Developing partial least square (PLS) internal parameters of apple (*Malus sylvestris* L.) cv. Manalagi by means of UV/Vis spectroscopy

Z Iqbal¹, I P Adiyaksa¹, A N Komariyah², R Damayanti¹, M A Kamal³, L C Hawa¹ and Y Hendrawan¹

¹ Department of Agricultural Engineering, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia
² Department of Bioprocess Engineering, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia
³ Department of Agro-industrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia

E-mail: zaqluliqbal@ub.ac.id

Abstract. In order to determine the ripening stages of apple (*Malus sylvestris* L), local farmer in Malang still utilized a traditional method by examining its size and appearance. However, this method needs worker’s extensive experience causing a non-standard ripeness level that may lead to lower the quality of crop. Thus, to solve this problem, fast and quantitative prediction method need to be developed. UV/Vis spectroscopy has shown its capability to provide a robust prediction of several internal attributes such as, Soluble Solid Content (SSC) and moisture content. The aim of this research is to develop a Partial Least Square (PLS) regression to predict internal parameter contained in apple. Fifty sample of apples were taken from local plantation in Bumiaji district. There were 3 stages to complete the research: (1) spectral data acquisition ranging from wavelength 200nm-1100nm; (2) Psychochemical measurement (moisture content, SSC and firmness); (3) Performing PLS regression based on spectral data and internal parameters. The result showed that PLS model of firmness could provide promising result where $R^2$ calibration and validation were 0.658 and 0.635 respectively. On the other hand, the model could not predict both moisture content and SSC resulting $R^2$ calibration 0.57 and 0.308 for $R^2$ calibration. Further enhancement needs to be addressed to the firmness model for improving its capability of prediction.

1. Introduction

Apple cv. Manalagi (*Malus sylvestris* L.) is one of valuable fruit in Malang area, mostly cultivated in Batu City, Poncokusumo, and Bumiaji district [1]. A fresh apple is commonly sold as a typical fruit from Malang which available in all period. Before distributing to the market, the farmers harvest the apple at certain level of ripeness by identifying its physical properties (e.g, size and colour) [2]. Size is preferable because it is easy to examine for field practical measurement and it associates with ripeness level [3]. Ripeness prediction stage is very crucial because it determines optimum quality of fruit and enhances long-term storage [4]. Harvesting unripe apple does not develop its full ripeness after storage, which leads to a small size, starchy flavor and a weak aroma. Additionally, picking overripe apple will
increase mechanical and biological damage [5].

However, this ripeness prediction method depends on farmer’s extensive experience that may provide different result among others. Thus, the uniformity of ripe apple could not be achieved that will lead to lower product quality in terms of organoleptic quality and storability [6]. Therefore, an objective and quantitative measurement need to be employed to replace the conventional method in order to maintain a good quality fruit. Several study regarding apple grading has been conducted such as, vision system [7,8] and aerial video [9]. Yet, they require a sophisticated set-up and high investment which is not suitable for field measurement and middle-low level plantation. Moreover, while apple cv. Manalagi does not change in color when ripening, the methods using image-base prediction will find a difficulty in ripening estimation.

As a fast measurement, UV/Vis spectroscopy has shown its capability to predict several parameter of crops, such as, soluble solid content (SSC), firmness and acidity [10]. Spectral data from UV/Vis wavelength will provide signature fingerprints of fruit that responsible to the change of certain chemical or internal parameters of fruit [11]. Thus, by simply identifying its spectral data using sensor probe, combined with multivariate prediction method, such as partial least square (PLS), the spectral data could be predictor to estimate its internal quality [10,11]. This method creates the possibility to develop a rapid and non-destructive measurement for monitoring ripening stage of apple. The aim of this research is to develop PLS regression as a basic study to develop quantitative measurement. In the following section, we provide a basic study to develop PLS regression on several internal parameters of apple.

2. Materials and Method
2.1. Materials
The total 50 fresh samples of apple cv. Manalagi were taken from apple private plantation, Bumiaji, Batu City, Indonesia. The total sample were derived from 4 different ripening stages (3, 4, 5 and 6 months). After harvesting, all sample was then transported to Faculty of Agricultural Technology laboratory for further examination.

2.2. Sample preparation
All sample from plantation was then cleaned and sorted. The fruit was divided onto two sampling, a solid sliced sample for measuring moisture content and firmness and extracted sample for Soluble Solid Content (SSC) measurement. Sliced sample was derived by simply cut a part of fruit; a cube shape for firmness and a random slice for moisture content. Meanwhile, extracted sample was made by using fruit juicer; aliquot of juice was placed on centrifuge tube. To reduce solid content, the sample was put on centrifuge at 6000 rpm for 15 minutes; a clear liquid sample in the middle of the tube was obtained for measurement.

2.3. Spectrum acquisition
UV/Vis spectrum was collected from extracted sample and measured by using UV-1280 Shimadzu spectrophotometer. The juice was put on cuvette and measured as absorbance of wavelength ranging from 200 nm to 1100 nm. Spectral data was collected as .CSV file for developing prediction model which then explained on the next section.

2.4. Internal parameter measurement
Parallel to spectrum acquisition, sliced and extracted sample was prepared for several internal parameter measurement as follow.

2.4.1. Moisture content
Oven drying method [12] was used in moisture content measurement. 5 g of sliced sample was cut from fresh apple. The fresh sample was weight (M1) and put on aluminium cup and dried at 105°C for 21 hours. The sample was then put on desiccator and weight (without aluminium cup) (M2). Moisture content was calculated using Equation 1 on a wet weight basis (ww).
2.4.2. **Soluble solid content (SSC)**
Soluble Solid Content (SSC) was measured by preparing 5 ml of extracted sample and it was put on hand refractometer; the value of SSC appeared on the instrument as °Brix [13].

2.4.3. **Firmness**
An apple solid sample was cut and put on penetrometer; the probe of penetrometer will penetrate the sample until fracture occurred. Firmness was defined as kilogram-force (kgf).

2.5. **Chemometric analysis**
PLS were utilized to build prediction model by using The Unscrambler X 10.3 Trial version software. Spectral data were set as predictor and internal parameter became response. This method would derive an important information from a hundred spectral data into several new variables called Principal Component (PC) / factor. The less factor built the more efficient prediction process would be. The process would provide calibration model which then used as prediction model; and validation model for validating the calibration model. To evaluate the model, several parameters need to be examined: coefficient of determination (R²), error and factor.

3. **Results and Discussion**
3.1. **Characteristic of internal parameter of apple**
3.1.1. **Moisture content and SSC**
The measurement showed that moisture content varied from 82.8 % ww to 87.3 % ww. Figure 1 illustrated that the increase of ripening stages equated to an increase of moisture content, this phenomenon has also been proved by other research which revealed the same trend; during the ripening stage of fruit, moisture content will then increase inside the product [14]. The increase of moisture content occurred by degradation of undissolved propectin into dissolved pectin. Production of pectin was responsible to the emergence of polygalacturonic and water as a secondary product. This process involving polygalacturonokinase acid and methyl acetate [15].

\[
\% \text{ Moisture} = \frac{M_i - M_e}{M_i} \times 100
\]  

(1)

![Figure 1](image-url) **Figure 1.** (a) moisture content and (b) SSC vs ripening stages

SSC of apple will increase over ripening period [2]. Some study showed that SSC was responsible to the sweetness level of the fruit. During ripening, enzymatic process broke insoluble polysaccharides (starch) into sugar which responsible to the increase of solid content [9,16]. Moreover, hydrolysis of
starch will fasten the production of glucose and fructose [17], making the SSC of apple would also increase. This trend was same as the graph showed in Figure 2b. In month 3, SSC varied from 0 °brix to 2 °brix until month 6 it reached up to 7.5 °brix. However, in the initial growth, glucose synthesizing was high and gradually decreased until the fruit reach ripe period [2].

3.1.2. Firmness
Ripening process involved structural change of fruit resulting softening of flesh, for instance, occurred during maturity stage [5, 7]. This phenomenon associated with the decrease of firmness of fruit. Figure 2 showed that average firmness level decreased from 2.443 kgf in the initial picked period to 0.725 kgf at month 6; overall, it varied from 0.7 kgf to 2.4 kgf. Firmness is responsible to apple optimum ripeness; According to P. Icka and R. Damo, the firmness level decreased while the starch index increased, and at certain level where the two parameters reached the breaking point, it was defined as an optimum harvesting time [6]. The change of firmness was affected by water content inside [14]. Water in fruit will penetrate wall cell making the increase of internal pressure of the wall which responsible to firmness level of fruit [18].

![Figure 2. Firmness vs ripening stages](image)

3.2. UV/Vis spectrum of apple and PLS analysis
As seen on Figure 3, the graph showed absorbance spectrum of apple from 350 nm to 1100 nm; spectrum from 200 nm to 350 nm was discarded because of lots of noise which could worsen model development. The UV/Vis spectrophotometer was only optimized in measuring ultraviolet to visible spectrum (350-780 nm), even it could also measure in the range of near infrared (780-1100 nm). However, in this case, all range spectrum was also used and compared with partial spectrum (which in the range of ultraviolet to visible). There was only a slight peak around 350 nm to 500 nm while other range did not provide a response. A clear pale-yellowish apple extract provided a high transmissivity making almost UV/Vis light from spectrometer pass through the apple juice, therefore, only a minimum light absorbed by the juice. However, there were a difference absorbance between samples, allowing the spectral data could be used as a predictor of several parameters. A total 50 set data were used for both calibration development and validation. Cross validation was set in order to manage a small group of spectral data. Full and partial spectrum were analysed and compared. As for partial spectrum, only an important range of wavelength was used; this spectrum depended on x-loading where a unique peak indicated an important wavelength that provide an influence for model development (Figure 4-6).
Coefficient of determination (R²) was one of important parameter to develop robust PLS model. R² > 80-95% illustrates a high correlation which could be used as prediction while R² in the range of 66-80% could indicated quantitative measurement to prediction the reference value [19]. Table 1 showed that the best PLS model was developed using partial spectrum while full spectrum generated lower parameters. Partial spectrum only used the most influenced absorbance appeared by interaction between sample and light emission making noise from other outranges spectrum been removed. From Figure 7-9, only firmness level showed a promising result where R² calibration and validation were 0.658 and 0.635 respectively, while SSC and moisture content were 0.57 and 0.308 for R² calibration. Even though
The firmness calibration model provided higher correlation among others, for practical use, it resulted unstable prediction for future measurement. Therefore, when spectrum data of the new other sample was obtained, this PLS model could produce high variation of reference value. Low $R^2$ developed by PLS might be caused by a low number of sample and low of range value of internal parameters.

![Figure 7. PLS model of moisture content](image1)

![Figure 8. PLS model of SSC](image2)

![Figure 9. PLS model of firmness](image3)

| Parameter     | Spectrum       | Factor | $R^2$ calibratio | $R^2$ validation | RMSEC | RMSECV |
|---------------|----------------|--------|-----------------|-----------------|-------|--------|
| Firmness      | Full           | 2      | 0.597           | 0.538           | 0.265 | 0.288  |
|               | Partial (373-385nm) | 2      | 0.658           | 0.635           | 0.244 | 0.244  |
|               | Full           | 2      | 0.410           | 0.260           | 1.220 | 1.389  |
| SSC           | Partial (374-393nm) | 2      | 0.578           | 0.568           | 1.036 | 1.076  |
| Moisture content | Full          | 2      | 0.279           | 0.184           | 0.709 | 0.775  |
|               | Partial (370-390nm) | 2      | 0.308           | 0.217           | 0.695 | 0.217  |
4. Conclusions
PLS calibration model for several parameters were developed. From statistical description, partial spectrum model development delivered the best result than full spectrum. As for firmness prediction model, it provided 0.658 R² calibration, 0.635 R² validation, 0.244 RMSEC and 0.244 RMSECV while other provide lower value for practical prediction. However, firmness PLS model needs to be improved for better prediction by adding more samples in a wider range of value.

References
[1] Sellitasari S, Ainurasyid, Suryanto A 2013 Differences in the production of apple crop (Malus sylvestris mill.) in different agro-climatic (case studies on the apple crop center production in batu city and malang regency) J. Produksi Tanaman 1 1 1-8. [In Indonesia]
[2] Hazarika S, Hebbl R L, Rizvi S S H 2017 Prediction of ripening stage of cameo apple using fourier-transform infrared spectroscopy Int. J. Fruit Sci. 1 1 1–11.
[3] Blažek J, Pišteková I Prediction of the harvesting time for four apple cultivars on the basis of beginning of flowering and attaining of t-stage of fruitlets and dependence of diameter of fruitlets at t-stage and fruits at ripening stage J. Hort. Res. 25 1 55–59.
[4] Watkins C B 2003 Principles and practices of postharvest handling and stress CAB Publishing.
[5] Skic A, Szymańska-Chargot M, Kruk B, Chylina M, Pieczynswy P M, Kurenda A, Zdunek A, Rutkowski K P 2016 Determination of the optimum harvest window for apples using the non-destructive biospeckle method Sensor 16 661 1-15.
[6] Icka P, Damo R 2014 Assessment of harvest time for red delicious cv. though harvest indexes in albania Bulgarian J. Agri. Sci. 20 3 628–632.
[7] Cárdenas-Pérez S, Channa-Pérez J, Méndez-Méndez J V, Calderón-Domínguez G, López-Santiago R, Perea-Flores M J, Arzate-Vázquez I 2017 Evaluation of the ripening stages of apple (Golden delicious) by means of computer vision system Bio. Eng. 159 1 46-58.
[8] Cheng H, Damerow L, Sun Y, Blanke M 2017 Early yield prediction using image analysis of apple fruit and tree canopy features with neural networks J. Imaging 3 6 1-13.
[9] Szalay L, Orndige M, Ficzek G, Hadley P, Tóth M, Battey N M 2013 Grouping of 24 apple cultivars on the basis of starch degradation rate and their fruit pattern Hort. Sci. (Prague). 40 3 93–10.1
[10] Wang H, Peng J, Xie C, Bao Y, He Y 2015 Fruit quality evaluation using spectroscopy technology: a review Sensor 15 1 11889–11927.
[11] Seifert B, Pfanz M, Zude M 2014 Spectral shift as advanced index for fruit chlorophyll breakdown Food Bioprocess Technol. 7 1 2050–2059.
[12] Nielsen S S 2010 Food analysis Springer International Publishing AG.
[13] Muchtadi T R, Sugiyono, Ayustaningworo F 1991 Food science Alfabeta. [In Indonesian]
[14] Lawalata V N, Budiastra I W, Haryanto B 2004 Exalcation of nutrition, organoleptic properties and physical properties of sagu mutiara by canary fruit (Canarium ovatum) addition Agritech 24 1 9-16.
[15] Imaduddin A H, Susanto W H, Wijayanti N 2017 The influence of ripeness level of starfruit (Averrhoa carambola L.) and addition of sugar proportion on physicochemistry and organoleptic properties of starfruit lempok J. Pangan Agroindustr 5 2 45–57. [In Indonesian]
[16] Ng J K T, Schröder R, Sutherland P W, Hallett I C, Hall M I, Prakash R, Smith B G, Melton L D, Johnston J W 2013 Cell wall structures leading to cultivar differences in softening rates develop early during apple (Malus x domestica) fruit growth BMC Plant Biol. 13 183 1-16.
[17] Anggraini R, Hasbullah R, Sutrisno 2015 Study of degreening stage on keprok madu terigas cv. orange in West Kalimantan J. Penelitian Pascapanen Pertanian 12 1 35-44. [In Indonesian]
[18] Blahovec J 2007 Role of water content in food and product texture Int. Agrophysics 21 1 209-215.
[19] Karoui R, Mouazen A M, Dufour E, Pillonel L, Schaller E, Baerdemaeker J D, Bosset J O 2006
Chemical Characterisation of European emmental cheeses by near infrared spectroscopy using chemometric tools *Int. Dairy J.* **16** 1 1211-1217.