Prediction of Piperine content in white pepper by NIR spectroscopy using Partial Least Square (PLS) method

S N Athfiyah¹², I W Budiastra¹, Sutrisno¹, Y A Purwanto¹
¹Department of Mechanical and Biosystem Engineering, IPB University, Indonesia
²Corresponding author, e-mail: athfiyah@apps.ipb.ac.id

Abstract. Pepper (Piper nigrum L.) is one of the prime spices and has great potential in improving the Indonesian economy. Pepper has an identity compound that has a spicy and savory taste that is piperine. Piperine is usually measured using a chemical method, which is time-consuming and rather high cost. So in this study, a faster method using NIRS is assessed to predict the piperine content of white pepper. Fifty samples of white pepper powder (30 grams each) were prepared for the reflectance and piperine measurements. The reflectance of samples were measured by FT-NIR spectrometers at wavelengths of 1000-1500 nm, and the piperine content of samples was determined by the chemical method. For the data processing, the NIR spectra were processed by several NIR data pretreatments to improve the accuracy of prediction, and the piperine content was calibrated by using the PLS method. The results of this study showed that the piperine content of white pepper powder could be predicted well using 7 factors of PLS without NIR data pretreatment with the statistical evaluation values of r(0.91), CV(4.68%), RPD(1.67), and consistency(80%).

Keywords: NIR, pepper, PLS

1. Introduction

Pepper (Piper nigrum L.) is one of the prime commodities in the crop estate commodities and has considerable potential in Indonesia’s economic growth because it contributes to the country’s foreign exchange [1]. Indonesia has been known as a major producer of pepper in the world with a kind of varieties and types of pepper [2]. In Indonesia, in 2015, the amount of pepper production was 81,501 tons, and the export volume was 58,075 tons, with a value of US $ 319,824,000 [3]. The use of pepper is generally in the food industry sector in the form of powder and seeds. However, powdered products are more attractive to consumers because they are so simple to use.

The quality of pepper can be determined from the content of piperine, which is an identity compound of pepper that makes pepper taste is spicy and savory. During this time, the determination of the piperine content of pepper is still done by chemical methods in the laboratory that needed time to find out the results, expensive costs due to the use of tools and chemicals, and requires preparation of samples that are not simple. An alternative faster and simple method is required; one of the potential methods is NIRS (Near Infrared Reflectance Spectroscopy).

NIRS research has been carried out in Indonesia, especially in the estate crop commodities such as on the caffeine content of Gayo arabica coffee [4], but it has not been done on white pepper from Indonesia. In other studies, the use of NIRS has been applied for the rapid determination of the piperine content of black and white ground pepper from Germany [5].
Therefore, studies about NIRS application on pepper from Indonesia are needed. In this research, NIRS was assessed to determine the piperine content of white pepper powder from Bangka Belitung, Indonesia, using the PLS method.

2. Materials and Method

2.1. Materials and Tools
White pepper from Bangka Belitung was ground to 100 mesh as the particle size of powder by using disk mill in F-Technopark, IPB University. Tools used in this research are NIR spectrometer type of NIRflex N-500, unscrambler software v 10.4 (CAMO), and Microsoft Excel in a personal computer, digital scale, petri dish, and Tyler sieve.

2.2. NIRS Measurement
A sample of white pepper powder was weighed about 30 g for NIR measurement. The sample was put in a petri dish and arranged into a flat (thickness of the sample was 0.5 cm). Measurement of NIR spectra was conducted using the NIR spectrometer in the wavelength of 1000-1500 nm with a 4/cm interval and a scan speed of 3 scans/s (Temperature around 22–25 °C). The number of samples for NIR testing was 50 samples.

2.3. Piperine Content Analysis
The concentration of piperine in white pepper powder was determined destructively. Based on [6] on SNI 0004:2013 to analyze of the piperine content of white pepper is using extraction with ethanol and measuring absorbance at 343 nm wavelength with an ultraviolet spectrophotometer. The tools that will be used must be wrapped in aluminum foil or tin foil. The sample was weighed 0.5 gr into the boiling flask and added 50 ml ethanol and several boiling stones. The instrument was installed in such a way and heated for 3 hours, then cooled and filtered into a 100 ml measuring flask, the volume of the solution in the measuring flask is adjusted to mark the line with ethanol (solution A). Solution A was taken as much as 5 ml using a pipette and transferred into a 50 ml measuring flask and then diluted to mark the line with ethanol (solution B). Solution B was taken as much as 5 ml using a pipette and transferred into a 25 ml measuring flask and then diluted to mark the line with ethanol (solution C). Solution C was absorbed using a spectrophotometer at a wavelength of 343 nm using ethanol as a blank. Piperine levels are expressed as weight percentages based on dry weight as follows (equation 1).

\[
Piperin (\%) = \frac{A}{A_{1} cm^{1\%}} \times \frac{50}{5} \times \frac{25}{5} \times \frac{100}{M} \times \frac{100}{100-KA}
\]

Note:
\[
M = \text{weight of sample (g)}
KA = \text{water content of the sample (%)}
A = \text{absorbance of the sample's solution}
A_{1} cm^{1\%} = \text{absorbance in 343 nm from 1 % of the piperine solution and cell 1 cm is 1238}
\]

2.4. NIR Data Processing and Analysis
The result of the NIR reflectance spectra of the sample measured with NIRFlex N-500 was transformed into absorbance spectra (log (1/R)). To get the best result for prediction, several pretreatments were used that is multiple scatter correlation (MSC), second derivative (dg2) of Savitzky-Golay, and the combination of MSC and dg2 of Savitzky-Golay. The calibration model was developed by using the PLS method in the unscrambler software version 10.4 (CAMO, Norway). The sample divided into 1/3 data for validation (34 samples) and 2/3 data for calibration (66 samples). Statistical parameters that used for evaluation of best prediction are correlation regression (r), the
standard of error (SE), coefficient of variation (CV), ratio performance to standard deviation (RPD), and consistency.

3. Result and discussion

3.1. Spectrum of NIR and Piperine Concentration of White Pepper Powder

The original absorbance spectrum of white pepper powder at a wavelength of 1000-1500 nm is shown in figure 1a. The obtained spectrum shows a wide range with 856 spectrum data that has several peaks and valleys. Absorbance data were obtained using logarithmic transformation from reflectance data. This transformation is carried out because the chemical composition of a material has a linear relationship with the NIR absorbance data [7].

The original spectra have information about the chemical content of a product. The chemical content with small concentrations requires pretreatment data to improve the prediction results of the womb [8]. The absorbance spectrum of three different pretreatment data can be seen in figures 1b, 1c, and 1d. The multiple scatter correlation (MSC) pretreatment data method was performed to remove scattering and baseline shifts in the obtained spectra (figure 1b), which simplifies the appearance of spectra and clarifies the peaks, but the peak absorption in the spectra is still the same as the original absorbance spectra. The second derivative (dg$_2$) pretreatment data method works to separate overlapping spectra on the original spectrum so that the resulting reflectance value will be reduced to smaller to clarify each peak and valley on the spectrum [9] (figure 1c). According to [10], the use of a combination of pretreatment data gives a better effect than separate pretreatment data. In figure 1d, a combination of MSC and dg$_2$ shows that the absorption peak is increasingly clear, which means that the chemical content is read more and more.

In some researches, it shows that the smaller the concentration of the predicted material, the pretreatment is needed that can clarify the absorption peak in the spectra.

![Figure 1](image-url)

**Figure 1.** Absorbance spectrum with data processing (a) Original; (b) MSC; (c) dg$_2$; (d) Combination
The piperine concentration in white pepper ranged around 3.84–5.35 % (Table 1). This concentration was similar to data of [11] that the minimum of piperine concentration on white pepper is 4% from ASTA (American Spice Trade Association).

### Table 1. Piperine concentration of white pepper powder

|          | Mean (%) | Range (%) | Standard of deviation (%) |
|----------|----------|-----------|---------------------------|
| Validation | 4.68     | 4.19-4.98 | 0.22                      |
| Calibration | 4.69     | 3.84-5.35 | 0.43                      |

3.2. Result of Calibration and Validation

The best result of piperine content prediction in white pepper powder is the use of original spectra with a factor of 7 which has an r-value of 0.91, SEC and SEP of 0.18% and 0.22%, CV of 4.68%, RPD of 1.68, and 80% as the consistency (Table 2). The use of pretreatment data types does not produce better accuracy than the original data. In the use of MSC, an r-value of 0.84 is obtained, which is no greater compared to the original data, but removing scatter and baseline in this pretreatment can increase the consistency to be 107.04%. Using \(d_2\) and the combination (MSC; \(d_2\)) brings a low consistency value (under 80%), so it is not feasible to be selected as the best treatment. The function of \(d_2\) and the combination can make the spectra show more the peak of absorbance, meaning that more chemical contents of the sample were detected. But the other chemical contents can close the information about piperine content so that can not to determine so clearly by using this pretreatment.

In some cases, no need to do a pretreatment data it can cause many factors. But on other research [12] about the prediction of the caffeine concentration on java preanger coffee bean, the best calibration model (r= 0.946, CV= 1.54%, RPD= 2.28 and consistency 87.57%) was obtained from the combination (first derivative and MSC) as the pretreatment and using 7 PLS factors. There are many differences between this research and others. Sample, particle size, the properties concentration of samples used for the calibration and validation are also different. The variation of particle size can make a difference because it can affect the diffusion of NIR radiation. The bigger the particle size, the greater the apparent absorbance [13].

### Table 2. Result of calibration and validation

| Data processing | Factor | r  | SEC (%) | SEP (%) | CV (%) | RPD   | Consistency (%) |
|-----------------|--------|----|---------|---------|--------|-------|-----------------|
| Original        | 7      | 0.91 | 0.18    | 0.22    | 4.68   | 1.68  | 80.00           |
| MSC             | 5      | 0.84 | 0.23    | 0.21    | 4.50   | 1.74  | 107.04          |
| \(d_2\)        | 2      | 0.92 | 0.16    | 0.26    | 5.52   | 1.42  | 63.61           |
| MSC;\(d_2\)    | 2      | 0.92 | 0.16    | 0.26    | 5.47   | 1.43  | 63.91           |

Figure 2 is a plot of piperine reference and piperine predicted by NIR by the best calibration, k for calibration data, and v for validation data. The distribution of calibration and validation data are equal; it means that is the good correlation. Moreover, the results indicate that the obtained calibration model by original spectra using 7 factors of the PLS method could be used to predict the piperine concentration in white pepper powder.
Figure 2. Plots of piperine reference vs. predicted

4. Conclusion

The best calibration model of PLS for good prediction of the piperine concentration of white pepper powder was obtained by original data spectra without data pretreatment using 7 PLS factors in 1000-1500 nm wavelength. This study shows that NIR spectroscopy can be used to determine the piperine content in white pepper powder.

References

[1] Kementerian Pertanian 2013 Pedoman Teknis Pengembangan Tanaman Lada Tahun 2014. Jakarta: Direktorat Jenderal Perkebunan Kementerian Pertanian.
[2] Departemen Pertanian 2009 Pedoman Teknis Pengembangan Lada Organik. Jakarta: Direktorat Jenderal Perkebunan, Departemen Pertanian.
[3] Kementerian Pertanian 2017 Statistik perkebunan Indonesia: lada. p 3-4.
[4] Rosita R, Budiastra IW, Sutrisno 2016 J Keteknikan Pertanian 4 (2): 179-186 doi: 10.19028/jtep.04.2.179-186.
[5] Schulz H, Baranska M, Quilitzsch R, Schütze W, Lösing G 2005 Journal of Agricultural and Food Chemistry 53 (9): 3358–3363 doi:10.1021/jf048137m.
[6] Badan Standardisasi Nasional 2013 Standar Nasional Indonesia: Lada Putih. SNI 0004:2013.
[7] Mohsenin NM 1984 Electromagnetic Radiation Properties of Food and Agricultural Products. New York (US): Gordon dan Breach Science Publisher.
[8] Huck CW, W Guggenbichler and GK Bonn 2005 Journal Analytica Chimica Acta 538:195–203.
[9] Ozaki Y, Mc Clure WF, Christy AA 2007 Near-Infrared Spectroscopy in Food Science and Technology New Jersey (US): John Wiley & Sons, Inc.
[10] Chen H, Q Song, G Tang, Q Feng and L Lin 2013 ISRN Spectroscopy: 1-9.
[11] Warta Penelitian dan Pengembangan Tanaman Industri 2011 Harmonisasi standarmutu lada. 7(3): 29.
[12] I W Budiastra et al 2018 IOP Conf. Ser.: Earth Environ. Sci. 147 012004.
[13] Purningsih et al 2018 J Keteknikan Pertanian 6 (3): 271-278 doi: 10.19028/jtep.06.3.271-278.