Estimation of Catechins Concentration of Green Tea Using Hyperspectral Remote Sensing

Yusuke Sohara *. Chanseok Ryu **.
Masahiko Suguri *. Si-bum Park ****. Shigenobu Kishino *****.**

**Graduate School of Agriculture, Kyoto University, Kyoto, Japan*
(Tel:+81-75-753-6167; e-mail: sohara@elam.kais.kyoto-u.ac.jp)
*** Graduate School of Agriculture, Kyoto University, Kyoto, Japan*
(Tel:+81-75-753-6317; e-mail: ryu@elam.kais.kyoto-u.ac.jp)
**** Graduate School of Agriculture, Kyoto University, Kyoto, Japan*
(Tel:+81-75-753-6279; e-mail: suguri@elam.kais.kyoto-u.ac.jp)
***** Graduate School of Agriculture, Kyoto University, Kyoto, Japan,*
(Tel:+81-75-753-6114; e-mail: psb5203@kais.kyoto-u.ac.jp)
****** Graduate School of Agriculture, Kyoto University, Kyoto, Japan,*
(Tel:+81-75-753-6114; e-mail: kishino@kais.kyoto-u.ac.jp)

Abstract: Models for the estimation of the concentration of catechins concentration in new green tea shoots were established using ground-based hyperspectral remote sensing. The coefficient of determination (R^2) was determined to be more than 0.913, the root mean squared error of prediction (RMSEP) was determined to be less than 0.617 % and the relative error of prediction (REP) was determined to be less than 6.17%, except in the EGC model (R^2=0.512, RMSEP=0.272%, and REP=15.7%). The regression coefficients of the green, red edge and near infrared regions were all changed, indicating that those regions were important for the estimation of catechin concentration. A similar trend was noted for the regression coefficients of ECg and EGCg. Therefore, the X -loadings of the first latent variables of ECg and EGCg (ester-type catechins) and EC and EGC (free-type catechins) were compared and the similarities between each type of catechin were determind. Therefore, each type of PLS regression model was designed based on date of the ester- and free-type catechins. The accuracy of the free-type model was as follows: R^2=0.774, RMSEP=0.273% and REP=7.85%. The accuracy of ester-type model was as follows: R^2=0.869, RMSEP=0.991% and REP=6.99%. The regression coefficients of the free-type catechins differed from those of the ester-type catechins. Large changes to the regression coefficients of the green to red, and red edge regions were also demonstrated.

Keywords: catechins concentration, hyperspectral image, the reflectance of new shoots, PLS regression, Regression coefficient

1. INTRODUCTION

Tea is one of the world's most popular beverages and has been cultivated for thousands of years. Japan's production volume (PV) of tea, mainly green tea, is 0.1 million tons/year and the 8^{th} largest in the world (FAO, 2008). The main brands of green tea, are: Shizuoka (41% of PV), Kagoshima (18% of PV), and Uji (3% of PV) (MAFF, 2009). New shoots are generally harvested in the middle of May in Uji, where the best quality tea is harvested, although PV is less in Uji than in the other places. Recently, tea's beneficial medical properties, such as its possible ability to prevent cancer, have received considerable attention (Yang et al. 1993). As tea grows, the yield increases, but the quality deteriorates. Therefore it is necessary for farmers to harvest new shoots are harvested so that the yield and quality are well balanced. However, the timing of harvesting new shoots is depending on their experience, but it is ambiguous. Therefore it is necessary to constitute the invariant method to help determine the optimum plucking time so that the yield and quality will be well balances.

Generally, the quality of green tea has a negative correlation with its quantity (Saba et al. 1993). In the case of quality, the nitrogen concentrations (Goto et al. 1986), amino acids (Horie et al. 1988), crude fiber (Smiechowska et al. 2006) and catechins concentrations (Ikegaya et al. 1988) have been used as chemical constituents to maintain the standards of quality. The taste that is considered one of the standards of tea quality and it consists of the sweetness, Umami, bitterness, and astringency (Ruan et al. 2007). Although bitterness is mainly influenced by the concentration of catechins, it has received attention for the biological and pharmacological activities (Yang et al. 2002). Generally, there are four types of catechins in tea; epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECg) and epigallocatechingallate (EGCg). Although the typical methods for determining the concentration of catechins, such as Kjeldahl or HPLC, are precise, they are also time consuming and laborious. Therefore, several studies have been carried out on non destructive methods such as near-infrared (NIR) spectroscopy (Chen et al. 2006) and H-NMR spectroscopy (Gall et al. 2004).However, these studies were not based on the shoots that are currently growing, but ont tea powder of green tea. Therefore, the amount of nitrogen concentration in new shoots in the field was estimated by the hyperspectral image (Ryu et al. 2007). One of the advantages of hyperspectral imaging is its ability to possibly measure the reflectance of a target without receiving interference from any other parts of the plant. Therefore, the objectives of this study are to establish catechin concentration estimation model based on the reflectance of new shoots in the field, fetermine the accuracy of this model and find out the characteristics of catechins based on the reflectance of new shoots.

2. MATERIALS AND METHODS

2.1 Experimental field

The experimental field was located at Wazuka-Cho, Souraku-Gun, Kyoto prefecture, Japan (135°55'E, 34°46'N and 358 m above sea level). The cultivar of tea planted in this field was *Camellia sinensis L, Yabukita,* which is cultivated for "Matcha". Matcha field was covered with shade screen to slow the growth of the plants and deepen the color of their leaves as shown in Fig. 1. Chemical and organic fertilizers were applied in this field. The test crop was covered on 31 April with screen net and harvested on 20 May, in 2009. Five sampling points were used in this field and the experiment was carried out every three or four days.

Fig. 1. Experimental field of tea

2.2 Hyperspectral remote sensing

The images were taken at five sampling points using a QE-V10E hyperspectral camera (Specim), as shown in Fig. 2. This camera measured the reflectance of tea leaves placed in the circular frame (d= 300 mm). The camera was a line scan camera, and it was positioned about 1 m above the circular frame and the resolution at that position was approximately 2 mm × mm. Reflectance of the new shoots was measured in 1024 discrete and narrow bands between 400 and 1000 nm. A reflectance board was positioned on either sides of the circular frame in order to reduce the influence of variation in the intensity of incoming light.

2.3 Sampling

New shoots that had more than *"pokoe"* (a new shoot with two leaves and a bud) in the circular frame were sampled. The samples were parched by the 500W microwave oven and dried for 24 hours at 60°C by circulation oven drier. The dry mass of each sample was measured using an electronic scale. Samples were then finely ground using a pulverizing mill. (Ryu et al. 2007)

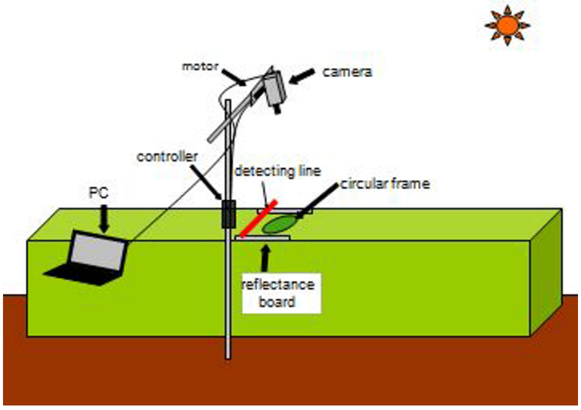

Fig. 2. Arrangement for taking pictures

2.4 High performance liquid chromatography (HPLC)

Approximately 50 mg of the powdered material is extracted with 5 ml 50% aqueous acetonitrile containing 0.085% phosphoric acid under ultrasonic treatment for 30 min. The extracts are centrifuged at 1,500 *g* for 10 min. The resultant solution was diluted two times with water and filtered through a 0.45 micro meter filter. The filtrated samples were separated by reverse-phase high-performance liquid chromatography using a Shimadzu LC-VP system (Shimadzu) equipped with a Develosil ODS-HG-5 column (4.6 mm x 150 mm, Nomura chemical) placed in a column oven set at 40℃ and a UV detector (SPD-20AV, Shimadzu) set at 230 nm. The separation of major catechins was carried out by using 0.085% phosphoric acid (elute A) and 40% acetonitrile in 0.085% phosphoric acid (elute B). The gradient used was: 0-10 min, 20% B; 10-30 min, linear gradient 20-49% B; 30-40 min, 75% B; 40-55 min, 20% B. The flow rate was 1.0 ml/min. Individual peaks were identified by the comparison of their retention times with those of authentic standards. All experiments were carried out in triplicate. The catechins concentration was calculated as the percentage of catechins per unit dry weight.

2.5 Image processing

Environment for Visualizing Image software ENVI version 4.5 (Research Systems) was used to separated each image into two parts: 1) new shoots and 2) others (mainly old leaves), as shown in (1). The nitrogen concentrations of young leaves differs from that of old leaves and this difference can be estimated using NDVI and Green NDVI. Therefore, the difference between NDVI and Green NDVI was utilized to separate new shoots from others. The reflectance of new shoots was divided by the reflectance of the reflectance board and defined it "Reflectance", as shown in equation (2).

$$GreenNDVI - NDVI = \frac{R_{NIR} - R_{Green}}{R_{NIR} + R_{Green}} - \frac{R_{NIR} - R_{Red}}{R_{NIR} + R_{Red}} \quad (1)$$