Development of calibration model for determination of sweeteners additives in Indonesia rice flour-based food by FT-NIR spectroscopy

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Abstract. Cane sugar and other artificial sweeteners are usually used as a food additive to provide sweetness in food. Sweetener additives can be consumed safely by considering the acceptable daily intake (ADI). This research was aimed to determine the type of sweeteners and the level of sweeteners added in food with regard to the ADI. The food sample used in this study was geplak, traditional Indonesian food, made of rice flour, coconut, and sugar, or other sweeteners. The reflectance of geplak powder was measured using the NIRFlex N500 Fiber Optic Solids Cell at 4000 – 10,000 cm⁻¹. The reflectance spectra obtained were pre-treated and analyzed using The Matlab version R2018a. Principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were used for data exploration and qualitative classification. PCA model was able to classify food added with sugar, saccharin, and cyclamate. PLS-DA calibration model using the 2nd derivative Savitzky-Golay as the spectral pretreatment achieved 100% accuracy in predicting high sucrose and high saccharin, as well as low cyclamate and low saccharin, while achieved a slightly low accuracy of 90.0 and 85.7% in predicting low sucrose and high cyclamate, respectively. PLS-DA prediction model, which also applies the 2nd derivative Savitzky-Golay pretreatment spectra, achieved 100% accuracy in predicting high and low sucrose, low cyclamate, and high saccharin.

1. Introduction
Food additives are ingredients added to enhance or retain food flavor or appearance [1]. The food additive is permitted as long as its use does not exceed the acceptable daily intake (ADI), which is the amount of a food additive that can be consumed daily over a lifetime without adverse effect [2]. As an additive, artificial sweeteners are used to substitute or alternate sugar due to their sweet taste but low calorie [3]. Sweeteners permitted in many countries are Aspartame, Acesulfame-K, Neotame, Saccharin, Sucralose, Cyclamate, and Alitame. Although they are safe, consumption of those sweeteners should consider the ADI.

There are various analytical techniques to detect food sweetener in food [4]. Sweeteners are detected using capillary electrophoresis [5], liquid chromatography/tandem mass chromatography (LC-MS/MS)
[6], or gas chromatography-electron capture detector (GC-ECD) [7]. Those methods are accurate yet require high-priced equipment and long sample chemical preparation. Therefore, for a routine and a large number of samples, analytical techniques that are fast and reliable to detect or determine sweeteners in food is of great importance.

One method of fast detection of food composition is using near-infrared (NIR) spectroscopy. The NIR spectrum is located in the spectrum range of 800-2500 nm or 4000-12,500 cm\(^{-1}\) at which hydrogen bond formation affects the overtones and combination tones [8]. It is one of the vibrational spectroscopic methods enabling complex chemical information to be determined [9]. NIR spectroscopy is used to determine material composition such as protein [10], total soluble solid [11], or acidity [12], as well as to detect adulteration in food [13]. The NIR analysis will be beneficial in providing sweetener information as the NIR spectrum triggers overtones and a combination of C, O, H, and N molecule vibrations of sweetener. It is also applied to determine various sugar and sweeteners [14]. However, the current studies only detected pure or mixed sweeteners, which were not mixed with other substances as food additives. This research aimed to apply FT-NIR spectroscopy to determine sweeteners additives in food made of rice flour using PCA and PLS-DA.

2. Materials and methods

2.1. Sample preparation

Geplak is a traditional food in Indonesia commonly found in Java Island, which is made of rice flour, shredded coconut, and sugar. Rice flour was sieved and roasted until dry, then shredded coconut was added. Sweetener and salt were melted in water. The solution was then added to the mix of flour and shredded coconut. The dough was then air-dried, and geplak was produced. No food colorant was added.

In this research, three sweeteners were used, i.e., table sugar (in this paper is used interchangeably with sucrose), saccharin, and cyclamate. One food sample weighed 30 g containing one concentration of sweetener. Sweetener concentration levels were determined based on the permitted maximum ratio of sweeteners (mg) per body weight (kg) according to Indonesian regulations, which were 170, 250, and 7500 mg for saccharin, cyclamate, and sugar, respectively. For this research, it was assumed that body weight was 40 kg, and there were 200 pieces of samples (each weighed 30 g). Therefore, 34, 50, and 1500 mg of saccharin, cyclamate, and sugar, were regarded as the acceptable daily intake (ADI) of each sweetener in each sample. A wide range of concentrations with 30 - 37 variations of each sweetener was applied to obtain a robust model. Concentrations range of sugar, cyclamate, and saccharin used in this research were 150 - 2760 mg, 5 – 200 mg, and 6 - 200 mg, respectively.

2.2. Spectral measurement

A geplak powder sample was weighed and transferred to a glass sample container (with a height of 10 mm and a diameter of 2 cm). To maintain uniformity of sample density during spectral measurement, each sample was loaded gently into the 2.5 cm diameter and 3 cm height cylindrical glass without press beforehand. NIR spectra of the samples were obtained in the reflectance mode using a NIRFlex N500 spectrometer operated in Fiber Optic configuration (Büchi Labortechnik AG, Flawil, Switzerland) with InGaAs detector. Spectra were acquired in diffuse reflectance mode in the long-wavelength region of 4000 – 10,000 cm\(^{-1}\) (2500 – 1000 nm) with a 4 cm\(^{-1}\) sampling interval resulted in total 1501 wavenumbers. The NIRS were recorded at ambient temperature, and a total of 133 spectra of samples were collected.

2.3. Data analysis

Raw spectra in reflectance mode were stored in MS Excel® (.csv file) for chemometric analysis. The Matlab version R2018a (The MathWorks, Inc., Natick, MA, USA) was used for spectra preprocessing and chemometrics analysis. For developing the PLS-DA model, the NIR spectra were preprocessed through normalization methods (minimum, maximum, and range), standard normal variate transformation (SNV), multiple scatter correlation (MSC), and Savitzky-Golay (SG) smoothing (1\(^{st}\) and 2\(^{nd}\) derivatives). Two chemometrics methods for classification were applied: principal component analysis (PCA) and partial linear square discriminant analysis (PLS-DA). PCA is an unsupervised
method used to reduce multi-variables in spectroscopic data to cluster samples based on principal components (PCs) scores [15]. PLS-DA, on the other hand, is a supervised method to classify samples based on the known classes [16].

Table 1. Classification of High and Low sweetener additives in geplak food (per 30 g)

| Sweetener type | High (mg) | Low (mg) |
|----------------|-----------|----------|
| Sugar (sucrose)| >1500     | <1500    |
| Cyclamate      | >50       | <50      |
| Saccharin      | >30       | <30      |

In this research, the PLS-DA model was based on levels of sweeteners added in food. The level was classified into ’High’ and ’Low’ related to the composition of sweeteners either higher or lower than the ADI for particular sweeteners. As mention above in sample preparation, the ADI for saccharin, cyclamate, and sugar added to one piece of food of 30 g were 34, 50, and 1500 mg. The classification of ‘High’ and ‘Low’ samples is shown in Table 1 and used in the PLS-DA model. For building the model, all wavelengths of 1501 variables were used.

3. Results and Discussion

3.1. Exploratory of spectral profile

Figure 1 shows the original and preprocessed NIR spectra of food added with sucrose, cyclamate, and saccharin in the spectra range of 4000 – 10,000 cm⁻¹. Original spectra of food, which consist of several ingredients such as rice-flour, coconut, water, and salt, with various additives, are shown in Figure 1a in which all spectra are widely dispersed showing no difference between samples. Therefore, it is difficult to differentiate samples using those original spectra. By applying a Savitzky-Golay 1st derivative spectra, several distinct peaks can be observed, as illustrated in Figure 1b. The differences in spectra were apparent in the spectral range of 4100-4200 cm⁻¹, 4400-5200 cm⁻¹, 5400 cm⁻¹, 7100-7200 cm⁻¹, and 8200 cm⁻¹, which are resulted from chemical composition difference between samples.
The Savitzky-Golay 1st derivative spectra transformation made the grouping of foods noticeable (figure 2a). The first two principal components explained 89.3% of the total variance. In general, this model is able to determine the type of sweetener added in food, although there are some samples with high saccharin overlapped with samples with cyclamate.

Figure 1. FT-NIR spectra of food added with sweeteners: (a) original spectra, (b) Savitzky-Golay 1st derivative spectra

3.2. Principle component analysis of samples
The Principal Component Analysis (PCA) is applied for dimensional reduction and exploration of data distribution with regard to sweeteners (sucrose, saccharin, and cyclamate) added in food samples. The Savitzky-Golay 1st derivative spectra transformation made the grouping of foods noticeable (figure 2a). The first two principal components explained 89.3% of the total variance. In general, this model is able to determine the type of sweetener added in food, although there are some samples with high saccharin overlapped with samples with cyclamate.
Figure 2. PCA plot of 1st Savitzky-Golay derivative spectra: (a) PC-1 and PC-2 score plot and (b) Loadings plot

Figure 2b shows the PCA loadings plot developed using the first two components (PC-1 and PC-2) explaining the weight of the variables. Separation of samples based on sweetener additives is generated by using the loading information. There are clear peaks at 5184 cm⁻¹, 5102 cm⁻¹, and 7352 cm⁻¹, which are assigned to amylose and starch [17], and seem to be responsible for distinguishing samples; therefore, it can be further analyzed using PLS-DA model. Amylose and starch are the main constituents in rice [18] used as an ingredient in the samples.
3.3. PLS-DA to determine high and low levels of sweeteners in food.

The PLS-DA classification results using several spectra preprocessing methods are described in Table 2. It can be seen from Table 2 that by using the original spectra and MSC Normalization method result in low accuracy for classifying samples. Overall, the percentages of accuracy are less than 75% for both calibration and prediction models. Other Normalization methods such as Mean, Maximum, Range, and SNV methods also have low accuracy in classification, therefore excluded from this discussion. By using Derivative methods such as the first and second Savitzky-Golay (SG) improve the accuracy, but 2nd SG performs better compared to the 1st SG method. The 2nd SG method has high accuracy in the calibration model with 85.7-100% accuracy.

Table 2. PLS-DA accuracy results (%) for various spectra preprocessing

| Prepossessing method | Calibration (72 samples) | Prediction (31 samples) |
|----------------------|--------------------------|-------------------------|
|                      | HSuc | LSuc | HCyc | LCyc | HSac | LSac | HSuc | LSuc | HCyc | LCyc | HSac | LSac |
| Original             | 50   | 63.6 | 71.4 | 54.5 | 60   | 54.5 | 50   | 50   | 66.7 | 40   | 57.1 | 25   |
| MSC                  | 60   | 63.6 | 64.3 | 63.6 | 53.3 | 63.6 | 75   | 75   | 50   | 60   | 71.4 | 25   |
| SG1                  | 90   | 100  | 78.6 | 90.9 | 86.7 | 90.9 | 100  | 100  | 33.3 | 100  | 100  | 50   |
| SG2                  | 100  | 90.9 | 85.7 | 100  | 100  | 100  | 100  | 100  | 66.7 | 100  | 100  | 50   |

MSC = Multiplicative scatter correction  
SG1 = Savitzky-Golay first derivative  
SG2 = Savitzky-Golay second derivative  
H = High, L = Low  
Suc = sucrose, Cyc = cyclamate, Sac = saccharin

However, when the model is applied to the prediction samples, low accuracies are shown in samples with high cyclamate and low saccharin. This result is in line with PCA classification shown in figure 2a in which some samples with a high level of cyclamate are separated from cyclamate group. When the best PLS-DA calibration model using the 2nd SG is plotted, the sample distribution can be seen in figure 3, which shows clear grouping except for some sample with high cyclamate overlaps with samples with low cyclamate. However, other samples with sucrose and saccharin are noticeably classified.

Figure 3. Classification plot of PLS-DA model for calibration using 2nd Savitzky-Golay derivative method
4. Conclusions
The results demonstrate that NIR spectroscopy and chemometrics analysis can be used to determine the level of sweeteners added in food with regard to the acceptable daily intake (ADI). Calibration model of PLS-DA applying the Savitzky-Golay 2nd derivative at spectral pretreatment obtained 100% accuracy in predicting high sucrose and high saccharin, as well as low cyclamate and low saccharin, while obtained 90.0% and 85.7% accuracy in predicting low sucrose and high cyclamate, respectively. PLS-DA prediction model using the Savitzky-Golay 2nd derivative pretreatment spectra obtained 100% accuracy in predicting high and low sucrose, low cyclamate, and high saccharin.

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6. References
[1] Pandey RM, Upadhyay SK. Food additive. In: El-Samragy Y, editor. Food additive. IntechOpen; 2016.
[2] Benford D. The acceptable daily intake a tool for ensuring food safety. Belgium; 2000. (ILSI EUROPE CONCISE MONOGRAPH SERIES).
[3] Chattopadhyay S, Raychaudhuri U. Artificial sweeteners – a review. J Food Sci Technol. 2014;51(April):611–21.
[4] Zygler A, Wasik A, Namies J. Analytical methodologies for determination of artificial sweeteners in foodstuffs. 2009;28(9).
[5] Beatriz A, Alberto J, Pereira D, Jesus D. Simultaneous determination of aspartame, cyclamate, saccharin and acesulfame-K in soft drinks and tabletop sweetener formulations by capillary electrophoresis with capacitively coupled contactless conductivity detection. Food Chem. 2011;124(4):1714–7.
[6] Chang C-S, Yeh TS. Detection of 10 sweeteners in various foods by liquid chromatography / tandem mass spectrometry. J Food Drug Anal. 2014;22(3):318–28.
[7] Yu S, Zhu B, Lv F, Li S, Huang W. Rapid analysis of cyclamate in foods and beverages by gas chromatography-electron capture detector (GC-ECD). Food Chem. 2012;134(4):2424–9.
[8] Sándorfy C, Buchet R, Lachenal G. Principles of molecular vibrations for near-infrared spectroscopy. In: Ozaki Y, McClure WF, Christy AA, editors. Near-infrared spectroscopy on food science and technology. New Jersey: John Wiley & Sons, Inc.; 2007. p. 11–46.
[9] Lohumi S, Lee S, Lee H, Cho BK. A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. Trends Food Sci Technol. 2015;46(1):85–98.
[10] Chen J, Zhu S, Zhao G. Rapid determination of total protein and wet gluten in commercial wheat flour using siSVR-NIR. Food Chem. 2017;221:1939–46.
[11] Wang A, Xie L. Technology using near infrared spectroscopic and multivariate analysis to determine the soluble solids content of citrus fruit. J Food Eng. 2014;143:17–24.
[12] Masithoh R, Haff R, Kawano S. Determination of soluble solids content and titratable acidity of intact fruit and juice of satsuma mandarin using a hand-held near infrared instrument in transmittance mode. J Near Infrared Spectrosc. 2016;24(1):83.
[13] Sørensen KM, Khakimov B, Engelsen SB. The use of rapid spectroscopic screening methods to detect adulteration of food raw materials and ingredients. Curr Opin Food Sci. 2016;10:45–51.
[14] Guven B, Velioğlu SD, Boyaci IH. Rapid identification of some sweeteners and sugars by attenuated total reflectance-fourier transform infrared (ATR-FTIR), near-infrared (NIR) and raman spectroscopy. GIDA J Food. 2019;44(2):274–90.
[15] Mees C, Souard F, Delporte C, Deconinck E, Sto P, Stévigny C, et al. Identification of coffee leaves using FT-NIR spectroscopy and SIMCA. Talanta. 2018;177:4–11.
[16] Esteki M, Simal-gandara J, Shahsavari Z, Zandbaaf S, Dashtaki E. A review on the application of chromatographic methods, coupled to chemometrics, for food authentication. Food Control. 2018;93(April):165–82.

[17] Aenugu HPR, Sathis Kumar D, Srisudharson, Parthiban N, Ghosh SS, Banji D. Near infra red spectroscopy- An overview. Int J ChemTech Res. 2011;3(2):825–36.

[18] Osborne BG, Fearn T, Hindle PT. Practical NIR spectroscopy with applications in food and beverage analysis. Longman Scientific & Technical, Harlow.; 1993.