dried in an oven (103 °C) for one hour and cooled in a chamber for one hour. To have an accurate determination of oil content, two measurements for each sample were run and the average of the oil content was calculated. The soxhlet extraction, as a standard method, was used for determination of oil content in eight randomly selected lines to validate the accuracy and the precision of the ASE procedure.

Near infra-red analysis

Near infrared reflectance spectroscopy was used to estimate the oil content in 1740 ground achenes samples. The ASE was used for determination of oil content in 326 samples which were used as the reference values for the prediction of oil content in NIRS samples. Analyses of NIRS were carried out using NIRVIS1038 instrument (INF2000 v3.00-07/98/ NVD-driver version 4.25 Build 335) which scanned the spectral range of 4596-9996 nm. Three scans for each ground sample were recorded and a total of the 978, 495 and 489 spectral scans were managed to develop combined calibration, annual

calibration of 2004 and of 2005, respectively. The spectral and reference data were managed and pretreated with the software Nirxal 4.21 (Büchi Labortechnik AG, Flawil, Switzerland). Two thirds of the samples were used for calibrations set and one third for the validations set. The calibration set contained the extreme spectra to define the limits of acceptance. The 3 spectra of the same sample remained together and designated to either the calibration or the validation set. The detected spectral outliers were omitted. All spectral data were mean-centered prior to calibration. In each calibration model the optimal number of factors, pretreatments and the wavenumber range were automatically determined by the software. The number of factors to be used in each case was determined by the predicted residual error sum of squares (PRESS) that shows the sum of squares of deviation between predicted values (y_n) and reference values (x_n). The selected spectra were matched with the reference data and NIRS calibration was developed using the partial least squares (PLS) regression technique.

Statistical parameters

In order to assess the developed calibrations, different statistical parameters were used. The extent of variability and distribution in calibration and validation data were measured by coefficient of variation (CV), standard deviation (SD) and range. The calibration quality value (Q-value), a specific index used to give the overall quality of a NIR calibration. A Q-value higher than 0.50 is acceptable for quantitative analysis. However, Q-value over 0.70 is considered robust. The highest Q-value was used as criteria for selection of the best calibration model (Nirxal 4.21 manual, 2002). The accuracy of the correlation (Bias) is the systematic difference between the predicted values (y_n) and the measured values (x_n). The value of the bias should be close to 0 for accurate calibration model.

$$\text{Bias} = \frac{1}{N} \sum_{i=1}^N (x_i - y_i)$$

The precision of calibration is represented by the standard error of estimation (SEE) and the standard error of prediction (SEP). The consistency, which is the ratio of the SEE and SEP, should be as close as possible to 100. The consistency is used to ensure that the number of factors selected for developing the best calibration model is optimum. The regression coefficient (r) expresses the relationship between the measured and the predicted values and describes the quality of quantitative calibration (Williams, 2004). A value of r larger than 0.91 indicates a good correlation. The coefficient of determination (R^2) shows the proportion of the variance in reference data that can be explained by the variance in the predicted data. When the value of R^2 is higher than 0.83, the robustness of the prediction of calibration model is maintained. The relative prediction deviation (RPD) is a ratio of SEP to SD of the reference values in the validation set. Value of 2.5 and higher are satisfactory for screening in breeding programs (Williams and Sobering, 1993).

$$\text{SEE} = \sqrt{\frac{1}{N} \sum_{i=1}^N (x_i - y_i - \text{Bias})^2}$$

where N is the number of spectra in the calibration set for estimating SEE. Similar formula was used for estimating SEP in the validation set.

Results and Discussion

Validation of ASE Method

The results of extracted oil content by Soxhlet and ASE methods are shown in Figure 1. Accelerated solvent extraction is a new extraction technique that is similar in principle to Soxhlet extraction, but the use of elevated temperature and pressure with ASE allows the extraction to be completed within a short time and with a small quantity of solvent (Schaefer, 1998). In this experiment, oil content was determined for a set of randomly selected eight lines to compare both methods. The total extraction by ASE was obtained in about 40 minutes under elevated temperature of 80 °C and pressure of 1015 mbar. The high temperature and pressure increase the diffusion rates of the analytes and hence the extraction rates would be improved (Matthäus and Brühl, 2001). The regression coefficient ($r=0.95$) and the Bias (0.08) indicated a good agreement between these two methods. Thus, the ASE was accurate and precise method for measuring oil content and could be used as an alternative extraction technique to Soxhlet for the conventional determination of oil content.

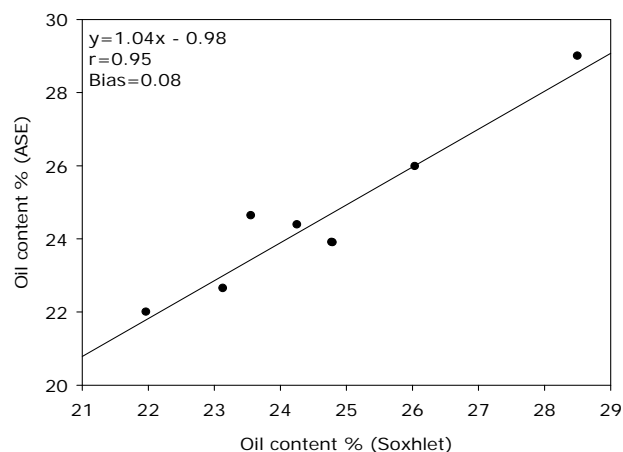


Figure 1. Comparison of Accelerated Solvent Extraction and Soxhlet methods for determination of oil content in randomly selected eight safflower lines.

Development of NIRS Calibration

The distribution of the reference values in the calibration and validation sets covered a great variability as demonstrated by the broad ranges and coefficients of variation observed in both sets (Table 1). The calibration and validation sets within combined and annual models had similar CV. However, both sets from 2004 had the lowest CV compared to sets from 2005 and combined years. The large variability observed in 2005 could be ascribed to differential adaptability of accessions to prevailing cool temperatures during early vegetative stages and humid conditions at reproductive stages that resulted in epidemic head rot and severe infestation of ALS. However, the prevailing conditions in 2004 were

relatively suitable for growing safflower compared to 2005; since the rainfall was well distributed during the growth stages giving accessions a better chance for expressing their potential. The wide range of variation in each calibration and validation set suggested that the two sample sets were representative of the overall genetic diversity in safflower in terms of oil content. The small differences in means, ranges, and SD between the calibration set and the validation set in combined and annual models (Table 1) demonstrated that the selected calibration set was suitable for NIRS calibration. The variation of the oil content in the samples covers the range reported in Germany for safflower grown under organic or chemical-free conditions (Reinbrecht et al., 2004; Elfadl et al., 2005; Rodulphi, 2007; Wurl et al., 2007).

Table 1. Calibration and validation statistics in NIRS models for estimation of oil content (%) in ground seed of safflower for combined and individual years.

| Year | Combined Years | | 2004 | | 2005 | |
|----------------|----------------|------------|-------------|------------|-------------|------------|
| | Calibration | Validation | Calibration | Validation | Calibration | Validation |
| N | 217 | 109 | 109 | 54 | 107 | 53 |
| Factor | 12 | | 10 | | 14 | |
| Mean | 19.3 | 19.3 | 22.0 | 21.5 | 16.7 | 16.1 |
| Range | 4.8-31.0 | 4.9-31.0 | 12.7-31.0 | 12.9-29.0 | 4.8-31.0 | 4.9-26.1 |
| SD | 4.6 | 4.6 | 3.4 | 3.5 | 4.20 | 3.8 |
| CV | 23.6 | 23.9 | 15.5 | 16.2 | 25.1 | 23.3 |
| Q-value | 0.64 | | 0.66 | | 0.66 | |
| r | 0.95 | 0.95 | 0.91 | 0.91 | 0.95 | 0.95 |
| R ² | 0.90 | 0.90 | 0.83 | 0.83 | 0.90 | 0.90 |
| SEE | 1.40 | | 1.42 | | 1.35 | |
| SEP | | 1.43 | | 1.42 | | 1.36 |
| Consistency% | 97.9 | | 100.1 | | 98.7 | |
| Bias | 0.0 | -0.3 | 0.0 | -0.03 | 0.0 | 0.03 |
| RPD | | 3.20 | | 2.50 | | 2.75 |

N, SD, CV, r, R² are assigned for number of samples, standard of deviation, coefficient of variation, regression coefficient, and coefficient of determination, respectively. SEE and SEP are standard error of estimation and of prediction. RPD is relative prediction deviation. Q is quality of calibration.

Different oilseeds have different wavelengths assigned to oil bands due to interactions between constituents, particle size in ground seed, and chain length and degree of saturation of fatty acids (Bhatty, 1991). Broadly, the wavelengths for oil in ground safflower achene obtained in this study range between 1000 and 2175 nm (4596-9996 cm⁻¹). Within this range, Panford et al. (1988) reported 1230, 1806, and 2170 nm as optimum wavelengths for estimation of oil content in ground safflower achene by using NIR scanning monochrometer. The NIR spectrum does not only depend on the chemical composition of samples but also on the physical characteristics of the samples, which are usually observed as the background and noise in the spectrum (Chen et al., 2007). Figure 2 shows the original (A) and pretreated (B) spectra of combined years recorded for the 326 ground samples of safflower. A parallel shift of the spectra can be seen, which is particularly pronounced between 4596 and 7000 cm⁻¹. This shift could be due to spectral influence of particle size. To eliminate this scatter, a data pretreatment was carried out with full multiplicative scatter correction (MSC). The recorded spectra for year of 2004 (495) were pretreated with MSC to remove the noise and then the first derivative (BCAP) was calculated to minimize shifts in the baseline. For best calibration in a year of 2005, the smoothing way of Savitzky-Golay with a gap of 9 data points was used to correct the shifts in the original spectra.

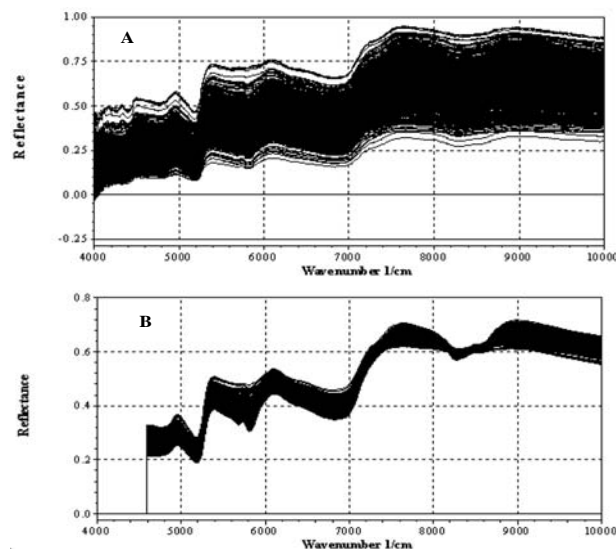


Figure 2. Original (A) and pretreated (B) spectra of 326 ground safflower samples collected during 2004 and 2005 growing season. Pretreatments were mean centering and full multiplicative scatter correction.

The statistics of the developed and validated NIRS calibrations for the estimation of oil content in ground safflower achenes are given in Table 1. In general, the PLS results showed that calibration and validation statistics were mostly similar for combined and annual models. Although the combined samples contained both samples from 2004 and 2005, the Q-values, a measure of the quality of a calibration, for annual calibrations (0.66 for both) were slightly higher than the Q-value (0.64) for combined calibration (Table 1). This result indicates that adding more samples may not significantly improve oil content prediction. However, the addition of samples with oil content outside the range obtained in this study may improve prediction more than just adding samples. Including more variability into calibration set improves the prediction accuracy as long as a typical data is not included and the number of factors is not too large (Peirs et al., 2003). The NIRS spectra do not contain only analytical information but also spectral interference that can degrade the performance of calibration model when the number of samples is too large (Centner et al., 1996). Also the cost and time spent in the reference analysis of large samples are often not affordable. Thus, the concentration range to be covered by the samples and the distribution within this range should be thoroughly considered for building calibration set of optimum size.

Optimum number of factors for calibration was selected based on the PRESS, which should be minimized, along with the high R^2 . (Sivakesava and Irudayaraj, 2002) The PRESS showed that 12, 10, and 14 factors were needed for developing the best calibration model for combined years, year of 2004, and year of 2005, respectively (Table 1). The overfitting, number of factors being too large, is avoided when the differences between standard errors and between coefficients of determination for calibration and validation sets are small (Garca-Alvarez et al., 2000). The primary parameters for determining the precision of the calibration

model are the SEE and SEP. The value of R^2 (Table 1), SEE and SEP (Figure 3) for combined model were 0.90, 1.40 and 1.43, respectively. Previous calibrations for oil content in ground and intact safflower achenes, collected in 2003 and 2004 (203 samples), were developed by Rudolphi et al. (2005). The oil content prediction was more accurate in ground achenes than in intact achenes as shown by lower SEE (0.77) and standard error of cross validation (0.90) and higher R^2 (0.96). The values of the consistency, a ratio between SEE and SEP, were 97.9%, 100.1%, and 98.7% for calibrations developed from samples of combined years, year of 2004, and year of 2005, respectively (Table 1). The obtained consistencies confirmed that the optimal number of PLS factors has been retained in combined and annual calibration models. The combined calibration model showed a good agreement between reference and NIRS predicted values. As shown in Figure 3 (A and B), the high value of r (0.95) indicates a good correlation between the predicted and the reference values for calibration and validation model. The Bias as a measure of the overall accuracy of the NIRS was 0 for calibration and was -0.3 for validation set implying that the calibration accuracy is maintained for the prediction of oil content. These results prove the ability of combined calibration to estimate the oil content precisely.

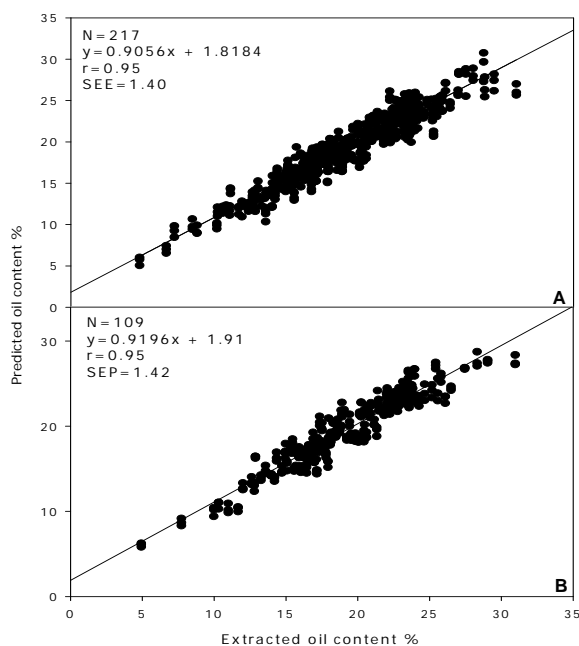


Figure 3. Extracted (ASE) versus predicted (NIRS) values of oil content in ground safflower seeds samples collected during 2004 and 2005 growing seasons for developing combined calibration (A) and validation (B) models.

The influence of years may decrease the NIRS prediction accuracy when the agro-climatic conditions under which the samples were collected are significantly different (Kopra et al., 2006). A close relationship between ASE and NIRS values for oil content was obtained in both calibration and validation set in annual models as indicated by high r

values (Figures 4 and 5). Bias was almost 0 for calibration set and close to 0 in validation set for both annual models (Table 1). Hence, there is hardly any systematic difference between the predicted and measured values. The calibration and validation models developed from the samples collected in 2004 predicted oil content with R^2 of 0.83, SEE of 1.42 and SEP of 1.42 as shown in Figure 4 (A and B).

Regarding the annual calibration of 2005, the PLS regression between measured and predicted values resulted in a high similar r (0.95) and R^2 (0.90) for both calibration and validation model. The standard errors of estimation and of prediction were 1.35 and 1.36, respectively (Figure 5A and 5B). The combined calibration and annual calibration of 2005 precisely estimated oil content compared to annual calibration of 2004 as indicated by R^2 , SEE, and SEP (Figures 4 and 5). Williams and Sobering (1996) suggest that the values for R^2 and RPD cannot be very high when the variance in the reference data is low. The relative prediction deviation (RPD) is a measurement of the ability of an NIRS model to predict a constituent efficiently (Williams and Norris, 2001). The results showed that the RPD for combined calibration, and annual calibration of 2005 and 2004 were 3.2, 2.75 and 2.5, respectively (Table 1). The combined calibration model was more efficient than those obtained from annual samples as indicated by RPD greater than 3. This might be attributed to large variability attained in the validation samples as shown by high SD and CV values (Table 1). This level of precision is sufficient to use NIRS as a tool for screening of oil content in a diverse safflower germplasm in the obtained range. A similar result in analysis of whole seed canola by NIRS demonstrated that the combined calibration developed from samples collected across years was more precise for estimation of oil content than the annual calibrations (Greenwood et al., 1999).

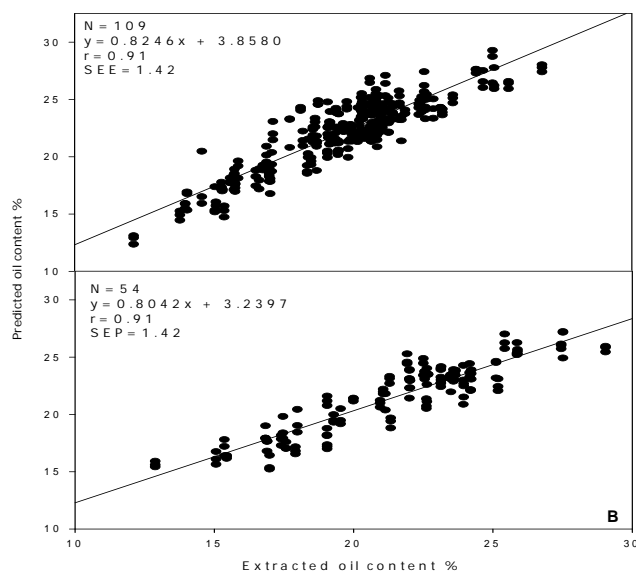


Figure 4. Extracted (ASE) versus predicted (NIRS) values of oil content in ground safflower seeds samples collected during 2004 growing season for developing annual calibration (A) and validation (B) models.

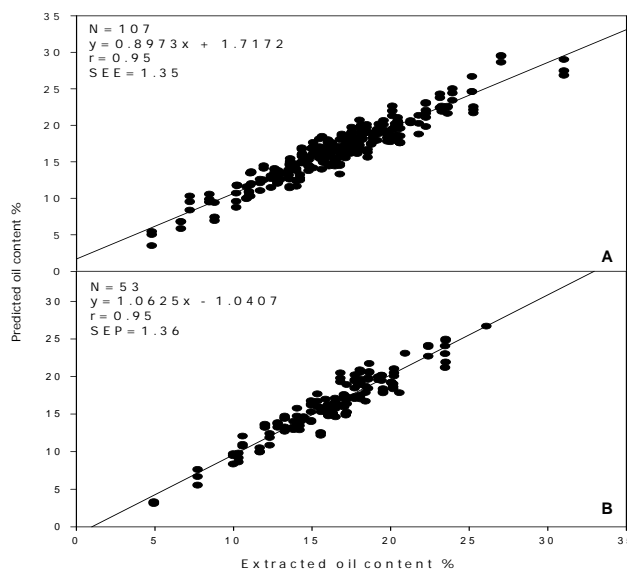


Figure 5. Extracted (ASE) versus predicted (NIRS) values of oil content in ground safflower seeds samples collected during 2005 growing season for developing annual calibration (A) and validation (B) models.

Conclusions

In summary, the accelerated solvent extraction could be used as an alternative method to Soxhlet for conventional oil extraction. The estimation of oil content in ground safflower achenes could be accomplished by NIRS with a high R^2 (0.90), similar SEE (1.40) and SEP (1.43) and reasonable RPD (3.2). This level of precision and accuracy in combined calibration model is sufficient to use NIRS as screening tool for oil content in safflower breeding programs. An increase of variability of the reference data improved the precision of the estimated oil NIRS model. In order to improve the calibration model, the range of oil content to be covered by the samples and the distribution within this range should be thoroughly considered for developing calibration set of optimum size. Further studies should be carried out for developing NIRS calibration for estimation of oil content in intact achenes as a non-destructive and cost-effective analysis and for validating the obtained calibration models with independent samples from other locations.

Acknowledgements

We are here highly appreciating and acknowledge the financial support provided by Pfister foundation, Bern, Switzerland and Vater & Sohn Eiselen-Stiftung, Ulm, Germany. The analyses described herein were performed at the Food Analysis Laboratory in University of Hohenheim. In recognition of this, we gratefully acknowledge the assistance of Prof. Dr. Isengard, Mr. Merkh and Ms. Erika Denzel. Special thanks go to Ms. M. Schnieder and Ms. A. Richter for assistance with samples preparation and data acquisition.

References

- Bhatty, R.S., 1991. Measurement of oil in whole Flaxseed by Near-Infrared Reflectance Spectroscopy. *J. Am. Oil Chem. Soc.* 68: 1. 34-38.
- Centner, V., Massart, D.L., de Jong, S., Vandeginste, B.M., Sterna, C., 1996. Lamination of uninformative variables for multivariate calibration. *Anal. Chem.* 68: 3851-3858.
- Chen, D., Cai, W., Shao, X., 2007. An adaptive strategy for selecting representative calibration samples in the continuous wavelet domain for near-infrared spectral analysis. *Anal. Bioanal. Chem.* 387: 1041-1048.
- Dardenne, P., 1996. Stability of NIR spectroscopy equations. *NIR News*, 7: 5. 8-9.
- Daun, J.K., Clear, K.M., Williams, P., 1994. Comparison of three whole seed near infrared analyzers for measuring quality components of canola seed. *J. Am. Oil Chem. Soc.* 71: 10. 1063-1068.
- DeBonte, L.R., 2008. Global supply of vegetable oils: Is our supply at risk?. *Proc. of the 7th Int. Safflower Conf.* Wagga Wagga, New South Wales, Australia.
- Elfadl, E.Y., Reinbrecht, C., Frick, C., von Witzke-Ehbrecht, S., Rudolphi, S., Claupein, W., 2005. Genotype by environment interaction in safflower (*Carthamus tinctorius* L.) grown under organic farming system in Central Europe. In: Esendal, E. (Ed.), *A unique crop for oil, spices and health, consequently a better life for you.* *Proc. of the 6th Int. Safflower Conf.* Istanbul, Turkey, June, 06-10. 2005, Pp: 39-43.
- FAOSTAT, 2006. <http://faostat.fao.org/site>.
- Garca-Alvarez, M., Huidobro, J.F., Hermida, M., Rodriguez-Otero, J.L., 2000. Major components of honey analysis by Near-Infrared Transflectance Spectroscopy. *J. Agric. Food Chem.* 48: 11. 5154-5158.
- Greenwood, C.F., Allen, J.A., Leong, A.S., Pallot, T.N., Golder, T.M., Golebiowski, T., 1999. An investigation of the stability of NIRS calibrations for the analysis of oil content in whole seed canola. *The 10th Int. Rapeseed Congress*, Canberra, Australia. <http://www.regional.org.au/au/gcirc/1/572b.htm>.
- Hom, N.H., Becker, H.C., Möllers, C., 2007. Non-destructive analysis of rapeseed quality by NIRS of small seed samples and single seeds. *Euphytica*, 153: 1. 27-34.
- Khan, M.A., Von Witzke-Ebrecht, S., Maass, B.L., Becker, H.C., 2004. Evaluation of a worldwide collection of safflower for morphological diversity and fatty acid composition. *On-farm-management von pflanzengenetischen ressourcen im rahmen der Pflanzenzüchtung.* *Gesellschaft für Pflanzenzüchtung e.* 62: 104-106.
- Kohel, R.J., 1998. Cotton improvement: Evaluation of near infrared reflectance for oil content of cottonseed. *The J. Cotton Sci.* 2: 23-26.
- Kopra, R., Nerusil, P., Kolovrat, O., Kucera, V., Kohoutek, A., 2006. Estimation of fatty acid content in intact seeds of oilseed rape (*Brassica napus* L.) lines using Near-Infrared Spectroscopy. *Czech J. Genet. Plant Breed.* 42: 4. 132-136.
- Leon, L., Garrido-Varo, A., Downey, G., 2004. Parent and harvest year effects on near-infrared reflectance spectroscopic analysis of olive (*Olea europaea* L.) fruit traits. *J. Agric. Food Chem.* 52: 16. 4957-4962.
- Matthäus, B., Brühl, L., 2001. Comparison of different methods for the determination of the oil content in oilseeds. *J. Am. Oil Chem. Soc.* 78: 1. 95-102.
- Nagaraj, G., 1993. Safflower seed composition and oil quality-a review. In: *3rd Int. Safflower Conf.* Beijing, Pp: 58-71.
- Nirxal 4.21 Manual, Büchi Labortechnik AG, CH-Flawil, 2002.
- Orman, B.A., Schumann, R.A., 1991. Comparison of near-infrared spectroscopy calibration methods for the prediction of protein, oil, and starch in maize grain. *J. Agric. Food Chem.* 39: 883-886.
- Panford, J.A., deMan, J.M., 1990. Determination of oil content of seeds by NIR: influence of fatty acid composition on wavelength selection. *J. Am. Oil Chem. Soc.* 67: 473-482.
- Panford, J.A., Williams, P.C., deMan, J.M., 1988. Analysis of oilseeds for protein, oil, fiber and moisture by Near-Infrared Reflectance Spectroscopy. *J. Am. Oil Chem. Soc.* 65: 10. 1627-1634.
- Peirs, A., Tirry, J., Verlinden, B., Darius, P., Nicolai, B.M., 2003. Effect of biological variability on robustness of NIR models for soluble solids content of apples. *Postharvest Biol. Technol.* 28: 269-280.
- Perez-Vich, B., Velasco, L., Fernandez-Martinez, J.M., 1998. Determination of seed oil content and fatty acid composition in sunflower through the analysis of intact seeds, husked seeds, meals and oil by near-infrared reflectance spectroscopy. *J. Am. Oil Chem Soc.* 75: 547-555.
- Reinbrecht, C., Barth, S., Von Witzke-Ebrecht, S., Khan, M.A., Becker, H.C., Kahnt, G., Claupein, W., 2004. Evaluierung von Safflor-Akzessionen für den Ökologischen Landbau (In German). *On-farm-management von pflanzengenetischen ressourcen im rahmen der Pflanzenzüchtung.* *Gesellschaft für Pflanzenzüchtung*, 62: 101-103.

- Rudolphi, S., 2007. Methodische Grundlagen der Züchtung von Saflor (*Carthamus tinctorius* L.) für den ökologischen Landbau. Dissertation (In German), Georg-August-Universität Göttingen. [http:// webdoc. sub. gwdg.de/diss/2007/rudolphi/](http://webdoc.sub.gwdg.de/diss/2007/rudolphi/).
- Rudolphi, S., Von Witzke-Ebrecht, S., Becker, H.C., 2005. Estimation of oil content in safflower by Near Infrared Reflectance Spectroscopy. In: Esendal, E. (Ed.), A unique crop for oil, spices and health, consequently a better life for you. Proc. of the 6th Int. Safflower Conf. Istanbul, Turkey, June, 06-10, Pp: 344-347.
- Schaefer, K., 1998. Accelerated solvent extraction of lipids for determining the fatty acid composition of biological material. *Anal. Chim. Acta.* 358: 69-77.
- Singh, R.J. (Ed.), 2006. Landmark research in oil seed crop. In: Singh, R.J. (Ed.), Genetic resources, chromosome engineering, and crop improvement: Oilseeds crop. CRC Press, 4: 2.
- Sivakesava, S., Irudayaraj, J., 2002. Rapid determination of Tetracycline in milk by FT-MIR and FT-NIR Spectroscopy. *J. Dairy Sci.* 85: 487-493.
- Shenk, J.S., Westerhaus, M.O., 1991. Population structuring of near infrared spectra and modified partial least squares regression. *Crop Sci.* 31: 1548-1555.
- Tao, S., Guo, L.Q., Wang, X., Liu, W.X., Ju, T.Z., Dawson, R., Cao, J., Xu, F.L., Li, B.G., 2004. Use of sequential ASE extraction to evaluate the bioavailability of DDT and its metabolites to wheat roots in soils with various organic carbon contents. *Sci. Total Environ.* 320: 1-9.
- Vasishtha, A.K., Bajpai, M., Singh, C., 1994. Variation in the characteristics and oil composition in different genetic varieties of safflower. *J. Oil Technol. Assoc. India*, 26: 59-63.
- Velasco, L., Fernandez-Martinez, J.M., 2002. Progress in breeding modified tocopherol content and composition in safflower. *Sesame and Safflower Newsletter*, 17: 98.
- Williams, P. (Ed.), 2004. Near-infrared technology-Getting the best out of light. Nanaimo, Canada, 2.0 Edition, Value Added Wheat CRC Ltd.
- Williams, P.C., Sobering, D.C., 1993. Comparison of commercial Near-Infrared Transmittance and Reflectance Instruments for analysis of whole grains and seeds. *J. Near Infrared Spectrosc.* 1: 25-32.
- Williams, P.C., Sobering, D.C. 1996. How we do it?: a brief summary of the methods we use in developing near infrared calibrations. In: Davies, A.M.C., Williams, P.C. (Eds.), *Infrared Spectroscopy: The future waves.* NIR Publications, Chichester, Pp: 185-188.
- Williams, P.C., Norris, K. (Eds.), 2001. Near-infrared technology in the agricultural and food industries. American Association of Cereal Chemists, St Paul, MN, 296p.
- Wu, J.G., Shi, C.H., Zhang, X.M., 2002. Estimating the amino acid composition in the milled rice powder by near-infrared reflectance. *Field Crops Res.* 75: 1-7.
- Wurl, G., Biertümpfel, A., Graf, T., 2007. Entwicklung ertragssicherer Saflor-Stämme mit hohen Ölgehalten für den ökologischen Anbau (In German). Organic eprints, http://orgprints.org/13320/01/13320-03OE628_2-ttl-graf-2007-saflor-taemme.pdf.