Available online at www.sciencedirect.com

ScienceDirect

IFAC-PapersOnLine 49-16 (2016) 096–099

Measuring and evaluating anthocyanin in lettuce leaf based on color information

Xiao Yang*#, Jingjin Zhang*#, Doudou Guo*, Xin Xiong*, Liying Chang*, Qingliang Niu*and Danfeng Huang**

** School of Agriculture and Biology, Shanghai Jiao Tong University, Key Laboratory of Urban Agriculture (South), Ministry of Agriculture, Shanghai, P.R. China, 200240. (e-mail: yalxd1988@sjtu.edu.cn Xiao Yang; jj.zhang@sjtu.edu.cn Jingjin Zhang; adoutx@sjtu.edu.cn Doudou Guo; xiongxin1989@126.com Xin Xiong; changly@sjtu.edu.cn Liying Chang; qlniu@sjtu.edu.cn Qingliang Niu)*

 # Contributed equally

*** Corresponding author (Tel: 86-021- 34206943; e-mail: hdf@sjtu.edu.cn); School of Agriculture and Biology, Shanghai Jiao Tong University, Key Laboratory of Urban Agriculture (South), Ministry of Agriculture, Shanghai, P.R. China. 200240.*

Abstract: Anthocyanin, one of the major secondary metabolites in red wave lettuce, is beneficial for human health and improvement of visual functions in salad. While the anthocyanin measurement in plant mainly relies on chemical analysis to date. In order to explore the possibility of digital image in non-destructive anthocyanin prediction, a quadratic model based on color parameters (red-green-blue color space and hue-saturation-intensity color space, RGB and HSI color space) of lettuce leaf was investigated. The results suggested that the characteristics of different color components combinations such as R/G, G/R, B/G, G/(R + B), G/(R + G + B), H, I/H, S/H, H/S, R/(R + G + B) - G/(R + G + B), G/(R + G + B) - B/(R + G + B), (G - R) /(G + R) and (G - B) /(G + B) were significantly correlated with anthocyanin content, and the correlation coefficient between S/H and anthocyanin content was the highest of 0.850. For quantitative prediction of anthocyanin content, B/G value was optimal color characteristic to forecast the anthocyanin content, with the function of $Y=aX+bX^2+c$ (R^2 of 0.781). This work demonstrates that the machine vision technique is promising non-destructive measurement of anthocyanin content in lettuce leaves.

Keywords: lettuce; anthocyanin; non-destructive measurement; machine vision method; color characteristic.

1. INTRODUCTION

Epidemiological and intervention studies have suggested that dietary fruits and vegetables are beneficial for human health, and the benefits have been attributed at least in part to secondary metabolites, such as anthocyanins, flavonoids and phenolic acids(Tsuda, 2012; Nijveldt et al., 2001). Effects of anthocyanin in obesity control, diabetes control, cardiovascular disease (CVD) prevention, and improvement of visual functions have attracted an increasing number of attentions (Tsuda, 2012).

Lettuce (*Lactuca sativa* L.), one of the most important crop consumed worldwide, contains rich anthocyanin, flavonoid, vitamins, carotenoids, and fiber (Nicolle et al., 2004). According to United Nations Food and Agriculture Association, the production of lettuce (and chicory) all over the world was about 25 million tons with a gross profit of over $14 billion in 2013(United Nations Food and Agriculture Association, 2013). For salad, the anthocyanin concentration contributed to not only the nutritive value but also the visual treat. Therefore, accurate measurement of anthocyanin is very useful for assessing the color and nutritional quality of raw lettuces as well as other rich anthocyanin foods (Giusti and Wrolstad, 2001).

Traditionally, experimental approaches to determine anthocyanin content in plant mainly rely on chemical analysis, which is high cost, time-consuming, tedious, and destructive. At present, the non-destructive detection measurements are applied widely due to its advantage of rapid assessment, such as near infrared spectroscopy (Huang et al., 2008), biosensor technology (Terry et al., 2005), nuclear magnetic resonance technique (Clark et al., 1997), ultrasonic detection (Mizrach, 2008), mechanics detection (García-Ramos et al., 2005), machine vision (Cubero et al., 2011), X-ray CT technology (Haff and Toyofuku, 2008), etc. Among these non-destructive detection methods, the machine vision has been more and more widely employed to measure and evaluate crop features, such as color information, shape and size, damaged condition and texture in recent years, as it is a fast, efficient, low-cost and convenient technique (Cubero et al., 2011). However, most of the research focused on automatic classification of crops (Blasco et al., 2007; 2009a; Zou et al., 2007), yield estimation (Blasco et al., 2009b; Okamoto and Lee, 2009; Safren et al., 2007), of which very few devoted to the internal quality attributes. Our previous study suggested that based on machine vision measurement, prediction of fruit interior quality from fruit surface color is feasible (data not shown).

In this study, we aim to measure and evaluate anthocyanin in lettuce by using machine vision technology. Based on image analysis, a quantitative prediction and a regression model of anthocyanin content in lettuce leaves were established.

2. MATERIAL AND METHODS

2405-8963 © 2016, IFAC (International Federation of Automatic Control) Hosting by Elsevier Ltd. All rights reserved.
Peer review under responsibility of International Federation of Automatic Control.
10.1016/j.ifacol.2016.10.018

2.1 Plant material

The lettuce seeds (*Lactuca sativa* L. cv. Lollo Rossca) were sown into culture dishes with wet filter paper for sowing seeds in 16th November 2015. After three days, lettuce seedlings were transferred to experimental flowerpots. After four weeks, the seedlings were planted and conducted in a glasshouse in Shanghai Sunqiao modern agricultural zones (Shanghai, China) from December 2015 to January 2016. The growth condition was set to 16°C at night and 25°C during the day in the glasshouse.

2.2 Image acquisition and anthocyanin assay

To acquire comparable images of lettuce, a phenotypic platform (LemnaTec Scanalyzer PL, Aachen, Germany) was employed to collect the images of 24 plants at 62 d after sowing. Inside the platform, a top-mounted camera was used to capture digital images with the illumination source in visible spectrum range (Fig. 1).

Fig. 1. The image acquisition platform.

For cyanin assay, regions of interests (ROI, circle of approximate 2 cm^2) were chosen and marked (Fig. 2). Effort was made to mark ROIs with large color difference. Then the concentration was measured by a Dualex 4 Scientific (6 mm diameter sensing surface, Dx4, FORCE-A, Orsay, France).

Fig. 2. The region of interest

2.3 Data analysis and quantitative prediction

There were 59 samples of lettuce leaves randomly selected, and divided into a training set (40 samples) and a validation set (19 samples). Leaf appearance characteristics in RGB and HSI color models were obtained from images using Matlab (version 2013, The MathWork, Inc., Natick, MA). Six color parameters were calculated for each region of interests (shown as circles in Fig. 2): red (R), green (G), blue (B), hue (H), saturation (S), and intensity (I). Then, the 6 color parameters were transformed into 37 mathematical combinations (shown in Table 1). Pearson Correlation was conducted to determine correlation coefficient by SPSS Statistics (version 22.0, The IBM Corporation, Armonk, NY).

For establishment of prediction models, a curve estimation method was taken to predict lettuce anthocyanin content based on color parameters. Then, these models were evaluated by normality test. The residuals as well as the confidence interval were calculated by SPSS, and a 5% significance level and image features of 19 samples (validation set) were set to test the regression models.

3. RESULTS AND DISCUSSION

3.1 Pearson Correlation analysis

The correlations between 43 color parameter combinations and anthocyanin content analysed by Pearson Correlation analysis are shown in Table 1. The correlations between color characteristics and anthocyanin content achieved in the combinations of R/G, G/R, B/G, G/(R + B), G/(R + G + B), H, I/H, S/H, H/S, R/(R + G + B) - G /(R + G + B), G/(R + G + B) - B/(R + G + B), (G - R) /(G + R) and (G - B) /(G + B) were stronger than other combinations ($|r| > 0.75$ for all). And the correlation coefficient (r) between S/H and anthocyanin content was the highest at 0.850.

Table 1. Correlation analysis of color characteristic and anthocyanin content in lettuce leaves

Color characteristic	r	Color characteristic	r
Blue (B)	-0.201	S/H	0.850**
Green (G)	-0.635**	S/I	0.498**
Red (R)	-0.287	H/S	-0.796**
Hue (H)	-0.815**	H/I	-0.704**
Saturation (S)	-0.559**	I/(H+S)	0.679**
Intensity (I)	-0.531**	S/(H+I)	0.528**
R/G	0.824**	I/(H+S+I)	0.593**
R/B	-0.420**	S/(H+S+I)	0.527**
G/B	-0.703**	H/(H+S+I)	-0.707**
G/R	-0.755**	2S-H-I	0.538**
B/G	0.832**	2S-H	0.639**
B/R	0.458**	R/(R+G+B)-G/(R+G+B)	0.765**
R/(G+B)	0.633**	R/(R+G+B)-B/(R+G+B)	-0.060
G/(R+B)	-0.764**	B/(R+G+B)	0.695**
R/(R+G+B)	0.626**	2G-R-B	-0.730**
G/(R+G+B)	-0.798**	2G-R	-0.692**
2G-B	-0.671**	G/(R+G+B)-B/(R+G+B)	0.792**
I/S	-0.468**	G-R	-0.695**