Feasibility study on the use of UV/Vis spectroscopy to measure total phenolic compound and pH in apple (Malus sylvestris L.) cv. Manalagi

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Abstract. This paper will report the feasibility study on the use of UV/Vis spectroscopy to determine apple quality based on Total Phenolic Compound (TPC) and pH. To achieve the conclusion, several stages had to be conducted. First, the total 50 sample of apples from 4 different ripening stages (3, 4, 5 and 6 month) were collected from local farmer in Bumiaji District and extracted. The juice from extraction was prepared for UV/Vis spectrum collection ranging from 200-1100 nm. TPC and pH were then measured. Afterwards, chemometrics analysis was performed to provide Partial Least Square (PLS) prediction model. And lastly, was identifying robustness of the model by analysing all the statistic parameter. The result showed that, partial spectrum of PLS model to predict absorbance of TPC provided good determination coefficient (R² calibration was 0.803 and R² validation was 0.710) while RMSEC and RMSECV were 0.070 and 0.088 respectively. As for pH model prediction, the best model was also obtained from the partial spectrum resulting R² calibration was 0.822 and R² validation was 0.797 while RMSEC and RMSECV were 0.56 and 0.61, respectively.

1. Introduction
Apple (Malus sylvestris L) widely cultivated in Malang City is an important commodity as raw material to produce several popular products such as, apple drink, vinegar and chips. This commodity contains antioxidant compound (phenolic) that play an important factor to increase potential storage and maintain quality attributes of fruit [1]. A correct set of optimal harvest time is especially important for long-term storage. If the harvest time is too early, the economic losses are significant because fruit weights are needlessly smaller. Besides, when the fruit is harvested too early, it provides inferior consumption quality, has less attractive coloration and the taste does not meet consumer preferences because low of sugars and other nutritious solids [2]. Additionally, a large amount of polyphenols in the final product of apple are highly demanded; the antioxidant activity provides beneficial health effect such as reducing the risk of cardiovascular disease [3], diabetes and total cholesterol [4]. Therefore, it is important to determine an optimum phenolic level which meet consumer preferences. Phenolic compounds,
antioxidants on apple, are closely associated with the sensory and nutritional quality of fresh and processed plants foods [5].

Several methods have been widely used to measure Total Phenolic Compound (TPC) on organic matter i.e. HPLC [6,7] and Folin-Ciocalteu method [8]. However, all these methods rely on laboratory instrumentation which need sample preparation and equipment that is not suitable for local retailers who conduct apple quality measurement. Therefore, in order to provide a fast measurement, UV/Vis spectroscopy combined with chemometrics will be proposed on this research since the method is very promising to predict internal quality of fruit [9,10]. Chemometrics involves statistic method to create robust regression model based on chemical parameter and spectrum data including pre-processing and selection of data. Alongside TPC, pH value of apple will also be studied; because it responsible to consumer preference as sour level on apple.

In the following section, the research will focus on developing PLS model as a basic method to predict TPC and pH level of apple. The use of partial spectrum will also be studied and compared with full spectrum configuration.

2. Materials and Method
2.1. Material
The total 50 sample of apple cv. Manalagi were taken from apple local plantation, Bumiaji, Batu City. The total sample were derived from 4 different ripening stages (3, 4, 5 and 6 month).

2.2. Sample preparation
All sample was transported to Faculty of Agricultural Technology Laboratory, cleaned and sorted. The sample was then extracted by using juicer and around 20 mL of juice was placed on centrifuge tube. Then, it was put on centrifuge for 15 minutes at 6000 rpm to obtained a clear liquid extract.

2.3. UV/Vis spectral data acquisition
UV/Vis spectrum from apple juice sample was collected by using UV-1280 Shimadzu spectrophotometer which operates from 200 nm to 1100 nm. The apple extract was put on cuvette before scanning.

2.4. Phenolic compound measurement
Folin-Ciocalteau method was used to determine Total Phenolic Compound (TPC) [8,11] with gallic acid as a standard. The standard curve [Figure 1] was plotted by mixing different concentration (10, 30, 50, 70, and 90 ppm) of gallic acid with 2.5 mL of Folin-Ciocalteau 10% reagent and 2 mL sodium carbonate 7.5%. The absorbance was measure at 765 nm. By using Equation 1, TPC was then determined.

\[
TPC = \frac{A - b}{a} \times df \times \frac{V}{M}
\]

Where:
- TPC: Total phenolic content (mg GAE/g)
- A: absorbance at 765 nm
- a: a value on standard curve regression (y = ax + b)
- b: b value on standard curve regression (y = ax + b)
- df: dilution factor
- V: volume of extracted apple (L)
- M: mass of extracted apple (g)
2.5. pH measurement

pH value was measured by using pH meter Crison Basic 20. The probe of pH meter was put to the apple extract until the instrument read and displayed the pH value. For the next other measurement, the probe was then washed by demineralized water. pH meter was calibrated using buffer solutions before measurement [12].

2.6. Chemometrics

To obtain robust calibration model several statistic procedures need to be conducted; called chemometrics. Using The Unscrambler X.10.3 Trial Version, this analysis involves multivariate regression based on spectrum data and internal parameters (TPC and pH). Partial Least Square (PLS) was chosen as modelling method since it could manage big data and provided informative result. PLS would perform Principal Component Analysis (PCA) on spectrum data and the internal parameters; then the regression would be develop based on them. Several statistic parameters would be analysed, i.e. coefficient of determination (R²), Root Mean Square Error (RMSE) and Principal Component (PC) / factor.

3. Results and Discussion

3.1. Total phenolic content (TPC) and pH in several ripening stage of apple

TPC value from third month to sixth month varied from 13.4 mg GEA/g to 7.9 mg GEA/g. Figure 2 showed that average TPC value gradually decreased from third month (13.4 ± 11 mg GEA/g) to fifth month (5.1 ± 3.4 mg GEA/g) and slightly increase in sixth month (7.9 ± 4.6 mg GEA/g). Fruit maturation is one of factor that influence the concentration of particular phenolic content. One study revealed that young apple fruits contain high dihydrochalcones and after 14 weeks of maturation, the level of dihydrochalcones, flavonols and chlorogenic acid in apple gradually decrease [13]; enzymes activity is also responsible to the change of phenolic content [14]. Similar study reported that TPC of several varieties of apple decrease over days after full bloom where at the beginning of ripening stages the TPC reached the highest level [15]. From the graph, the variation of measurement on each month was very high; this phenomenon might occur by fruit position in the apple tree crown influence the TPC since random sampling process applied on this research. Moreover, non-standard ripening prediction by the farmer affect the variation of TPC in every month.
The average pH of apple from Figure 2 showed an increase over ripening period. The average value starting from 4.015 ± 0.076 in the third month and the following month increased to 4.022 ± 0.059, 4.214 ± 0.102 and 4.249 ± 0.01 in month 4, 5, and 6 respectively. Perez [16] reported that total acid will decrease over ripening period of apple making the pH value will increase. The increase of pH was affected by chemical activity during ripening process; at the first three month, apple contained more organic acids and it all converted into sugar for the next month. Over ripening stages, fruit would become sweeter and less sour because degredation of H$^+$ ion on oragnic acid while OH$^-$ increase making the pH value in the fruit gradually increased [17].

3.2. Partial least square of TPC and pH
To develop calibration model, this research only used 50 set data for calibration and validation where every set sample consist of spectral data and internal chemical compound (TPC and pH). Spectral data was used as predictor while pH and TPC as a response and to create a robust prediction model in a small set of data, cross validation was selected. Direct prediction of TPC might lower R$^2$ value; calculation (using Equation 1) to obtained TPC from absorbance and other variable measurement might give higher error. Therefore, in this analysis, absorbance value of TPC was set first. The research also performed both full and partial spectrum analysis. Even though the spectrum instrument measured in the range of near infrared (780 nm to 1100 nm), the optimum acquisition data was ultraviolet to visible range; in this research, because of lots of noise in the range of 200 nm to 350 nm, full spectrum was defined in the range of 350 nm to 1100 nm. In partial spectrum analysis, only the most influenced wavelength was applied for PLS calculation. Determination of partial spectrum depended on x-loading (Figure 3) Partial spectrum was used ranging from 353 nm to 384 nm for TPC and 372 nm to 385 nm for pH. Determination of PLS model to predict both pH and TPC absorbance was carried out by analyzing several parameters comprising R$^2$, RMSEC, RMSECV, and factor which was summarized in Table 1.
From Table 1, it could be seen that the best model of absorbance of TPC was developed by partial spectrum. The model created high R² calibration (0.797) and R² validation (0.686) while RMSEC, RMSECV were 3.23 and 4.04 respectively. The model also provided low error which meet the standard for PLS model [18] moreover R² in the range of 70-80% is acceptable for rough estimation in agricultural crops even it was less accurate [19]. For both R², they resulted high value (70-80%) making this model could only be used for general prediction. Some studies also reported that in several agricultural crops, R² in the range of 70-80% was commonly achieved [20, 21].

In order to get prediction value of TPC, one step calculation need to be conducted by involving predicted absorbance of TPC. As seen of Figure 5, the result showed that statistic parameter was similar with absorbance of TPC; however, the result provided higher error than absorbance of TPC model. Standard curve of galic-acid (R²=0.9913) will provide a slight error since its coefficient of determination did not reach 1. Therefore, one step calculation for obtaining predicted TPC will increase the error.

As for pH model prediction, the best model was also obtained from the partial spectrum. From the results, it was found that pH model provided R² calibration value accounted for 0.822 while R² validation was 0.797. For the value of RMSEC and RMSECV were 0.56 and 0.61, respectively. From these result, the model has a potential in predicting pH value similar with TPC model. Both R² for calibration and validation had high correlation between prediction and actual of pH, but for R² validation was highly lower than that of calibration. Therefore, there would be inconsistence in future prediction on other sample.

| Parameter          | Spectrum       | Factor | R² calibration | R² validation | RMSEC | RMSECV |
|--------------------|----------------|--------|----------------|---------------|-------|--------|
| Absorbance of TPC  | Full           | 4      | 0.694          | 0.557         | 0.087 | 0.106  |
| TPC (mg GAE/g)     | Partial (370-384nm) | 4      | 0.803          | 0.710         | 0.070 | 0.088  |
| pH                 | Full           | 4      | 0.652          | 0.522         | 4.25  | 5.06   |
|                    | Partial (370-384nm) | 4      | 0.797          | 0.686         | 3.23  | 4.04   |
|                    | Full           | 3      | 0.800          | 0.743         | 0.059 | 0.068  |
|                    | Partial (372-385nm) | 3      | 0.822          | 0.797         | 0.560 | 0.610  |
4. Conclusions
In developing PLS model, two spectrum configurations were compared. The best prediction model of both absorbance of TPC and pH was obtained by using partial spectrum resulting high $R^2$ but still had a wide range between calibration and validation. Further enhancement needs to be developed by adding more sample in a wide range of value.

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