APPLICATION OF HYPERSPECTRAL IMAGING FOR RAPID AND NON-INVASIVE QUANTIFICATION OF QUALITY OF MULBERRY FRUIT

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ABSTRACT

This study investigated the potential of using hyperspectral imaging working in near infrared region (850-1750 nm) for rapid and non-invasive determination of the total flavonoid in mulberry fruit. The spectra of samples were extracted according to the shape information of fruit contained in the hyperspectral images. Partial least-squares regression was used to calibrate the total flavonoid content of fruit samples with their corresponding spectral data. Results showed that a good correlation was obtained between the frozen days and spectral information. It indicates that it is possible for rapid and non-invasive quantification of quality of mulberry fruit with hyperspectral imaging technique.

Keywords: mulberry fruit, hyperspectral imaging, total flavonoid

1 Introduction

Mulberry fruit comes from the mulberry tree (*Morus* sp.), a genus of family Moraceae and distributed in a wide area. Mulberry can grow in a wide range of climatic, topographical and soil conditions (Ercisli and Orhan, 2007). The mulberry fruit is a multiple fruit with a length of 2-3 cm. The immature mulberry fruits are white or green to pale yellow with pink edges. When the fruits are ripening, the color becomes red, later dark purple and finally black.

Mulberry fruit with its sweet flavor is widely used in jam, pies, tarts, wines, and liquor, and is a delicacy among humans and birds alike. Total flavonoid content is often considered an important quality index of mulberry fruit [1].The quality evaluation of mulberry is usually determined by chemical or sensory analysis (Chian, 2013). The chemical analysis intends to extract various flavonoid compounds from mulberry fruit tissues using organic reagents, then high performance liquid chromatography (HPLC) (Zhang et al., 2010, Chen et al, 2008), or gas chromatographic (GC) (Gao et al., 2010), or ultraviolet (UV). However these methods are not capable of fast determination and real-time monitoring. Some of them are destructive, some take a long time to obtain the testing result for one fruit from the preparation to the end, and some need professional operators to finish the analysis (López-Posadas et al., 2008, Sadeghipour et al., 2005).

With the combination of the main advantages of spectroscopy and computer vision, hyperspectral imaging technique can simultaneously acquire spectral and spatial information in one system. Such ability let hyperspectral imaging be able to determine the inherent chemical and physical properties of the specimen as well as their spatial distribution simultaneously, which is critical for the quality prediction of agricultural and food products in a detailed way. There are lots of researches on the quality determination of many fruits using hyperspectral imaging technique, e.g. apple, citrus, grape, mango, and banana.

However, it has not reported to determine the total flavonoid content of mulberry fruit and to generate the content distribution within the fruit using hyperspectral imaging. In this work, the feasibility of using hyperspectral imaging technique for rapid and nondestructive determination of total flavonoid content in fresh mulberry fruits was investigated.

2 Materials and methods

2.1 Mulberry fruit samples

Fresh mulberry fruits were taken for experiment. All investigated mulberry fruit samples were collected at Huzhou Academy of Agricultural Sciences in China from the 1th April to 10th May, 2013. During this time, mulberry fruit changes its color from green to red, to dark. The flavonoid concentrations in mulberry fruit consequently changed with the color of the fruit. In total, 45 samples (10 mulberry fruits in each sample) were collected from mulberry trees. The samples were stored at -20 ^{o}C. Spectra collection and total flavonoid determination were carried out on the same day.

2.2 Hyperspectral imaging equipment

In this work, a hyperspectral image of each fruit was acquired by using a pushbroom line-scanning HSI instrument. The system mainly consisted of an ImSpectorV17E imaging spectrograph covering the spectral range of 850-1750 nm, a high-resolution single piece digital camera, a camera lens, a specially assembled light unit consists of two 150w quartz tungsten halogen lamps as the light source, and a conveyer belt operated by a stepper motor. Each fruit was placed on the moving table and then was scanned line by line to build a hyperspectral image (R_0) called 'hypercube' with a dimension of (x, y, λ). A 2-D image (y, λ) with the whole spectral dimension (λ) with one spatial dimension (y) was acquired at a time. A complete hyperspectral cube was taken as the line was scanned along the direction of x dimension, and was stored in a band-interleaved-by-line (BIL) format. The dimension of the first hypercube was 672 pixels in x-direction, n-pixels in y-direction (based on the length of the sample) and 256 bands in λ-direction. Average spectra of each fruit were extracted from its hyperspectral image using the Region of Interests Function (ROI) of ENVI v4.6 software.

2.3 Hyperspectral image correction

Before hyperspectral image acquisition, the hyperspectral imaging equipment needs black and white board correction to eliminate the noise caused by dark current and uneven light intensity. In the same acquisition environment, the white correction image W_λ was collected with a standard white board, and the black correction image B_λ was collected when the camera lens was covered. The mulberry fruits were put on the conveyer belt, and the original hyperspectral image I_λ was collected. The corrected image R_λ was got after the calculation of formula (1).

$$R_\lambda = \frac{I_\lambda - B_\lambda}{W_\lambda - B_\lambda} \quad (1)$$

2.4 Determination of total flavonoid content

Immediately after spectra acquisition, mulberry fruit samples were used to determine total flavonoid content by the ultraviolet-visible spectrometry method. Briefly, fresh mulberry fruits were frozen with the liquid nitrogen. After freezing, each sample including 10 mulberry fruits were immediately grinded. 10 mL 70% aqueous ethanol solution was used to extract flavonoid from1 g of the grinded fine powder at supersonic condition for 30 min. Then the sample was centrifuged at 3000 × g for 10 min, the supernatant liquid was transferred to 50 mL volumetric flask, the precipitate was re-extracted 2 times as described above. At time zero, 1 mL supernatant liquid was transferred to volumetric flask; 75 uL of 5% $NaNO_2$ was added; then 150uL of 10% $AlCl_3$ was added; 0.5 mL of 1 M NaOH was added and the solution was fixed to 2.5 mL using 70% aqueous ethanol solution. The solution was used to detect absorbance value at 510 nm using a spectrophotometer. The Quercetin was used as the standard for a calibration curve. The flavonoid content was calculated using the following linear equation based on the calibration curve:

$A = 2.6758C + 0.008$, $r^2 = 0.9975$

where A is the absorbance and C is the flavonoid content in mg/ mL.

2.5 Partial least-squares regression

Partial least-squares regression (PLSR) was used to relate the extracted spectral data and the total flavonoid content. PLSR can decompose both the spectral (independent variables) and concentration (dependent variables) information simultaneously, resulting in extracting a set of orthogonal factors called latent variables (LVs). In the decomposition process, dependent variables are actively considered in estimating the LVs to ensure that the first several LVs are most related for predicting dependent variables. Results showed that a good correlation was obtained between the reference total flavonoid content and spectral information.

3 Results and discussion

3.1 Total flavonoid content of mulberry fruits

All of the 45 samples were separated into two group. 30 samples were

randomly picked for calibration, and the rest of 15 samples were set for prediction. The standard deviation of total flavonoid in total samples was 0.0554, 0.0610 in calibration set, and 0.0440 in prediction set.

3.2 Analysis of hyperspectral reflectance curve

In the spectra of 45 mulberry fruit samples obtained from raw data, it could be found that there were many reflection peaks in near infrared region (850-1750 nm), which would be a close relationship with the chemical composition in samples. However, the original hyperspectral has more noise in the head and the tail. So the spectral information in 900-1700 nm region was picked up for detail research, combining with chemometrics to find the relationship between the spectral data and the total flavonoid content in mulberry fruit.

3.3 Partial least-squares regression model

Partial least squares (PLS) analysis is widely used for calibration in current chemometric analysis. PLS is performed to establish a regression model to predict physiological concentrations. PLS finds the fundamental relations between the variable matrix Y (the properties of interest) and the variable matrix X (the spectra). PLS is particularly suited when the number of variables is greater than the samples, and when there is multicollinearity among *X* values.

In the partial least-squares regression model, the spectral data was set as *X*, and the total flavonoid content values of mulberry fruit samples were set as *Y*. In calibration set, the correlation coefficient (*r*) is 0.8867, and the root mean square error of calibration (RMSEC) is 0.0201. In the prediction set, the correlation coefficient (*r*) is 0.7482, and the root mean square error of prediction (RMSEP) is 0.0312. It indicates that the detection of total flavonoid content of mulberry fruits with hyperspectral imaging technique was feasible.

4 Conclusion

In this study, the feasibility of rapid and nondestructive determination of total flavonoid content in fresh mulberry fruit with hyperspectral imaging technique was preliminarily researched. The hyperspectral image of mulberry fruit was gained, and the total flavonoid content of mulberry fruit was detected immediately. The PLS model was built to find the relationship between the hyperspectral image information and the total flavonoid content. The results showed the correlation coefficient (*r*) was 0.8867 and 0.7482 in calibration set and prediction set separately. It indicates that the detection of total flavonoid

content of mulberry fruits with hyperspectral imaging technique was feasible, and the hyperspectral imaging technique colud be used for nondestructive detection of other chemical composition content in mulberry fruit.

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