

Non-destructive measurement of antioxidant activity and water content in chili powder (*Capsicum annuum* L.) using near-infrared spectroscopy

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Abstract

Chili (*Capsicum annuum* L.) is a potential source of antioxidants due to its high vitamin and lutein contents. The information on antioxidant activity and water content of chili are needed by the chili-based industries which utilise chili's natural antioxidant content for various purposes in health, food, or herbal products. These information are often obtained through destructive testing which damages the tested materials, and also requires a relatively long analysis time. Therefore, the present work aimed to non-destructively measure the antioxidant activity and water content of chili powder using near-infrared spectroscopy (NIS) combined with partial least square (PLS) analysis. A total of 84 samples of chili powder from four different colour groups were used in the present work. Approximately 70% of the samples were used for calibration, while the rest were used for validation. Samples' reflectance data were acquired using NIRFlex N500 Fiber Optic Solids Cells at wavelengths of 1000 - 2500 nm. The antioxidants activity and water content were also analysed destructively for comparison/reference data. PLS was then performed on the spectrum and reference using the Unscrambler X version 11.0 software. There were also three pre-treatments used to improve the spectrum, *i.e.*, multiplicative scatter correction, first derivative Savitzky Golay, and de-trending. The best calibration model was successfully obtained when the antioxidant activity spectrum was processed using the de-trending pre-treatment with six PLS factors. The coefficient of determination (R^2) and residual predictive deviation (RPD) values for this were 0.83 and 2.60, respectively. Additionally, the best calibration model was successfully obtained when the water content spectrum was processed using the first derivative Savitzky Golay pre-treatment with five PLS factors. The R^2 and RPD values for this were 0.96 and 2.25, respectively. Both models were observed to be accurate, and have the potential to be used for measuring the antioxidant activity and water content of chili powder samples.

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Introduction

Chili (*Capsicum annuum* L.) is one of Indonesia's horticultural products, and a staple seasoning used in the country's various cuisines. The chemical composition of fresh chili includes calories, proteins, fats, carbohydrates, calcium, phosphorus, iron, vitamins A, B, and C, and water. Besides that, chili also contains alkaloid compounds such as capsaanthin, carotenoids, alkaloids, resins, and essential oils. Most of these contents are sources of antioxidants. The United Nations Food and Agriculture Organization has announced chili as the most important crop in the genus *Capsicum* in the world (FAO, 2011).

With the development of edible material processing technology, the use of chili powder to

replace its fresh products is increasing in both food and non-food industries. Food industries such as instant noodles, spices, seasonings, and canned fish utilise chili powder as one of the main ingredients in their products. The non-food industries such as the pharmaceutical and drug companies also utilise chili powder as a raw material. As compared to fresh chili, chili powder with a maximum water content of 13% has its advantages when stored for a long period of time such as price stability and ease of distribution. Furthermore, lutein in chili is one of the sources of antioxidants. Antioxidants in foods have an essential role in maintaining the product quality, preventing rancidity and changes in nutritional values, colour, and aroma, and other physical damages caused by oxidation reaction (Basanta *et al.*, 2018).

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Industries that utilise chili powder are required to first determine its water content to meet standards prescribed by the Directorate of Agricultural Product Processing (Ministry of Agriculture, 2010) on chili processing. The water content analysis is complex, and takes a long time to complete. Therefore, the present work proposed a faster technique by using near-infrared (NIR) technology which has the ability to detect various chemical contents through the spectrum. The advantages of using the NIR method include non-destructive, easy preparation of samples, no chemicals are required, and able to simultaneously detect several quality parameters of the product. Therefore, the NIR method is widely researched and applied in the field of agriculture (Munawar *et al.*, 2016).

Presently, based on the measurement of their peel colour using a chromameter, research on chemical content prediction of red chili has been developed. The study showed that vitamin C and carotene in chili were predicted accurately (Khuriyati *et al.*, 2018). However, capsaicin was not predicted due to the fact that the R^2 value was very small (< 0.08), and unlikely to be expressed by a mathematical model (Khuriyati *et al.*, 2019). Lim *et al.* (2015) successfully developed a predictive model of capsaicin content in red chili powder using NIR with an R^2 value of 0.985. Martinez *et al.* (2014) conducted a research on the determination of capsaicin, ascorbic acid, total phenolics, and antioxidant activity of *C. annuum* L. var. serrano (serrano peppers) using the mid infrared spectroscopy (MIF-FTIR). The R^2 of the prediction model was 0.913, thus showing that the technique was excellent and trustworthy. Jiang *et al.* (2018) were also able to make predictive models for quality measurements of curly chili pepper using the NIR method with multivariate analysis.

Since the testing of such antioxidant activity has not been carried out in chili powder as compared to fresh chilli, it is thus necessary to develop a measurement model with the application of the NIR technology. The testing with the NIR spectroscopy is a way to reduce the measurement time, and is carried out without damaging the tested samples (non-destructive).

Materials and methods

Sample preparation

Fresh chili samples were obtained from farmers in the special region of Yogyakarta,

Indonesia, and visually categorised into four colour groups namely light green, dark green, red tinge, and red. To obtain powder, the fresh chili samples were dried using a cabinet dryer at 60°C for 15 h, ground using a food processor, and sieved using a 60-mesh sieve. A total of 84 chili powder samples were obtained, and grouped based on visual colour groups of fresh chili; light green ($n = 21$), dark green ($n = 21$), red tinge ($n = 22$), and red ($n = 20$).

Spectra acquisition

Spectra acquisition was carried out for each chili powder sample using near-infrared spectroscopy (NIR). Spectra were recorded at wavelengths of 1000 - 2500 nm using NIRFlex N500 Fiber Optic Solids Cell (Büchi Labortechnik AG, Flawil, Switzerland). Briefly, sample was added into vial tube which was rotated at 120° from the starting point. The spectrum total of each sample was used to build the calibration model.

Determination of antioxidant activity

Antioxidant activity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Payet *et al.*, 2005). The DPPH assay measures the free radical scavenging capacity of a substrate. The DPPH reagent offers a convenient and accurate method for titrating the oxidisable groups of natural antioxidants. Briefly, chili powder samples were dissolved in methanol at 0.1 - 12.8 µL/mL concentrations. Next, 2 mL of chili powder solution was added into a reaction tube, with 280 µL of 0.004% DPPH methanolic solution. The mixture was incubated at 30°C for 1 h, and the absorbance was measured with a UV-VIS spectroscopy at 525 nm. The inhibition percentage of the radical scavenging activity was calculated using Eq. 1:

$$\text{inhibition} = 100 - 100 \frac{A_s}{A_o} \quad (\text{Eq. 1})$$

where, A_o = the absorbance of the blank, and A_s = the absorbance of the sample.

Determination of water content

Thermogravimetric method was used to determine the water content by weighing the sample before and after drying, and obtaining the difference (AOAC, 1999). Briefly, an empty dish was dried in the oven at 105°C for 30 min, then transferred to a desiccator to cool. Afterwards, it was weighed, filled with 3 g of sample, and lidded. The sample was

spread using a spatula, which was later dried in the oven for 3 h at 105°C. Afterwards, the lidded dish was transferred to the desiccator to cool, and it was reweighed with the dried sample. After that process, all samples were weighed until their weight stability was attained. The water content was calculated using Eq. 2:

$$\text{Water content (\%)} = \frac{W_2 - W_1}{W_1} \times 100 \quad (\text{Eq. 2})$$

where, W_1 = the weight (g) of sample before drying, and W_2 = the weight (g) of sample after drying.

Spectra pre-treatment

The raw spectra acquired from NIR spectrometer were often observed to contain noises (Chen *et al.*, 2012). Therefore, pre-treatment should be carried out before building the calibration model in order to minimise the noises, and improve the quality of the spectra. Three pre-treatments provided in the Unscrambler X Version 11.0 Camo Analytics software such as multiplicative scatter correction, first derivative Savitzky Golay, and de-trending were applied on the raw spectra.

Data processing

The spectra data were analysed using Unscrambler X Version 11.0 Camo Analytics software. Approximately, 70% of the samples were used for calibration, while the rest were utilised for validation. The calibration aimed to build a prediction model with the validation correcting the built technique.

The parameter of the model prediction was coefficient determination (R^2), residual predictive deviation (RPD), and consistency. When R^2 was lesser than 0.8 ($R^2 < 0.8$), the built model was considered bad, and *vice versa* (Elfadl *et al.*, 2010; Calegari *et al.*, 2018). When the RPD value ranged between 1.5 to 2.0, the model discriminated between minors and the highest values of the response. However, when the RPD value ranged between 2.5 and 3.0, the model had a good prediction accuracy (William and Norris, 2001; Kumar, 2015). Therefore, RPD value became the main determinant when R^2 was greater than 0.8 ($R^2 > 0.8$).

Results and discussion

Spectra analysis

Spectra acquisition were recorded between 1000 and 2500 nm for a total of 84 samples, which were categorised into four colour groups namely light green, dark green, red tinge, and red. Some peaks of each spectra indicated the presence of chemical bond, as antioxidant containing C-H bond was absorbed at wavelength of 1434 and 1635 nm. However, other wavelengths of 1395 - 1452, 1755 - 1775, 2060 - 2090, 2370 - 2390, and 1520 nm were indicated by O-G str. 1st, C-H 1st, O-H combination, O-H def. 2nd, and N-H str. 1st overtones, respectively. Water content was detected at wavelengths of 1454 and 1932 nm, and indicated by O-H stretching and combination, respectively (Nawrocka and Lamorska, 2013).

Figure 1 shows the differentiation between raw spectra and three pre-treatments used namely multiplicative scatter correction, first derivative Savitzky Golay, and de-trending. The multiplicative scatter correction (MSC) was able to reduce basic variations, and eliminate scattering effect. Additionally, it was also able to cut the number of latent variables as compared to non-pre-treatment methods. The results of the MSC pre-treatment are shown in Figure 1b, as it helped to improve the outcomes of the spectrum, thus making it smoother and denser as compared to the raw spectra.

First derivative Savitzky Golay is a method for reducing noise without removing important information from the data, decreasing base effects, and increasing smaller absorption peaks, with the production of an ordinate linear offset. Savitzky Golay's algorithm matches polynomials for each successive curve segment as it replaces original values with more regular variations. Through the analysis of first derivative, changes were observed with the original data as the results shown in Figure 1c had a very significant difference as compared to the raw spectra.

The de-trending pre-treatment can remove non-linear trends in spectroscopy data. The pre-treatment also calculates the baseline function as least squares fit of a polynomial for the sample spectrum data. De-trending was further applied to the individual spectrum. As the polynomial order de-trending increased, additional baseline effects were removed (Cen and He, 2007; Munawar, 2014). Zero, first, and second orders were observed to correct the offset, offset and tilt, with offset, slope, and curvature, respectively. These results showed very significant differences as compared to the raw spectra as there

was a change between the valley shape and peaks produced from the spectrum of chili powder. The results of de-trending pre-treatment to the raw sample spectra data are depicted in Figure 1d.

Antioxidant activity and water content analysis

Table 1 showed that chili powders with light green and red colours had the highest and lowest antioxidant activities, with an average of 49.6 and 34.3%, respectively. These results corresponded with the research of Hamed *et al.* (2019) which observed that green and red chilies had antioxidant activity values of 61 and 59%, respectively. Therefore, the chili powder samples had a standard water content in the range of 9 - 12%.

ANOVA was also applied to determine significant differences in both antioxidant activity

and water content between the four groups of the tested chili powder. Table 1 showed that the four samples of chili powder had no significant difference with water content ($p > 0.05$) in all groups, as a comparison of the antioxidant activity values between the classifications were made directly. Table 1 also showed that there was a significant difference ($p \leq 0.05$) with the amount of the chili powder antioxidant activity. In order to detect the groups that significantly differed, a *posthoc* test was further applied. The results further showed that there was no significant difference ($p > 0.05$) between light and dark green chili powder. However, there were significant differences between both red tinge and red chili powders with light and dark green samples ($p \leq 0.05$).

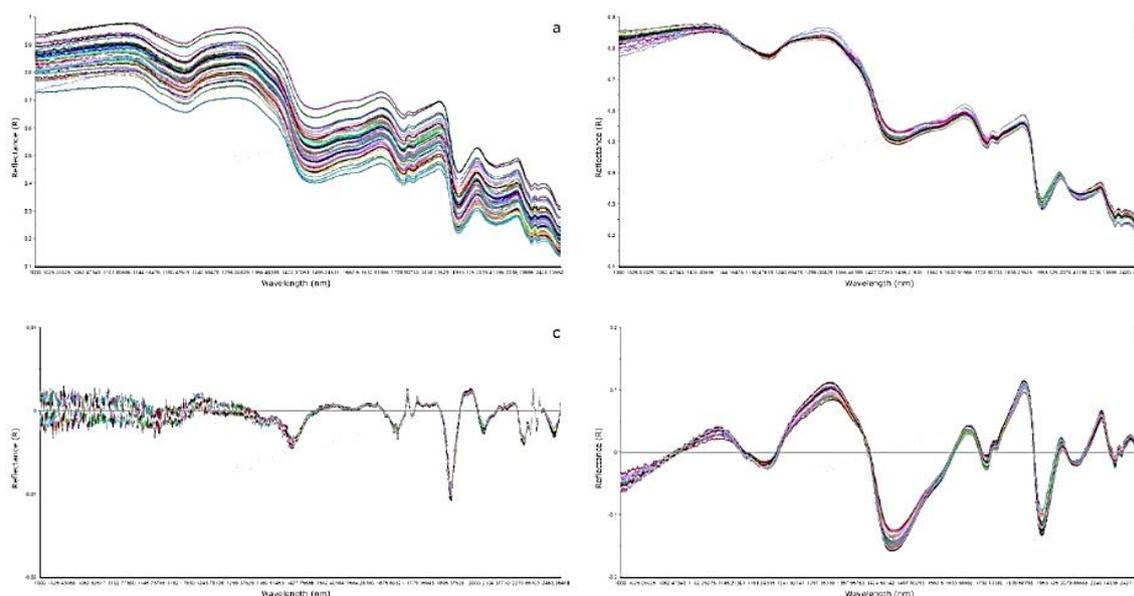


Figure 1. (a) raw spectra, (b) multiplicative scatter correction, (c) first derivative Savitzky Golay, and (d) de-trending.

Table 1. The antioxidant activity and water content of chili powder samples.

Colour of chili powder	Number of samples	Mean weight per sample (g)	Mean antioxidant activity (%)	Mean water content
Light green	21	3	49.66 ± 4.43 ^a	9.28 ± 2.06 ^a
Dark green	21	3	47.97 ± 5.5 ^a	9.43 ± 1.39 ^a
Red tinge	22	3	41.03 ± 3.10 ^b	11.55 ± 1.17 ^a
Red	20	3	34.32 ± 2.10 ^c	10.39 ± 1.02 ^a

Means with different lowercase superscripts within a column are significantly different at $p \leq 0.05$.

Calibration and validation of models

The antioxidant activity of chili powder samples obtained from destructive testing was further used as a reference for developing a calibration model, which determined the success of using NIR to estimate material quality parameters. From the total of 84 samples of the tested chili powder, 70 and 30% of them were used as calibration and validation data, respectively (Calegari *et al.*, 2018; Liu *et al.*, 2018). The detailed descriptive statistics of the calibration and validation data were shown in Table 2.

Regression model for predicting antioxidant activity

The process of building a calibration model was carried out using the partial least square (PLS) with data processing, as the number of optimal factors of PLS applied on the NIR spectrum was 7 (Andasuryani *et al.*, 2013). The calibration model was the correlation between the NIR reflectance and antioxidant activity. The best calibration model was also determined from the R^2 and RPD values (Lammertyn *et al.*, 2013). In predicting the antioxidant activity, all built models had achieved acceptable results, with the R_c and R_p values of all the techniques were higher than 0.80 (Jiang *et al.*, 2018). The regression model for predicting antioxidant activity is shown in Figure 2.

Table 2. Reference measurement results in the calibration and validation sets.

Parameter	Subset	Number of samples	Mean	SD	Max.	Min.
Antioxidant activity	Calibration set	59	43.18	± 7.16	59.19	30.43
	Validation set	25	43.68	± 7.47	57.28	31.66
Water content	Calibration set	59	10.12	± 1.69	13.00	6.50
	Validation set	25	10.20	± 1.69	13.00	7.00

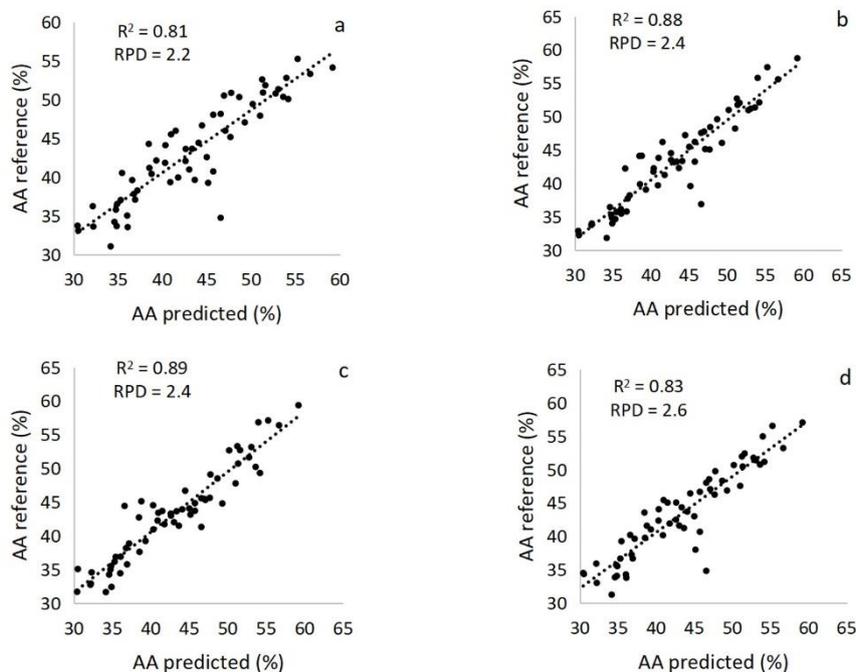


Figure 2. Regression model of prediction for antioxidant activity of (a) raw spectra, (b) multiplicative scatter correction, (c) first derivative Savitzky Golay, and (d) de-trending.

The best pre-treatment for predicting antioxidant activity was de-trending pre-treatment with six PLS factors. This pre-treatment seemed to remove non-linear trends in spectroscopy data as it

also operated on individual spectra (Sila *et al.*, 2016). Furthermore, the pre-treatment also calculated the baseline function as a least-square fit of a polynomial for the sample spectrum data, and was also applied to

the individual spectrum. The second order of the polynomial was selected in processing the data due to its ability to correct the offset, slope, and curvature of the data. When compared with raw spectra or results of the other pre-treatments, the outcome had significant differences.

Regression model for predicting water content

As explained earlier, the calibration model with the correlation between the NIR reflectance data and water content was carried out using the PLS method with data processing. Also, the number of optimal factors of PLS applied on the NIR spectrum was 7 (Andasuryani *et al.*, 2013), and was best determined from R^2 and the RPD values (Lammertyn *et al.*, 2013). The regression model for predicting the water content is shown in Figure 3.

The best pre-treatment to predict water content was first derivative Savitzky Golay pre-treatment

with five PLS factors. This method was used for reducing noise without removing important information from the data, decreasing base effects, and also increasing smaller absorption peaks, with the production of an ordinate linear offset (Savitzky and Golay, 1964). Savitzky Golay's algorithm matched polynomials for each successive curve segment as it replaced the original value with more regular variations. Through analysis of the first derivative, changes were observed when compared with the original data. These results had a very significant difference when compared with the raw spectra. First derivative Savitzky Golay had processed the data by standardising the first and last column of the spectrum to zero, in order for the result values of the other spectrum to be negative and positive. Moreover, there was a change of shape between the valley and the peak which resulted from the chili powder spectra.

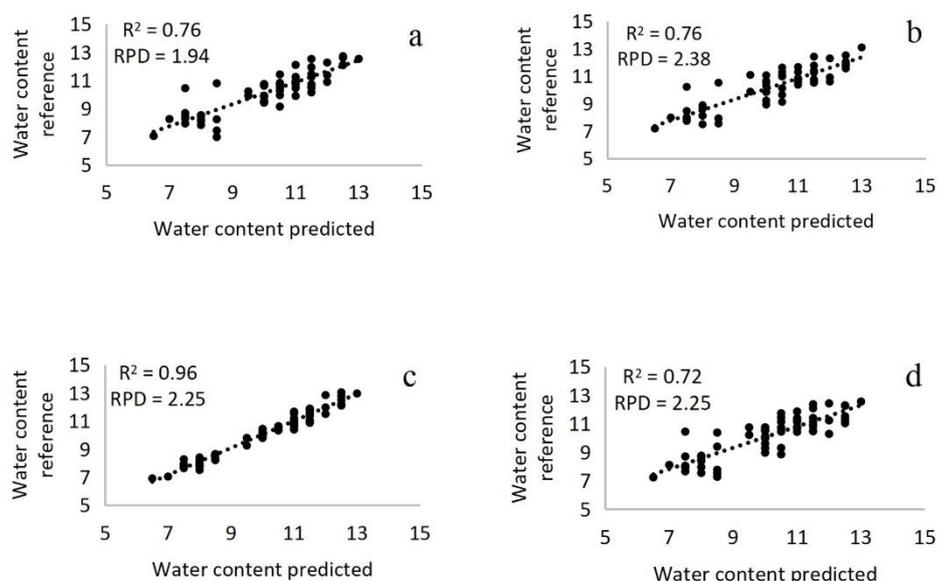


Figure 3. Regression model of prediction for water content of (a) raw spectra, (b) multiplicative scatter correction, (c) first derivative Savitzky Golay, and (d) de-trending.

Conclusion

The use of de-trending and derivative Savitzky Golay pre-treatments with six and five PLS factors on antioxidant activity and water content spectra successfully predicted the quality parameter values of chili powder samples, respectively. The prediction accuracy of the two models were good, as indicated by the determination coefficient (R^2) and RPD values

of both antioxidant activity (0.83 and 2.60) and water content (0.96 and 2.25), respectively. Therefore, the model was accurate, and could potentially be used for measuring antioxidant activity in chili powder.

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