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Rapid Determination of the Oil and Moisture Contents in *Camellia Gauchowensis* Chang and *Camellia Semiserrata* Chi Seeds Kernels by Near-Infrared Reflectance Spectroscopy

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Abstract

A fast and effective determination method of different species of vegetable seeds oil is vital in the plant oil industry. The near-infrared reflectance spectroscopy (NIRS) method was developed in this study to massively analyze the oil and moisture contents of *Camellia gauchowensis* Chang and *C. semiserrata* Chi seeds kernels. In the prediction models of NIRS, the levels of accuracy obtained were sufficient for *C. gauchowensis* Chang and *C. semiserrata* Chi, the correlation coefficient of which oil were 0.983 and 0.962, respectively, while which of moisture were 0.937 and 0.907, respectively. The near infrared spectrum of crush seeds kernels was more precise compared to intact kernels. Based on the calibration models of the two *Camellia* species, the NIRS predictive oil contents of *C. gauchowensis* Chang and *C. semiserrata* Chi seeds kernels was developed in the traditional chemical measurement, the rapid, precise measurement of oil and moisture of *C. gauchowensis* Chang and *C. semiserrata* Chi seeds kernels with the traditional chemical measurement, the rapid, precise measurement of oil and moisture of *C. gauchowensis* Chang and *C. semiserrata* Chi seeds kernels with the traditional chemical measurement, the rapid, precise measurement of oil and moisture of *C. gauchowensis* Chang and *C. semiserrata* Chi seeds kernels can be actualized by NIRS method.

Keywords

Near infrared reflectance spectroscopy; *Camellia* seeds kernel; Oil content; Moisture content

1. Instruction

Camellia oleifera, native to China, one of the most important sources of high quality edible plant oil, has been more than 1000 years in southern China [1] and is widely distributed with more than 12 million acres in production [2]. *C. gauchowensis* Chang and *C. semiserrata* chi [3], two common woody species of *C. oleifera* indigenously to China, are mainly cultivated in mountain area. As cooking oil that has highly nutritional value for human health, the annual *Camellia* oil production exceeds 150 million kilograms [4,5]. It has 85–92% unsaturated fatty acids [5] including 75–85% oleic acid that plays important roles in reducing cholesterol and triglycerides in the blood [6,7], and a variety of other functional components such as vitamin E, phytosterols, squalene, and flavonoids [8].

For the proper utilization of *C. oleifera* oils in food and other industries, the quality characteristics of *C. oleifera* seeds such as the contents of oil, moisture and protein, and the compositions of fatty acid, should be evaluated quickly and reliably by analytical tools at the harvest, marketing and processing. Conventional analysis for oil and moisture are time-consuming, laborious, and required the use of toxic chemicals and expensive equipment [9]. The development of fast and effective methods becomes a necessity when the application needs are taken into consideration.

In recent years, there is a growing interest in fast, reliable and environmentally friendly technologies both in food production and food research [10]. Near-infrared reflectance spectroscopy (NIRS) technology is a simple and non-destructive method that can measure the quality and compositional attributes of many substances [11,12]. The NIRS has some important advantages, like short analysis time, minimal sampling process, and non-destructiveness with a performance comparable to chemistry analytical methods [13,14]. The NIRS is a spectroscopy method that uses the near-infrared region (400–2500 nm) of the electromagnetic spectrum [15] that can quantify organic compounds by the absorption of near-infrared light with the chemical bonds. Reflectance signatures are collected from sample materials with known nutrient concentrations, and then mathematical models are developed to estimate nutrient constituents of materials with unknown levels [16]. Automatic data collection

by NIRS allows the assessment of multiple traits in large sets of samples [14] with short duration and with minimal error [16].

Based on the resolution of the analytical and quality factors from food samples with correlation of electromagnetic absorption at aforementioned wavelength, NIRS is used routinely in sensory, physical and chemical analysis of food and agricultural products [17-19]. It has been widely used in different crops, such as sesame [20], maize [21], rice [22], soybean [23] and sunflower [24] to predict oil, moisture, protein, fatty acids, phenols or crude fiber. However, the ability of NIRS for prediction of *C. oleifera* oil and moisture content, especially C. *gauchowensis* Chang and *C. semiserrata* chi, has not been reported.

It is necessary to rapidly, substantially and reliably determine oil and moisture contents in *C. oleifera* seeds, which plays a key role in national production, importation as well as in food processing and breeding programs. Since analyses based on NIRS do not require labor intensive sample pre-treatment and processing, samples are measured with simple grinding or as a whole. In this study, we developed four quantitative spectral analysis models by NIRS for *C. gauchowensis* Chang and *C. semiserrata* Chi seeds to fast determine the oil and moisture contents, respectively.

2. Materials and methods

2.1 Materials

Each of 110 samples of *Camellia gauchowensis* Chang and *Camellia semiserrata* Chi seeds were obtained from the seed resource bank of Guangdong Academy of Forestry. To guarantee the reliability and applicability of the models, calibration and independent validation samples were selected from different regions in Guangdong province such as Gaozhou, Huizhou, Yangjiang, Lianping, and Zhaoqing to ensure a wide range of reference values. The seeds were collected and naturally air-dried after full maturity. The inner shells of seeds were peeled off to get the *Camellia* kernels. The intact *Camellia* kernels were selected and preserved.

Traditional chemical measurements for oil and moisture were Soxhlet extraction [7] and oven drying [23], respectively.

2.2 Near infrared spectroscopy

A DA7200 NIRS analyzer (Perten Instruments AB, Huddinge, Sweden) equipped with simultaneous detection of two beams of halogen and mercury lamp was used for spectral measurement. Fixed holographic grating partial light and indium arsenic diode array detection technology with electric refrigeration constant temperature, full spectrum was simultaneously scanned. Spectral data were acquired in the 900 to 1,700 nm range with 5 nm resolution at the collection data of 100 times per second. Simplicity software was integrated as a device manager.

- 2.3 Spectra Measurements
- 2.3.1 Sample pretreatment

The *Camellia* seeds kernels were openly placed in an air-conditioned thermostat room at $20 \pm 2 \,^{\circ}$ C for two weeks to keep their moisture content under the 10%. The kernels were loaded into a small round plastic cup specifically designed for detection of precious and minute quantities of samples. One batch of specimen was directly used for spectroscopic analysis and one batch of comminution was used for spectral decomposition.

2.3.2 Spectral feature data acquisition

The NIRS instrument was opened to preheat for 1 h. Before the spectrum collection, RE-SULT-Integration software was used to compile spectrum acquisition program. The working parameters of the instrument were set as the measuring wavelength range from 950 to 1,650 nm at 30 subsequently scanning times. The sampling mode was transmittance and the data acquisition were absorbance.

After writing the collection program, the processed samples were placed on the spectrometer sample tray in turn to be scanned. The near infrared spectra of samples were collected by the cup light probe. To reduce the error caused by inhomogeneous loading, each prototype was scanned 3 times from different angles. The all spectral characteristics were collected and recorded.

2.3.3 Calibration model construction

The average spectra of each sample were collected for smoothing pretreatment by Unscrambler classifier chemometrics software package. A quantitative spectral analysis model for *Camellia* seeds samples was established by partial least squares

(PLS) and full interactive validation. Based on the results of principal component analysis (PCA), the calibration model was determined.

3. Results and discussion

3.1 Comparison of *Camellia* seeds spectrograms between non-destruction and comminution

Vegetable oil contains massive fatty acids such as oleic acid, linoleic acid, stearic acid, and palmitic acid, hydrogen groups of which including C—H and O—H have strongly absorbance in the near-infrared spectrum. In Fig. 1, the absorptions of *Camellia* seeds kernels in different treatment were prominently different, reflecting varying oil at around 1,200 nm (C—H) [25] and moisture at up to 1,450 nm (O—H) [26]. The sample spectrum after crushing was more concentrated (Fig.1.B) than that of the untreated samples (Fig.1.A). The near-infrared reflectance spectrogram (NIRS) of *Camellia* seeds kernels could be used for the quantitative analysis and the selection of suitable sample types, in addition, the NIRS accuracy was higher when the *Camellia* seeds kernels were comminuted. Hence, a special adapter for selecting the smaller samples probably is suitable contrived for NIRS scanning [27].

3.2 NIRS analysis of *Camellia* seeds kernels

The light absorption causes vibration and oscillation between atoms to make the change of light energy [28]. Hence, the diverse spectrums in NIRS area indicated the differences in the moisture content, and the oil chemical composition and content for both *Camellia gauchowensis* Chang and *C. semiserrata* Chi seeds kernels.

3.2.1 NIRS analysis of Camellia oil

The calibration models had marvelous accuracy for the detection of *C. gauchowensis* Chang (Fig. 2) and *C. semiserrata* Chi (Fig. 3) kernels oil. Some spectral variables, including irrelevant information and unreliable prediction, were removed by suitable algorithms [29] from each 110 samples, wherein the effective quantities of tested samples were 106 of *C. gauchowensis* Chang and 104 of *C. semiserrata* Chi. From Fig.2.A and Fig.3.A, the principal component analysis (PCR) showed that the both centralities of scores were high. After the PCs from 0 to 20 were analyzed by residual validation variance (Fig.2.B and Fig.3.B), the regression coefficients were ideal when

the PCs were both selected at 5 (Fig.2.C and Fig.3.C). In Fig.2.D and Fig.3.D, the linear relationships were established between predicted and measured values, the calibration parameters of which were shown in Table 1.

The coefficient of correlation (R_c) was 0.983 for *C. gauchowensis* Chang kernels oil and 0.962 for *C. semiserrata* Chi oil. The good selected model should have high coefficient of calibration and low error, and should also have a small difference between root mean square error of calibration (R_{MSEC}) and standard error of calibration (SEC) [30]. The coefficient of determination (R_d) was high at 0.967 for *C. gauchowensis* Chang seeds kernels oil, while 1.564 of R_{MSEC} and 1.572 of SEC were little distinct. The R_d was also high at 0.926 for *C. semiserrata* Chi oil seeds kernels oil, while 1.714 of R_{MSEC} and 1.723 of SEC were extraordinary close. Compared with the two *Camellia* seeds kernels oil by R_d , R_{MSEC} , offset value, and SEC, the accuracy of predictive model of *C. gauchowensis* Chang was higher than *C. semiserrata* Chi. The calibration models for *C. gauchowensis* Chang and *C. semiserrata* Chi seeds kernels were both scientific and practicable to predict the oil contents.

3.2.2 NIRS analysis of *Camellia* moisture

Both of calibration models analysis for *C. gauchowensis* Chang and *C. semiserrata* Chi seeds kernels moisture were shown in Fig. 4 and Fig. 5, respectively. The scores showed high concentricity by PCR in Fig.4.A and Fig.5.A. The residual validation variance (Fig.4.B and Fig.5.B) and the regression coefficients (Fig.4.C and Fig.5.C) were analyzed to set up the predict models (Fig.4.D and Fig.5.D), the calibration parameters of which were also shown in Table 1.

Both of the R_c of *C. gauchowensis* Chang (0.937) and *C. semiserrata* Chi (0.907) seeds kernels moisture were high, while the R_d were up to 0.878 and 0.822, respectively. R_{MSEC} and SEC were almost same, 0.257 and 0.259 of *C. gauchowensis* Chang, 0.272 and 0.274 of *C. semiserrata* Chi, respectively. The offset values were generally low, 0.522 and 0.618, respectively. R_c , R_d , R_{MSEC} , SEC, and offset were all within the normal range making the calibration models of *C. gauchowensis* Chang and *C. semiserrata* Chi seeds kernels have good accuracy to predict the moisture contents.

2.3 NIRS predictive model verification

Calibration models must be validated before application in practice [31]. Hence, the NIRS content should be estimated with high correlation and good accuracy when the predicted results were compared to conventional measured results [32]. The comparative analysis of oil and moisture content of C. gauchowensis Chang and C. semiserrata Chi kernels by NIRS and standard laboratory method was shown in Table 2. In 106 C. gauchowensis Chang samples, the maximal oil content by NIRS method was 68.43%, the 23.85% of minimum, the 48.71 ± 8.94% of mean ± standard deviation (SD), 0.18 of variation coefficient (CV), respectively, the maximum of which by Soxhlet method was 56.82%, the 22.16% of minimum, the $45.32 \pm 7.57\%$ of mean ± SD, 0.17 of CV, respectively. In 104 C. semiserrata Chi kernels, compared with NIRS and Soxhlet method, the maximums of oil were 71.08% and 70.00%, respectively, 31.71% and 51.71% of minimum, 58.37 ± 7.39% and 62.73 ± 4.38% of mean ± SD, 0.13 and 0.07 of CV. The maximums, minimums, average values, SDs and CVs of both C. gauchowensis Chang and C. semiserrata Chi were accorded with the conventional measurement. Hence, the predict models of oil content by NIRS for C. gauchowensis Chang and C. semiserrata Chi seeds kernels, were provided with preferable accuracy and precision.

For moisture content analysis of 106 *C. gauchowensis* Chang seeds kernels, the maximums by NIRS and oven drying method were 9.02% and 9.00%, respectively, while 2.40% and 2.74% of minimum, 4.39 \pm 1.08% and 4.62 \pm 0.84% of mean \pm SD, 0.25 and 0.18 of CV, respectively. In 104 *C. semiserrata* Chi kernels, the maximal moisture contents by NIRS and oven drying were 6.37% and 5.14%, respectively, while 2.32% and 0.71% of minimum, 3.49 \pm 0.71% and 3.19 \pm 0.84% of mean \pm SD, 0.20 and 0.26 of CV, respectively. Accordingly, the two predict models of *C. gauchowensis* Chang and *C. semiserrata* Chi seeds kernels by NIRS could be available with rapid and effective determination of moisture contents, respectively. Compared with *C. gauchowensis* Chang and *C. semiserrata* Chi seeds kernels by NIRS, the oil content of *C. gauchowensis* Chang was lower 9.66% than *C.*

semiserrata Chi, and the moisture content was higher 0.90%. The moisture content

for its specific application is important with regard to the economics of *Camellia* trading, and keeps its quality with a low level preventing the growth of micro-organisms and mould spoilage [33]. *C. semiserrata* Chi seeds kernels had higher oil content and lower moisture, when it was under the same condition with *C. gauchowensis* Chang. Consequently, *C. semiserrata* Chi was more suitable for cultivate, storage, process, and application.

4. Conclusions

This study demonstrated that NIRS was a powerful technique to predict oil and moisture content of *Camellia gauchowensis* Chang and *Camellia semiserrata* Chi seeds kernels. High coefficient of calibrations showed that NIRS analysis, a fast and effective method, could be applied in *Camellia* oil industry. This predictive model had good accuracy and sufficient credibility. Compared with the traditional chemical measurement and analysis, the detection time by NIRS was effectively shortened, while the detection accuracy improved and the detection stability ensured. Under the establishment of a relatively accurate calibration model, it could be used as the reference basis for making the *C. gauchowensis* Chang and *C. semiserrata* Chi procurement price.

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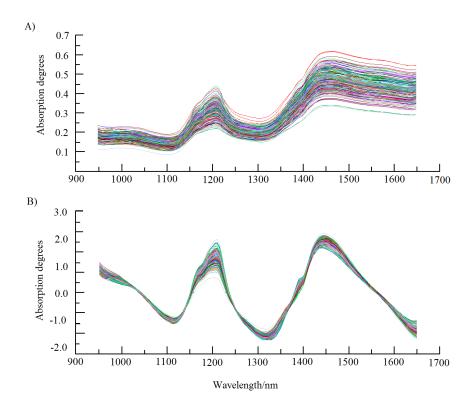


Fig.1 Near infrared spectrogram comparison of *Camellia* seeds kernels between non-destruction (A) and comminution (B)

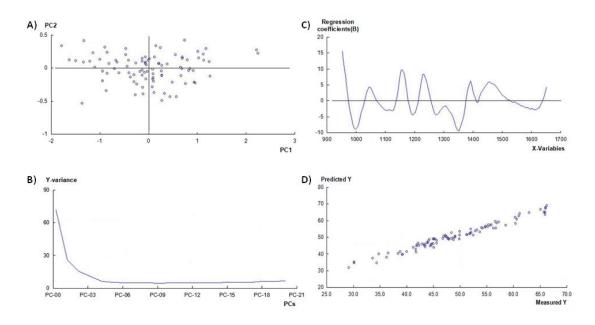


Fig.2 Near infrared spectrogram of Camellia gauchowensis Chang kernels oil

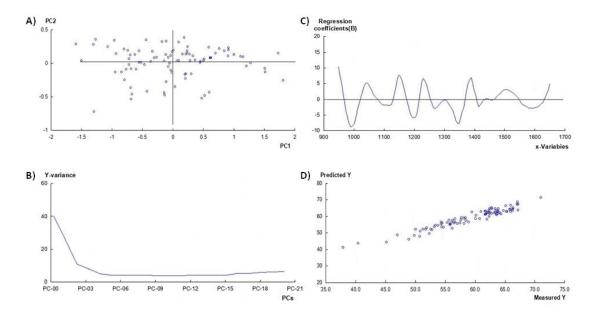


Fig.3 Near infrared spectrogram of Camellia semiserrata Chi kernels oil

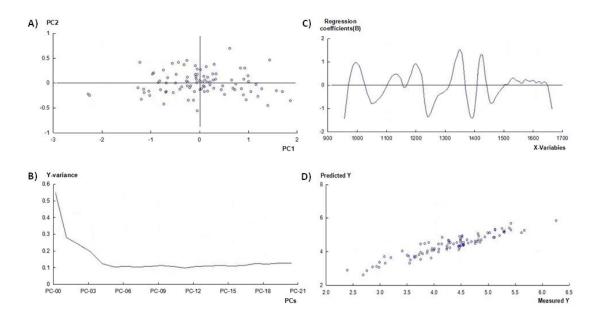


Fig.4 Near infrared spectrogram of Camellia gauchowensis Chang kernels moisture

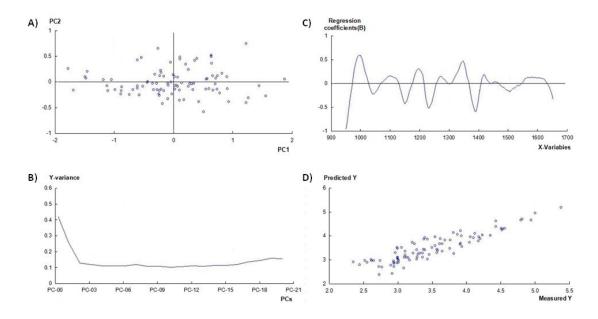


Fig.5 Near infrared spectrogram of Camellia semiserrata Chi kernels moisture

Indicator	Seeds kernels	R _c ¹	R_{d}^2	R_{MSEC^3}	SEC ⁴	Offset
Oil	Camellia gauchowensis Chang	0.983	0.967	1.564	1.572	1.616
	Camellia semiserrata Chi	0.962	0.926	1.714	1.723	4.375
Moisture	Camellia gauchowensis Chang	0.937	0.878	0.257	0.259	0.522
	Camellia semiserrata Chi	0.907	0.822	0.272	0.274	0.618

Table 1 Oil and moisture calibration parameters by NIRS analysis

Note ${}^{1}R_{c}$, Coefficient of correlation; ${}^{2}R_{d}$, Coefficient of determination; ${}^{3}R_{MSEC}$, Root mean square error of cross; ${}^{4}SEC$, Standard error of calibration

Table 2 Comparisons with NIRS and traditional measurement of oil and moisture

contents for C. gauchowensis Chang and C. semiserrata Chi kernels

Seeds kernels	Number	Method	Max ¹ /%	Min²/%	Mean ± SD ³ /%	CV ⁴
Camellia gauchowensis Chang	106	NIRS	68.43	23.85	48.71 ± 8.94	0.18
		Soxhlet	56.82	22.16	45.32 ± 7.57	0.17
<i>Camellia semiserrata</i> Chi	104	NIRS	71.08	31.71	58.37 ± 7.39	0.13
		Soxhlet	70.00	51.71	62.73 ± 4.38	0.07
Camellia gauchowensis Chang	106	NIRS	9.02	2.40	4.39 ± 1.08	0.25
		Oven	9.00	2.74	4.62 ± 0.84	0.18
<i>Camellia semiserrata</i> Chi	104	NIRS	6.37	2.32	3.49 ± 0.71	0.20
		Oven	5.14	0.71	3.19 ± 0.84	0.26
	Camellia gauchowensis Chang Camellia semiserrata Chi Camellia gauchowensis Chang	Camellia gauchowensis Chang106Camellia semiserrata Chi104Camellia gauchowensis Chang106	Camellia gauchowensis Chang106NIRS SoxhletCamellia semiserrata Chi104NIRSCamellia gauchowensis Chang106NIRSCamellia gauchowensis Chang106NIRSCamellia semiserrata Chi104NIRS	$\begin{array}{c} \mbox{Camellia gauchowensis Chang} & 106 & \\ \mbox{NIRS} & 68.43 \\ \mbox{Soxhlet} & 56.82 \\ \mbox{NIRS} & 71.08 \\ \mbox{Soxhlet} & 70.00 \\ \mbox{Soxhlet} & 70.00 \\ \mbox{Camellia gauchowensis Chang} & 106 & \\ \mbox{NIRS} & 9.02 \\ \mbox{Oven} & 9.00 \\ \mbox{NIRS} & 6.37 \end{array}$	$\begin{array}{c} \mbox{Camellia gauchowensis Chang} & 106 & NIRS & 68.43 & 23.85 \\ \mbox{Soxhlet} & 56.82 & 22.16 \\ \mbox{NIRS} & 71.08 & 31.71 \\ \mbox{Soxhlet} & 70.00 & 51.71 \\ \mbox{Soxhlet} & 70.00 & 51.71 \\ \mbox{Camellia gauchowensis Chang} & 106 & NIRS & 9.02 & 2.40 \\ \mbox{Oven} & 9.00 & 2.74 \\ \mbox{NIRS} & 6.37 & 2.32 \\ \mbox{Camellia semiserrata Chi} & 104 \end{array}$	$\begin{array}{c} \label{eq:camellia} Camellia gauchowensis Chang \\ Camellia semiserrata Chi \\ Camellia semiserrata Chi \\ Camellia gauchowensis Chang \\ Camellia gauchowensis Chang \\ Camellia gauchowensis Chang \\ Camellia gauchowensis Chang \\ Camellia semiserrata Chi \\ Camell$

Note ¹Max, Maximum; ²Min, Minimum; ³SD, standard deviation; ⁴CV, coefficient of variation.