Near-infrared spectroscopy for analysis of oil content and fatty acid profile in *Jatropha curcas L.* flour

Terren M.[1,2], Lecler B.[3], Baeten V.[3], Mergeai G.[2]

[1] Durabilis Foundation, Kortrijksesteenweg 930, B-9000 Gent, Belgium.

[2] Gembloux Agro-Bio Tech (ULg), Department of tropical crop husbandry and horticulture, Passage des Déportés 2, B-5030 Gembloux, Belgium

[3] Quality of Agricultural Products department, Walloon Agricultural Research Centre (CRA-W), B-5030 Gembloux, Belgium

Corresponding e-mail: m.terren@doct.ulg.ac.be

Introduction

Jatropha curcas L. (JCL) is a multipurpose tropical shrub that belongs to the Euphorbiaceae family. It has its centre of origin in Central America and grows in tropical and subtropical regions throughout Africa, India and South East Asia (Jongschaap et al., 2007). It is often planted as living fence, to reclaim wasteland or used to control soil erosion. JCL is rather drought resistant and is cultivated for its seeds that have high oil content (up to 50%) (Tatikonda et al., 2009). The oil contains several toxic compounds, thus it is non-edible and can be used as a high quality biodiesel, as bio pesticide, in soap production and in oil lamps (Achten et al., 2008; Kumar and Shamar, 2008). While JCL has ideal oil quality for biodiesel production, it is still an undomesticated plant and genetic variability and environmental conditions have a large impact on oil quality (Kaushik et al., 2007). As improved plant material is not widely available, it is all the more important to screen seeds with high oil content suitable for industrial application and to know their fatty acid composition for quality breeding. Conventional oilseeds methods and analyses used to measure quality are time-consuming, cost inefficient and require sample preparation and the use of chemical products. Fatty acid composition is usually determined by gas chromatography and the oil content is often determined by extraction methods with large amount of organic solvents that contribute to environmental problems. Indirect oil content analysis can be carried out using Near Infrared spectroscopy (NIRS) where the absorption of near infrared energy (1100-2500 nm) by the sample is measured. Sample throughput can be increased up to ten times in comparison to conventional methods. While the precision of NIR methods is not as precise as extraction method, it can be used as a quick and efficient method to determine oil content and fatty acid composition. NIRS has been used to analyse quality traits in numerous food and industrial crops, especially cereals and oilseeds such as rapeseed (Velasco et al., 1999), sunflower (Pérez-Vich et al., 1998) and sesame (Sato et al., 2003). Only few studies of NIRS to assess JCL seed quality traits have yet been conducted (Montes et al., 2013; Vaknin et al., 2011). The objective of this study was to find a quick way to measure oil content and fatty acid composition of JCL seed meal using near infrared spectroscopy.

Materials and methods

A total of 277 JCL seed samples were used to develop a NIRS prediction model for the determination of individual oil content and fatty acid composition. Seeds were harvested during 2010 from a multi local trial installed in 2009 in three different agro-ecological zones in Senegal. Each sample consisted of at least 10 g seeds from a single plant.

Each sample was ground using an ultracentrifugal grinding mill (Retsch ZM100, F. Kurt Retsch GmbH & Co., Germany) to pass a 1-mm ring sieve. All samples were scanned twice when placed in a 1/4-rectangular sample cell by near-infrared reflectance spectroscopy (Model 6500, Foss NIRsystems,

Silver Spring, MD). The spectral data were recorded as the logarithm of reciprocal of reflectance (log1/R) within the wavelength range 400-2498nm by 2 nm steps. 58 samples were randomly selected for external validation and analyzed by wet chemistry analyses. Soxtec Extraction System unit (Foss Tecator AB, Höganäs, Sweden) was used to measure oil content. Fatty acid profile was determined by gas chromatography. Before building the calibration model, raw spectra data was preprocessed using second derivate transformation (2,4,4,1), standard normal variate (SNV) and detrending procedures to eliminate scattering distortion. For calibration, only the spectral data from 1108-2498nm were used, since the inclusion of the segment from 400-1108 led to poorer results. Calibration equations were developed for oil content, palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (18:3) by multiple linear regression analysis (MLR) using WinISI IV software (Intrasoft International, LLC., Port Matilda, PA). Individual fatty acids were expressed as the percentage of total fatty acids. Critical H and T were used to identify outliers. A subset of 20 samples was used for external validation. The best calibration equations were selected by comparing the coefficient of determination (R^2), the ratio of performance to deviation (RPD), the standard error of calibration (SEC), the standard error of cross validation (SECV), and the standard error of prediction (SEP) for the external validation.

Results and discussion

The descriptive statistics including mean, range and standard deviation (SD) for oil content and individual fatty acid composition of JCL meal samples used in the calibration set are shown in Table 1. 99% of the oil is composed of four principal fatty acids including oleic, linoleic, palmitic and stearic acids. The two unsaturated fatty acids, oleic and linoleic, represented 78% of the oil content, the two saturated fatty acids, palmitic and stearic, 21%. The seeds showed large variability for oil content, ranging from 5,34 to 45,35% in calibration set. The range of values obtained for oil was lower than those reported by Montes et al. (2013) and Vaknin et al. (2011). Highest variability for fatty acids was observed for linoleic and oleic acid, ranging from 22,10-37,51% and 41,79-54,82% respectively, followed by palmitic, stearic, palmitoleic and least in linolenic acid.

Calibration results for oil content and fatty acid ratio in JCL meal are shown in table 2. For the oil content and the individual fatty acids, coefficients of determination (R^2) values from calibration statistics varied from 0,60 to 0,99. Reliable equations were developed for oil content (R^2 = 0,99; RPD = 9,42), linoleic acid (R^2 = 0,89; RPD = 2,85) and oleic acid (R^2 = 0,81; RPD = 2,19). These equations for oil content, linoleic and oleic acid in JCL meal indicate good accuracy of the calibration model, similar to those obtained in single intact JCL kernels (Vaknin et al., 2011). The high potential for determining oil content by NIRS can be of huge utility for screening a large number of samples to determine oil content and fatty acid composition (linoleic and oleic acid) in breeding programs for development of high quality JCL oil. The NIRS method performed considerably poorly for palmitic and stearic acids that occur in lower concentrations in the seeds, with values of R^2 of 0,60 and 0,62 respectively. These equations would still be reliable enough to identify seed variants with significantly different fatty acid compositions. A wider range of reference values is needed to obtain more accurate calibration equation of palmitic and stearic acid. The correlation between the total oil content and the individual fatty acids are shown in table 3. Oleic acid had strong negative correlation with linoleic acid, with correlation coefficient of -0,80.

Table 1. Fatty acid composition of the oil in the 58 samples of calibration set.

Trait	N	Mean	Range	SD
Oil content	55	25,35	5,34-45,35	6,67
Palmitic (C16:0)	56	14,54	11,17-17,92	1,12
Palmitoleic (C16:1)	58	1,00	0,42-1,58	0,19

Stearic (C18:0)	54	6,49	4,54-8,43	0,65
Oleic (C18:1)	57	48,30	41,79-54,82	2,17
Linoleic (C18:2)	56	29,80	22,10-37,51	2,57
Linolenic (C18:3)	53	0,25	0-0,53	0,09

Table 2. Calibration results for oil content and fatty acid ratio in JCL meal. N indicates number of samples, coefficient of determination (R2), standard errors of calibration (SEC) and of cross validation (SECV) and standard errors of prediction (SEP) of external validations.

	Calibration performance			External validation	
Trait	R2	SEC	SECV	SEP	R2
Oil content	0,99	0,69	0,71	0,80	0,99
Palmitic (C16:0)	0,60	0,71	0,74	0,82	0,67
Palmitoleic (C16:1)	0,66	0,11	0,12	0,15	0,44
Stearic (C18:0)	0,62	0,40	0,41	0,47	0,68
Oleic (C18:1)	0,81	0,95	0,99	1,02	0,84
Linoleic (C18:2)	0,89	0,85	0,90	1,06	0,93
Linolenic (C18:3)	0,78	0,04	0,04	0,05	0,87

Table 3. Correlations between the total oil content and individual fatty acids in JCL flour.

	Oil content	Palmitic	Palmitoléic	Stearic	Oleic	Linoleic	Linolenic
Oil content	...	-0,46	-0,53	-0,29	0,09	0,27	-0,66
Palmitic		...	0,67	0,06	-0,34	-0,24	0,55
Palmitoléic			...	0	-0,32	-0,11	0,51
Stearic				...	0,48	-0,71	-0,16
Oleic					...	-0,80	-0,44
Linoleic						...	0,10
Linolenic							...

Conclusion

NIRS could be a promising technique to determine oil content and quality traits of JCL seeds. It performed well to determine concentrations of oil, linoleic and oleic acids in JCL seed flour samples. Since NIRS allows simultaneous determination of different quality traits, it is a simple tool to reduce analytical time and cost and thus could be very helpful in analysis of a large number of samples in breeding programs and in industrial processing of the seeds and their by-products.

References

Achten WMJ, Verchot L, Franken YJ, Mathijs E, Singh VP, Aerts R, Muys B (2008). Jatropha bio-diesel production and use, Biomass and Bioenergy, 32 (12), 1063-1084

Jongschaap REE, Corré WJ, Bindraban PS, Brandenburg WA (2007). Claims and facts on *Jatropha curcas* L. International Report 158 (p.66) Wageningen: Plant Research

Kaushik N, Kumar K, Kumar S, Kaushik N, Roy S (2007) Genetic variability and divergence studies in seed traits and oil content of Jatropha (*Jatropha curcas* L.) accessions, Biomass and Bioenergy 31, 497–502

Kumar A, Sharma S (2008) An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review, Industrial Crops and Products, 28 (1), 1–10

Montes JM, Technow F, Bohlinger B, Becker K (2003). Grain quality determination by means of near infrared spectroscopy in *Jatropha curcas* L., Industrial Crops and Products, 43, 301–305

Pérez-Vich B, Velasco L, Fernández-Martínez JM (1998). Determination of seed oil content and fatty acid composition in sunflower through the analysis of intact seeds, husked seeds, meal and oil by near-infrared reflectance spectroscopy, Journal of the American Oil Chemists' Society, 75, 547-555

Sato T, Maw A, Katsuta M (2003). , NIR reflectance spectroscopic analysis of the FA composition in sesame (*Sesamum indicum* L.) seeds, Journal of American Oil Chemist Society, 80, 1157–1161

Tatikonda L, Wani SP, Kannan S, Beerelli N, Sreedevi TK, Hoisington DA, Devi P, Varshney RK (2009). AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L., a biofuel plant, Plant Science, 176, 505–513

Vaknin Y, Ghanim M, Samra S, Dvash L, Hendelsman E, Eisikowitch D, Samocha Y (2011). Predicting *Jatropha curcas* seed-oil content, oil composition and protein content using near-infrared spectroscopy – a quick and non- destructive method, Industrial Crops and Products, 34, 1029–1034

Velasco L, Moellers C, Becker HC (1999). Estimation of seed weight, oil content and fatty acid composition in intact single seeds of rapeseed (*Brassica napus* L.) by near infra-red reflectance spectroscopy, Euphytica, 106, 79–85