Application of Fourier Transform Near-Infrared (FT-NIR) spectroscopy for detection of adulteration in palm sugar

K Rismiwandira, F Roosmayanti, M F R Pahlawan and R E Masithoh

Department of Agricultural and Biosystems Engineering, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

Corresponding author: evi@ugm.ac.id

Abstract. The aim of this research was to detect adulteration in palm sugar by coconut sugar using FT-NIR spectroscopy with two chemometric methods namely partial least squares regression (PLSR) and principal component analysis (PCA). The absorbance spectra were taken using the FT-NIRFlex-500 Solid. Several spectral pre-processing methods used were the 1st Savitzky-Golay Derivative, Normalization, Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), and Baseline. Coconut sugar as adulterant with various concentration ranging from 0 to 100% were added to the palm sugar. A total of 77 spectra of pure and adulterated palm sugar samples were divided into two groups in which 51 samples used for developing calibration model and 26 samples used for developing prediction model. The spectral obtained were pre-processed and analyzed using The Unscrambler X version 10.4. The best transformation of PLSR was MSC with coefficient of determination (Rc²) of 0.93 and the root mean square error (RMSE) of 0.07 for calibration. By using prediction data sets, the model resulted in coefficient of determination of prediction (Rp²) of 0.91 and a root mean square error of prediction (RMSEP) of 0.09. Based on this result, FT-NIR spectroscopy combined with chemometrics is a promising method in food authentication.

1. Introduction
Nowadays, palm sugar is commonly consumed as sweeteners to alternate table-top sugar or sucrose. Palm sugar is becoming popular following the increase in healthy lifestyle due to the lower glycemic index (GI) of palm sugar (35-42) [1] compared to GI of sucrose from sugarcane (91) [2]. Palm sugar in crystal-shape can be made from coconut (Cocos nucifera) or sugar palm (Arenga pinnata) sap which is produced from thickens sap after boiling and then granulated. Both coconut and palm sugar have moderate similar color [3] and difficult to differentiate visually. Due to higher price of palm sugar made from sugar palm compared to coconut, adulteration might be unavoidable.

Adulteration is categorized as a food fraud including addition of low quality or dangerous substances into the main material motivated by economic purposes [4]. Several studies reported analytical methods in detecting adulteration by using HPLC [5,6] or 1H NMR spectroscopy [7,8]. Other research detected adulteration based on image processing [9, 10]. Another method in adulteration detection is vibrational spectroscopy using Near Infrared (NIR) and Mid Infrared (MIR), as well as Raman spectrometer [11]. NIR spectroscopy works at region of 1000-2500 nm or 10,000 – 4000 cm⁻¹ is the most popular method used for detection of adulteration [12]. Chemical bonds of atoms containing C-H, O-H, and N-H have high vibrational frequencies detected in the NIR region.
Therefore, sugar which comprises mainly C-H-O atoms can be easily detected using NIR spectrometer. Detection of adulteration using NIR method was conducted in onion powder [14], black pepper [15], herbal medicine [16], or coffee [17,18]. Study reported the use of FT-IR spectroscopy for detecting adulteration of palm sugar resulted in good performance [19]. However, study of adulteration detection in palm sugar using NIR spectroscopy is limited, therefore it was conducted in this research.

This research used FT-NIR spectroscopy method for detecting the addition of coarse coconut sugar to palm sugar in various concentrations. The method employed two chemometric methods namely partial least squares regression (PLSR) and principal component analysis (PCA). The findings will be beneficial to obtain high quality palm sugar products since any potential adulterations can be detected rapidly and non-destructively at early stage.

2. Materials and methods

2.1. Coconut and palm sugar sample

The main ingredient in this research was palm sugar made from Arenga pinnata sap, while the adulterant was coconut sugar from Cocos nucifera sap. Both sugars were in crystal-shape produced by boiling the saps until thickens. The samples were purchased from various sugar craftsmen in the local markets. Samples were stored in dry and air-tight containers to maintain sample quality prior to spectra acquisition. Coconut sugar as adulterants at 0 to 100% concentrations were added to palm sugar to obtain final concentrations of ±50 grams measured using an analytical balance (Ohaus Scout Pro SPS202F) as shown in Table 1.

Before spectra acquisition, samples were re-dried at 65-70°C for 12 hours to remove excessive water which can affect the spectra [13]. By using gravimetric method, water content of coconut sugar and palm sugar were calculated which resulted in 1.2% and 0.7% (w/w), respectively. In order to obtain size uniformity, the samples were sieved with an 80-mesh sieve (0.18 mm; ASTM Standard). To get a well mixture of coconut-palm sugar, each sample of coconut-palm sugar combination was placed in a bottle and put on a Vortex for mixing. Seven samples of each concentration combination were used resulting in total 77 samples. All samples were placed in the desiccator (Normax ISO 13130) before spectra acquisition.

| Adulteration Class | Coconut Sugar | Palm Sugar | Number of Samples |
|--------------------|---------------|------------|------------------|
| %                  | gram          | %          | gram             | Samples |
| 0%                 | 0             | 100        | 50               | 7       |
| 10%                | 10            | 90         | 45               | 7       |
| 20%                | 20            | 80         | 40               | 7       |
| 30%                | 30            | 70         | 35               | 7       |
| 40%                | 40            | 60         | 30               | 7       |
| 50%                | 50            | 50         | 25               | 7       |
| 60%                | 60            | 40         | 20               | 7       |
| 70%                | 70            | 30         | 15               | 7       |
| 80%                | 80            | 20         | 10               | 7       |
| 90%                | 90            | 10         | 5                | 7       |
| 100%               | 100           | 0          | 0                | 7       |

2.2. Spectra acquisition

Three grams of sample were put into sample a vial having diameter of 10-15 mm for scanning. NIR spectrum was obtained with the FT-NIR spectrophotometer (Buchi NIRFlex N-500 solid) equipped with six sample vials. Scanning spectra data were obtained using the NIRWare software. The spectra were obtained in reflectance mode in the wavelength region of 10,000-4000 cm\(^{-1}\) with a sampling
interval of 4 cm\(^{-1}\) resulted in a total of 1501 wavelength data. All measurements were done at room temperature.

2.3. Spectra preprocessing and chemometrics analysis
Spectra data from the NIRWare were converted into Excel file for further analysis. The original reflectance data obtained from the NIR instrument were then altered into absorbance data. The Unscrambler X version 10.4 software. CAMO Software US (Oslo, Norway) was used for spectra preprocessing and multivariate analysis. Of 77 spectra, 51 spectra were used for calibration and 26 spectra were used for prediction data sets. In this study, FT-NIR spectra data were analyzed using two chemometric methods, namely partial least squares regression (PLSR) and principal component analysis (PCA). Several preprocessing methods were carried out on the original data, including 1st Savitzky Golay Derivative, Normalization, Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), and Baseline.

3. Results and discussion

3.1. Interpretation of spectra data
All FT-NIR original spectra in Figure 1.a show similar trends and are difficult to distinguish spectra based on adulteration concentration, except pure palm sugar spectra which has the lowest absorbance value. After applying the 1st Savitzky-Golay derivatives preprocessing method to the original spectra, several peaks of spectra can be observed which may be related to the main chemical constituents of sugar from palm trees such moisture and sugar (sucrose, fructose, and glucose), as well as minor components such as amino acid, protein, mineral, and ash [20, 21].

Figure 1. (a) Original and (b) 1st Savitzky-Golay derivatives FT-NIR spectra of pure and adulterated palm sugar at different concentrations

Distinct peaks are noticeable at 4000 cm\(^{-1}\)-5000 cm\(^{-1}\), such as at 4060 cm\(^{-1}\) (C-H combination), 4150 cm\(^{-1}\) (C-H combination), 4270 cm\(^{-1}\) (C-H stretch/deformation), 4340 cm\(^{-1}\) (C-H bend 2\(^{nd}\) overtone), 4460 cm\(^{-1}\) (O-H stretch/C-O stretch combination), 4540 cm\(^{-1}\) (C-H stretch/C=O stretch combination), 4620 cm\(^{-1}\) (asymmetric C-H stretch/C-H deformation), 4750 cm\(^{-1}\) (O-H bending/C-O stretch combination; Asymmetric C-O-O stretch 3\(^{rd}\) overtone), 4892 cm\(^{-1}\) (N-H/N-H in-plane bend; C-
N stretch or N-H/C-N stretch; N-H in-plane bend or combination), and 4996 cm\(^{-1}\) (N-H stretch/N-H bend combination) [22]. Other peaks can be observed at 5260 cm\(^{-1}\) of C=O stretch 2\(^{nd}\) overtone, 5900 and 5944 cm\(^{-1}\) of C-H stretch 1\(^{st}\) overtone, 6936 cm\(^{-1}\) of N-H stretch 1\(^{st}\) overtone, and 6988 cm\(^{-1}\) of O-H stretch 1\(^{st}\) overtone or C=O stretch 3\(^{rd}\) overtone.

3.2. Principal component analysis (PCA)
Principal Component Analysis (PCA) is an unsupervised technique to explore differences between samples and adulterants based on spectra profiles. Figure 2 shows PCA score plot developed from MSC preprocessing spectra. By using PC-4 and PC-6, the palm sugar can be classified into three groups, namely low, medium, and high-level adulteration. The low level consisted of 0\%-20\% of coconut sugar, medium level consisted of 30\%-60\% of coconut sugar, and high level consisted of 70\%-100\% of coconut sugar. The low, medium, and high level of adulteration are located in the negative axis, around axis, and positive axis of PC-6, respectively. However, they are not clearly defined according to PC-4.

Figure 2. PCA of MSC pre-processed of FT-NIR spectra showing clustering based on adulteration level
Several peaks which show difference in loadings of PC-4 and PC-6 are peaks at 5430 cm\(^{-1}\) of O-H stretch/C-O stretch 2\(^{nd}\) overtone combination, 6970 cm\(^{-1}\) of C-H, R-OH 2\(^{nd}\) overtone combinations, and 7210 cm\(^{-1}\) of C-H combination [22, 23]. Low level adulteration showed from negative value of PC-6 means higher concentration of palm sugar compared to coconut sugar. Palm sugar have higher sucrose compared to coconut sugar [2], thus the loading at 6970 cm\(^{-1}\) may contributes to sucrose as also reported by Guven et al. [23]. Loading at 5430 cm\(^{-1}\) which is reflected from positive value of PC-6 (Figure 2) is associated with water owned by high level adulteration sugar or high concentration of coconut sugars. Water content of coconut sugar in this research is higher than palm sugar.
3.3. PLSR model for determining level of adulteration in palm sugar

PLSR method was used to develop a model to predict concentration of coconut sugar added to palm sugar. By using original and preprocessing spectra, the PLSR are developed. The performance of calibration models is determined from the determination coefficient of calibration ($R_c^2$) and root mean standard error of calibration (RMSEC). Model which has the highest $R_c^2$ and low RMSEC is selected. In this research, PLSR models were developed using original and several preprocessed spectra, namely 1st Savitzky Golay Derivative, Normalization, Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), and Baseline. The PLSR results are presented in Table 2. The best PLSR model is obtained by using the MSC method with $R_c^2$ of 0.93 and RMSEC of 7.83% meaning that the model is usable for quality control applications [24]. By using predictive data set, the model is able to predict the level of adulteration with $R_p^2$ of 0.91 and RMSEP of 9.13%.

Table 2. Calibration and prediction results by using original and pre-processing spectra at wavenumber of 4000 – 10000 cm$^{-1}$

| Method     | Calibration | Prediction |
|------------|-------------|------------|
|            | N  | $R_c^2$ | RMSEC (%) | N  | $R_p^2$ | RMSEP (%) |
| Original   | 51 | 0.85   | 11.87      | 26 | 0.61   | 19.78      |
| MSC        | 51 | 0.93   | 7.83       | 26 | 0.91   | 9.13       |
| Normalization | 51 | 0.91   | 9.20       | 26 | 0.85   | 12.30      |
| SNV        | 51 | 0.93   | 7.88       | 26 | 0.90   | 9.62       |
| Baseline   | 51 | 0.86   | 11.36      | 26 | 0.70   | 17.32      |
| SG-1       | 51 | 0.93   | 8.17       | 26 | NA     | 54.4       |

N: number of samples; MSC: Multiplicative Scatter Correction; SNV: Standard Normal Variate; SG-1: 1st Savitzky Golay Derivative; RMSEC/P: root mean square error of calibration/prediction
Figure 4 shows regression coefficient of PLSR model generated using MSC spectra. Regression coefficients can be used to relate the specific band to chemical content of samples [25]. Several peaks which are considered to be important in developing model for determining coconut sugar addition in palm sugar can be observed at 4308, 4590, 4670, 4970, 5250, 6850, 6960, and 8240 cm$^{-1}$. Those important wavelengths are related to the absorbance of several important chemical compositions in palm and coconut sugar. In general, wavelengths at 5800-5400 cm$^{-1}$ and 4600-4000 cm$^{-1}$ contribute to vibrations of sugar molecules [26]. Noticeable peaks at 4308 and 6960 cm$^{-1}$ are associated to sucrose [23] in which palm sugar has higher sucrose than coconut sugar [2]. Peak at 4308 and 8240 cm$^{-1}$ are assigned to glucose and fructose [23] which although present in coconut and palm sugar but in relatively low concentration [27]. Peaks appeared between 7000 and 6800 cm$^{-1}$ are related to water [26] which presents in both palm and coconut sugar. The peak at 4590, 4670, 4970, and 5250 cm$^{-1}$ are associated to protein [28, 29] which also presents in low concentration in both sugars [27].

![Regression coefficients](image)

**Figure 4.** Regression coefficients of PLSR model using MSC preprocessing spectra for predicting level of coconut sugar adulteration in palm sugar

4. Conclusion
This research studied the potential of FT-NIR spectroscopy to detect adulteration of coconut sugar in palm sugar at various concentrations. Using MSC preprocessing spectra to develop the PLSR model, the calibration model yielded $R_c^2$ and RMSEC of 0.93 and 7.83%. When applied to the predictive data set, the model yields $R_p^2$ of 0.91 and RMSEP 9.13%. The results confirm the potential of FT-NIR for detecting coconut sugar adulteration in palm sugar.
Acknowledgments
Great appreciation is delivered to Faculty of Agricultural Technology Universitas Gadjah Mada for financial support under Hibah Penelitian Inovatif 2019 and Tafip (UKSW) for his assistance in finishing this work.

References
[1] Haagen A and Lantican C B 2015 Arenga pinnata bioethanol data 1–62
[2] Srikaeo K, Sangkhia W and Likittrakulwong W 2019 Walailak J. Sci. Technol. 16 897–907
[3] Saputro A D, Walle D V D and Dewettinck K 2020 Food Biosci. 38 100780
[4] Manning L 2016 Curr. Opin. Food Sci. 10 16–21
[5] Domingues D S, Pauli E D, Abreu J E M, Massura F W, Cristiano V, Santos M J and Nixdorf S L 2014 Food Chem. 146 353–62
[6] Wang Q, Zhao H, Zhu M, Zhang J, Cheng N and Cao W 2020 LWT - Food Sci. Technol. 118 10863
[7] Bergana M M, Adams K M, Harnly J, Moore J C and Xie Z 2019 J. Food Compos. Anal. 78 49–58
[8] He C, Liu Y, Liu H, Zheng X, Shen G and Feng J 2020 Food Res. Int. 130 108936
[9] Pribadi W, Masithoh R E, Nugroho A P and Radii IOP Conf. Ser. Earth Environ. Sci. 355 012003
[10] Wasnik P G, Menon R R, Sivaram M, Nath B S, Balasubramanyam B V and Manjunatha M 2019 J. Food Sci. Technol. 56 2320–5
[11] Danezis G P, Tsagkaris A S, Camin F, Brusic V and Georgiou C A 2016 TrAC - Trends Anal. Chem. 85 123–32
[12] Esteki M, Shabasvari Z and Simal-Gandara J 2018 Food Control 91 100–12
[13] Osborne B G 2006 Near-Infrared Spectroscopy in Food Analysis Encyclopedia of Analytical Chemistry (John Wiley and Sons Ltd.) a1018
[14] Lohumi S et al. 2014 J. Agric. Food Chem. 62 9246–9251
[15] Wilde A S, Haughey S A, Galvin-king P and Elliott C T 2019 Food Control 100 1–7
[16] Zhao J, Cui P, Liu H, Wang C, Li G and Pan M 2020 Infrared Phys. Technol. 104 1–9
[17] Pauli E D et al. 2014 Food Res. Int. 61 112–119
[18] Ebrahimii-Najafabadi H, Leardi R, Oliveri P, Chiara, Casolino M, Jalali-Heravi M and Lanteri S 2012 Talanta 99 175–179
[19] Roosmayanti F, Rismiwindira K and Masithoh R E 2020 Detection of coconut (Cocos nucifera) sugar adulteration in palm (Arenga pinnata Merrill) sugar by Fourier Transform Infrared (FT-IR) Spectroscopy
[20] Tomomatsu A, Itoh T, Wijaya C H, Nasution Z, Kumendong J and Akila M 1996 J. Trop. Agric. 40 175–181
[21] Saputro A D, Walle D Van De and Dewettinck K 2019 Sugar Tech 21 862–867
[22] Shenk J S, Workman J J and Westerhaus M O 2008 Application of NIR spectroscopy to agricultural products Handbook of near-infrared analysis ed D A Burns and E W Ciurczak (Boca Raton: CRC Press)
[23] Guven B, Velioglu S D and Boyaci I H 2019 J. Food 44 274–290
[24] Williams P C 2001 Implementation of near-infrared technology, in Near-infrared technology ed The Agricultural and Food Industries 2nd ed. P C Williams and K Noris (Minnesota: The American Association of Cereal Chemist, Inc.)
[25] Masithoh R E, Lohumi S, Amanah H Z, Yoon W S and Cho B K 2020 Heliyon 6 e05099
[26] Simeone M L F, Parrella R A C, Schaffert R E, Damasceno C M B, Leal M C B and Pasquini C 2017 Microchem. J. 134 125–130
[27] Saputro A D, Walle D Van De and Dewettinck K 2020 Food Biosci. 38 100780
[28] Masithoh R E and Yuliyanda I 2019 IOP Conf. Ser. Earth Environ. Sci. 355 012004
[29] Magwaza L S, Messo, Naidoo S I, Laurie S M, Laing M D and Shimelis H, 2016 *Food Sci. Technol.* **72** 63–70