

## Classification of chili powder (*Capsicum annuum* L.) antioxidant activity based on near infrared spectra

Aprilia, Y.I., \*Khuriyati, N. and Sukartiko, A.C.

Department of Agroindustrial Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No.1 Bulaksumur Yogyakarta 55281 Indonesia

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### Abstract

Testing the antioxidant activity of chili powder is often destructive; these methods are expensive, complicated, and lengthy analysis time. Meanwhile, information on antioxidant activity is needed by the industry to determine its quality class in rapid and uncomplicated handling. Therefore, this study was aimed to measure the antioxidant activity of chili powder and classify it into three quality classes, namely high, medium, and low, using Near Infrared (NIR) spectroscopy at spectral wavelengths of 1000-2500 nm and combined with chemometric techniques. The antioxidant activity of the sample was evaluated using the DPPH assay. Processing of the data started with outlier detection using *Hotelling's T<sup>2</sup>*, then confirmed using *leverage analysis* and *influence plot*. The data were then processed with *Smoothing-Savitzky Golay*, *SNV*, and *De-Trending*. *Principal Component Analysis* (PCA) was performed for classifying the samples, which were validated with full cross-validation. The results showed that the antioxidant activity was detected at a range of 1395 - 2390 nm. De-Trending was the best pre-treatment that successfully classified the low and high levels of antioxidant activity with a success rate of 100% and classified a medium level of antioxidant activity with a success rate of 96.97%.

## 1. Introduction

Chili (*Capsicum annuum* L.) is an important spice needed throughout the world. Chili is one type of vegetable that has particularly rich in organic micro-component and antioxidant variations (Alam *et al.*, 2018). Chili is known as one richest source of the natural antioxidant substance that contains bioactive compounds that play a significant role in the preventions of free radicals and influence human health positively (Olatunji and Afolayan., 2018). Several studies have shown that these compounds have therapeutic benefits such as anti-cancer, anti-bacterial, analgesic, anti-inflammatory, antitumor, and antiviral (Sricharoen *et al.*, 2017).

Unfortunately, the detection of antioxidant activity in chilies has been carried out destructively so far. The method is damaging the material and has several disadvantages including being more expensive, complicated, time-consuming, and requires large solvent and reagent volumes. The potential technology for making fast, precise detection with more accurate results is using NIR spectroscopy (Samadi *et al.*, 2018). NIR has advantages such as rapid analysis, simultaneous determination of multicomponent samples, the analysis

uses non-destructive processes, the analysis is carried out in real-time, and has been used for food quality (Li *et al.*, 2019).

In a preliminary study conducted by Domínguez-Martínez *et al.* (2014), antioxidant activity measurements were carried out on fresh chili samples, while Ionică *et al.* (2017) used hot pepper fruits at different stages of growth and ripening as samples. Therefore, this research was developed to use chili powder as the object. Instead of fresh form, chili powder was chosen because the level of particle density and particle size is more uniform so that the results of NIR spectrum illumination are more optimal. Moreover, chili powder has benefits such as prices that tend to be stable, the quality which is not easily damaged, and longer shelf life.

This study is aimed to evaluate the antioxidant activity and classify the level of antioxidant activity in chili powder using a combination of the chemometric models based on the Principal Component Analysis (PCA) method with Near Infrared (NIR). This information will be beneficial to the industry, namely, to assist in the rapid determination of the quality class of the chili powder and help to provide a quick decision

\*Corresponding author.

Email: [nafis.khuriyati@ugm.ac.id](mailto:nafis.khuriyati@ugm.ac.id)

regarding the process of formulation of ingredients and their quality control. This will benefit the industry because it is more cost-effective, reduces the number of workers, and also time.

## 2. Materials and methods

### 2.1 Sample preparation

Samples were collected from four levels of fresh chili maturity which were then grouped according to their color, similar grouping procedure developed by Jiang *et al.* (2018), including light green, dark green, red tinge, and red. Fresh red chili from the field then undergone a process of sorting and washing. Chili stalks and also damaged parts were then removed and the drying process was carried out with a cabinet dryer at 60°C for 10-15 hrs. The dried chili was powdered using a blender and sieved using a 60-mesh sieve. The powder was packaged 10 g per sample in a zipper plastic as the primary packaging and aluminum foil as the secondary packaging. The samples were then stored at room temperature.

### 2.2 NIR spectra measurements

A sample of 0.5 g was placed on a petri dish to maintain uniformity in the shape and thickness of the sample. Next, the NIR radiation were emitted to the samples at wavelengths between 1000-2500 nm. Scans were performed on each sample, so a total of 84 chili powder spectrum data were obtained. The original spectrum was then stored in Ms Excel.

### 2.3 Determination of antioxidant activity

According to Apak *et al.* (2016), antioxidant activity is related to the action of antioxidant kinetics in extinguishing reactive species, usually expressed as reaction rates or scavenging percentages per unit time. DPPH method was chosen because this method is simple, rapid and sensitive to test the antioxidant activity of the natural compound (Sudewi *et al.*, 2017). This test used the DPPH test procedure developed by Piang-Siong *et al.* (2017), in which 280  $\mu\text{L}$  of 0.004% DPPH methanol solution were pipetted into microtiter plates, then followed by 20  $\mu\text{L}$  of sample solution or solvent for blanks. This mixture was incubated at 30°C for 1 hr. The absorbance measurements were carried out at a wavelength of 515 nm on a spectrophotometer every 5 mins for 90 mins. The percentage of inhibition (% radical scavenging activity) was calculated using the following Equation: (Piang-Siong *et al.*, 2017).

$$\% \text{ Inhibition} = 100(1 - A_s/A_0)$$

Where  $A_s$  is the absorbance of the sample at a wavelength of 515 nm and  $A_0$  is the absorbance of the

blank at a wavelength of 515 nm. A significant difference in antioxidant activity in the four sample groups was then observed using Games-Howell Post-hoc test.

### 2.4 Statistical analysis

Samples were classified into three groups namely low, medium, and high levels of antioxidant activity. The grouping was done using statistical methods namely Hierarchical Cluster Analysis (HCA) with agglomerative strategies.

### 2.5 Outliers prediction

In this study, identification of outliers was carried out using the *Hotelling T<sup>2</sup> Statistics* method and subsequently confirmed using the leverage analysis and influence plot method.

### 2.6 Chemometric study and analysis

Data obtained from NIR spectroscopy testing was in the form of reflectance spectra at certain sources. Then the data was converted until the absorbance value was obtained at a certain wavelength. After that, the data was imported into software to process the data, namely The Unscrambler X 11.0 version. Pre-treatments, such as Smoothing-Savitzky Golay, Standard Normal Variate (SNV), and De-Trending were applied during the data processing so that the presence of hydroxyl, aromatic, and alkene can be detected from the functional groups, as characteristics of the presence of antioxidant activities. Furthermore, the chemometric analysis was carried out with the Principal Component Analysis (PCA) method aiming to classify the level of antioxidant activity in the chili powder samples. This analysis was carried out to determine the variance values of PC-1 and PC-2, the success percentage score of the classification process, and determine the position of the research object in the PCA quadrants.

## 3. Results and discussion

### 3.1 Chemical data analysis

Chemical analysis of the sample's antioxidant activity, which was classified into color-based four groups was carried out using the DPPH method. A significant difference in antioxidant activity in the four sample groups ( $p < 0.05$ ) had been observed. Further testing using the Games-Howell Post-hoc test showed that there was no significant difference in antioxidant activity in the light (A) and dark green (B) sample groups. In contrast, there was a significant difference in antioxidant activity between the light and dark green groups and the Red Tinge (C) and Red (D) groups, as

shown in Table 1. The highest antioxidant activity was observed in light green chili powder, which was  $49.55 \pm 4.46\%$  and the value decreased in the dark green, red tinge, and red sample groups, respectively. These results are in accordance with Chakrabarty *et al.* (2017), that green chili contains high amounts of vitamin C which strengthens the immune system. In addition, the source of antioxidants, namely lutein, is found to be greatest in green chili. This lutein level will decrease with the level of maturity in the chili. The high level of antioxidant activity in green chilies is also supported by the presence of the main antioxidant carotenoids in yellow chili (near green) namely violaxanthin which accounts for 37-68% of the total carotenoid content.

Table 1. Chemical testing results for chili powder samples

Grouping	Color of Each Group	Sample	Antioxidant's Activity (%)
A	Light Green	21	$49.55 \pm 4.46^a$
B	Dark Green	21	$47.97 \pm 5.51^a$
C	Tinge	22	$41.01 \pm 3.09^b$
D	Red	20	$34.33 \pm 2.08^c$

Values with different superscript are significantly different ( $p \leq 0.05$ ).

Although the percentage of antioxidant activity decreased in color-based sample groups, statistically classification using Hierarchical Cluster Analysis (HCA) with agglomerative strategies, with the output of a dendrogram, resulted in only three groups with different antioxidant activities, as shown in Table 2. It demonstrated NIR's superiority in grouping chili powder samples over inspection by color.

Table 2. Classification of samples by level of antioxidant activity

Level of Antioxidant Activity	Number of Samples for Each Group			
	A	B	C	D
Low (<38%)	-	-	4	20
Medium (38-49%)	14	10	18	-
High (>49%)	7	11	-	-

### 3.2 Raw spectra analysis

According to Syahrul *et al.* (2018), absorption of infrared radiation by the constituent molecules of the material causes its single bond to vibrate. Absorption and the existence of harmony from the movement of chemical bonds cause molecular vibrations with low electronic energy transition absorption, overtones, and ribbon combinations through stretching (*str*) and deformation (*def*). The spectra output is reflectance (R) which is then converted to the absorption or absorbance value of NIR spectra using the  $\log_{10}(1/R)$  formula

(Masdar *et al.*, 2016). The peaks formed on the spectrum of the chili powder sample, as shown in Figure 1, indicated the presence of antioxidant activity in the sample. The highest absorbance level was observed in the light green chili powder, followed by dark green, red tinge, and red, respectively. The obtained raw spectra were evaluated for their antioxidant activity by taking into their absorbance characterization. The absorbance characterization used was adjusted to the type of sample (chili powder) according to the previous research conducted by Hwang *et al.* (2017) and Medina-Juárez *et al.* (2012). The loadings plot on the raw spectrum, as depicted in Figure 2, specifically showed the detection of antioxidant activity at wavelengths of 1395 - 2390 nm. The chemical bond in the form of O-H stretch 1<sup>st</sup> overtone, O-H combination, and O-H def. 2<sup>nd</sup> overtone occurs due to the presence of hydroxyl groups in the material. The hydroxyl group (-OH) is derived from several sources of antioxidants in red chili such as capsanthin, violaxanthin, lutein, capsaicin, sinapic acid, ferulic acid, and beta carotene. This chemical bond in the form of C-H stretch 1<sup>st</sup> overtone is due to the presence of aromatic and alkene functional groups which are also known to be constituents of lutein and capsaicin.

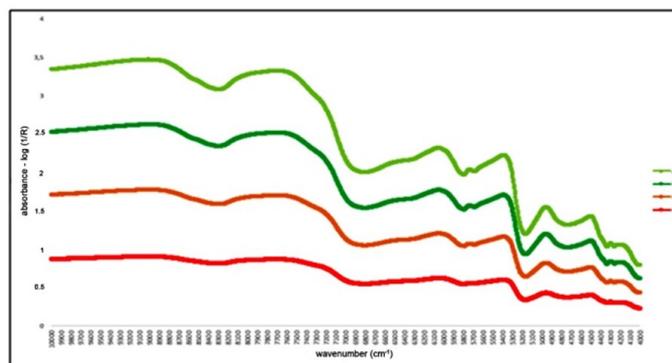


Figure 1. Raw spectra from chili powder

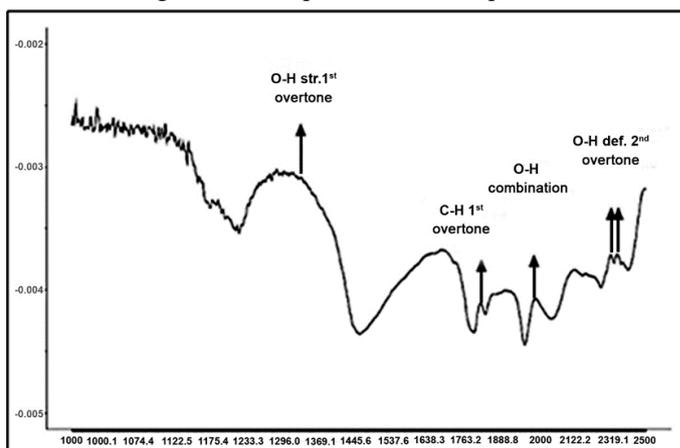


Figure 2. Loadings plot of raw spectra.

### 3.3 Preprocessing of spectral data

NIR spectrum is influenced by various parameters. The chemical variations and physical properties of the sample as well as the measurement process and the

changes to the spectrometer will influence the spectrum. This effect will primarily appear as a problem with overlapping, non-linearity, light scattering, and random noise (Buchi, 2016). One possibility to overcome this problem is using pre-treatments. Pre-treatment or preprocessing is a process to change the value of the data variables to reduce the influence of noise wave interference which causes the spectrum pool to become smoother and denser (Sari et al., 2020).

Through the application of some of these pre-treatments as a spectrum correction method, it can be concluded that Near Infrared (NIR) can detect antioxidant activity in the wavelength range of 1395-2390 nm. Chemical bonds that are read include O-H str. 1<sup>st</sup> overtone detected at wavelength range 1395-1452 nm, chemical bond C-H 1<sup>st</sup> overtone at range 1755 - 1775 nm, O-H combination at a wavelength of 2060 - 2090 nm, O-H def. 2<sup>nd</sup> overtone in the range 2370 - 2390 nm, and N-H str. 1<sup>st</sup> overtone at a wavelength of 1520 nm. The loadings plot from SNV pre-treatment shows that the presence of antioxidant activity contained in chili powder can only be detected through the position of hydroxyl-shaped functional groups only.

### 3.4 The existence of outliers

*Hotelling's T<sup>2</sup> Statistics* is a special processing tool available on *The Unscrambler X* software and allows outlier boundaries to be visualized as ellipse on the resulting score plot (Camo AS, 2006). Based on the results of identification of the outliers above, it can be concluded that the analysis using the leverage method produced four samples with the highest leverage value, then the influence plot analysis also showed that four samples were outside the boundary of the area that means outliers, and in the *Hotelling's T<sup>2</sup>* analysis, statistics show as many as four points out of the boundary area, where all four samples turned out to have the same sample code so that it proved to be outliers. According to Aulia and Atok (2017), outliers can have a

significant effect on identification result. Therefore, the four samples that were proven to be outliers were eliminated in the subsequent data processing, so that the classification process of antioxidant activity was carried out only on 80 chili powder samples.

### 3.5 Classification of antioxidant activity levels

Dimension reduction occurs in *Principal Component Analysis* (PCA) is done by making linear combinations of correlated variables to form a series of new uncorrelated variables with maximum variance called the Principal Component (PC) (Rich et al., 2020). *Full cross-validation* was selected to validate the data because it has the advantage of being able to increase relevance and also the strength of a more reliable analysis (Camo AS, 2006). In detail, the results of the PCA analysis of the whole group classifying antioxidant activity can be described more specifically in Table 3. The classification of the antioxidant activity level can be determined by interpreting the PCA score plot shown in Figure 3. According to Suhandy et al. (2018), if the amount of variance from PC<sub>1</sub> and PC<sub>2</sub> is greater than 70%, the score plot shows a good depiction of two-dimensional visualization.

## 4. Conclusion

The antioxidant activity of chili powder samples was successfully measured using near-infrared spectroscopy in the wavelength range from 1395 to 2390 nm through the detection of hydroxyl, aromatic, and alkene functional groups. The use of De-Trending in PCA analysis produced the best results in the classification of antioxidant activity of powdered chili samples into three levels, i.e., high, medium, and low, with success rates of 100%, 96.97%, and 100%, respectively. The combination of NIR and chemometrics, therefore, was able to detect and measure quickly, accurately, and non-destructively, the antioxidant activity of chili powder samples, which provide benefits for the industry.

Table 3. Overall PCA analysis of classification antioxidant activity of chili powders

		Pre-treatment			
		Raw data	Smoothing-Savitzky Golay	SNV	De-Trending
Varians	PC1 (%)	96	96	96	100
	PC2 (%)	4	4	3	0
Percentage of PCA Classification Success (%)	Low Antioxidant Level	100	100	100	100
	Medium Antioxidant Level	93.94	93.94	96.97	96.97
	High Antioxidant Level	91.67	91.67	95.83	100
Position in the PCA Quadrant	Low Antioxidant Level	Located to the right of the diagram: Quadrant 1 and Quadrant 4			
	Medium Antioxidant Level	Samples at the center of the diagram: Quadrants 1, 2, 3, and 4			
	High Antioxidant Level	Located to the left of the diagram: Quadrant 2 and Quadrant 3			

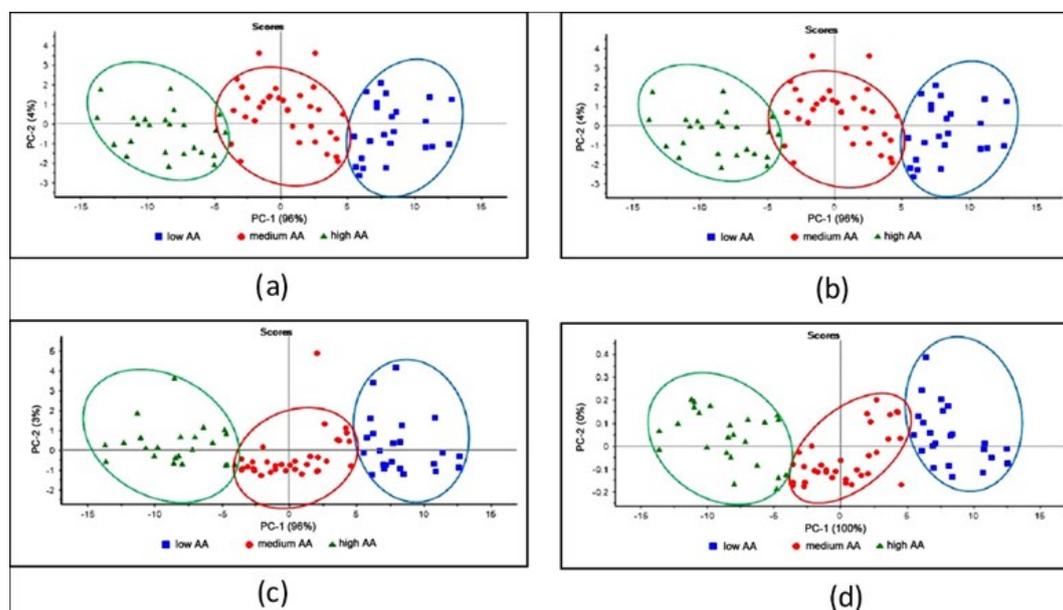


Figure 3. PCA score plot from overall pre-treatments: (a) without pre-treatment; (b) with smoothing-SG; (c) SNV; (d) Detrending pre-treatment

### Conflict of interest

The authors declare no conflict of interest.

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