Investigation of a Proper Sampling Position for Freshness Assessment of Cabbage by Spectrophotometric-Chemo Metric Approach

D Syukri¹, Wellyalina¹, R F Nanda¹
¹Department of Crop Technology, Faculty of Agricultural Technology Andalas University
Corresponding author’s email address: dsyukri@ae.unand.ac.id

Abstract. The objective of this study was to identify the differences of cabbage’s layers based on their responses to the environmental conditions. The differences between layers were observed according to the spectrophotometric evaluation. The three outer layers of a fresh cabbage were separated, sampled and put into extraction tubes. The metabolites inside the each layer were extracted by the mixture of isopropanol and hexane (2:1) and analyzed by a spectrophotometer with a scanning analytical mode from 200 nm to 800 nm. The obtained data were analyzed with a metabolomics statistical software. Based on the statistical results, it can be concluded that each layer of cabbage has a specific profile of metabolites distribution. Each layer has produced different responses to the environment after harvest where the first layer indicated more sensitive to the environmental conditions. Therefore, the selection of the first layer of cabbage is not proper for any evaluation relating to postharvest investigations.

Keywords: freshness evaluation, metabolomics, post-harvest, spectrophotometry

1. Introduction
Identification of freshness marker or indicator is an emerging subject on food distribution including postharvest science [1]. The utilization of metabolomics approach to identify a specific metabolite compounds as a freshness indicator that relate to senescence process has become a trending research topic on post-harvest of fresh produces [2]. Since the freshness indicator will be related to the senescence symptoms of measured fresh produces [3,4], therefore, determining the sampling position of the measured products that reflects the senescence process is very important[5].

In this study, a simple spectrophotometric- metabolomics screening for investigation of senescence symptom of a cabbage was done. Cabbage is a unique fresh produces due to its multi-layered property. Since the senescence symptom is strongly influenced by environmental conditions therefore the senescence process of each cabbage leaf might be different due to its different position. The result of this study would indicate the difference of cabbage leaves that might relate to the stress to the environment conditions after harvest. The most senescence leave would not be suitable for further freshness indicator investigation.
2. Material and methods

Fresh cabbages obtained from the local farmer for subsequent analysis in this study and all chemicals used were analytical grades.

2.1 Sample preparation and analysis

The collected fresh cabbages were transferred to the laboratory after purchase. The samples were then washed and dried gently. The three outer layers were separated, and each layer was measured as much as 5 g and immediately put into 15 ml test tubes. 10 mL of the mixture of isopropyl alcohol and hexane (2:1) was added to the sample. The mixture was homogenized and put into a sonicator for 15 minutes. The solutions were then filtered and measured with a spectrophotometer using a scanning method with wavelengths from 200 to 800 nm. The color observation of each outer leaf of cabbage was also conducted by using a Chroma meter.

All obtained data was calculated statistically in order to find any differential features between each leaf of cabbage. The multivariate statistical analyses including principal component analysis was conducted using R statistic.

3. Results and Discussion

Table 1 presents the L, a, b data of each outer leaf of cabbage. According to the data, it could be indicated that the color pattern of each leaf was slightly different. Leaf color will fade with depth of position of the leaf. Therefore, the metabolism of each leaf might also different according to the color properties. Shen et al. [6] mentioned that the difference of color among tea leaves has a correlation with the metabolic activities inside the leaves. Therefore, the spectrophotometric was then further analyzed to indicate for clarification of this opinion in point of cabbage’s leaves.

Table 1. Colour measurement of cabbage leaves

| Position of leaf | L   | a   | b   |
|------------------|-----|-----|-----|
| The First layer  | 70.5| -5.5| 12.5|
| The second layer | 69.1| -8.3| 17.8|
| The third layer  | 70.5| -5.5| 12.5|

Figure 1 indicates the spectrophotometric data of each leaf in UV-VIS observation wavelength. There is a close relationship between the structure of organic compounds and its absorption in UV-VIS spectrophotometric region [7]. Based on the absorbance profile of each cabbage leaves, it seemed that the pattern was similar. However, since the previous Chroma metric data indicate the differences among the leaves, the clustering statistical calculation need to further conducted.

In this study, PCA calculation was used. The PCA is a statistical method that reduces the dimensionality of the acquired data by variables (principal components) [8]. This methodology reveals group sample relationships and similarities based on their spatial proximity. This classification procedure yields the maximum separation between known classes of samples and makes them easier to be distinguished.
Figure 1. The spectrophotometric data of cabbage leaves

Figure 2 shows the PCA result of obtained spectrophotometric data. It can be shown that there was a separation of organic compounds distribution among the leaves. There were three clusters that were also circled and positioned in different areas of the score plot to discriminate differences between groups as a function of then position of cabbage leaves. From the score plot, it can be concluded that after PCA analysis, the distribution of metabolites in each layer was different to each other. The difference in metabolites distribution between each layer might indicate that there was a difference in responses to environmental stress among layers. The score plot of the second layer and third layer was in negative values while the value of the first layer of cabbage leaf was positive which mean it’s might be related to the present stress condition. It can be suggested that the first layer might exposure hardly by the environments compared to the inner leaves. For identification of the freshness indicator of cabbage, the utilization of the first layer would not recommend. Ideally, the freshness indicator should be not present in initial state of fresh produces which mean the stress level of fresh produces should also be present in the lowest level.
4. Conclusion
The stress condition on each cabbage leaves was different according to the environmental exposure. The study suggested that the first layer of cabbage might experience the most stress levels compared to the inner leaves. Since, spectrophotometric analysis was not suitable for structure prediction on the metabolite that indicate the level of stress, further analytical technique such as chromatography-mass spectrometry need to be conducted.

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