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Noninvasive Evaluation of Fructose, Glucose, and Sucrose Contents in Fig Fruits during Development Using Chlorophyll Fluorescence and Chemometrics

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ABSTRACT

The use of chlorophyll fluorescence (ChlF) technique was evaluated on nondestructive measurement of sugar content during fruit development. Multivariate models, principal component analysis (PCA), and partial least-squares regression (PLSR), were developed for the classification and prediction of fructose, glucose, and sucrose in fig fruits. The results of this study showed a significant correlation between fluorescence parameters and sugar content during fruit development. The PCA-ChlF can be used as a fast screening method for discriminating the degree of maturity based on sugar content. In addition, the root mean squared error (*RMSE*) and coefficient of determination (R^2) of PLSR-ChlF for predicting sugar content were 2.01 g 100 g^{-1} DW and 0.96 for fructose, 1.03 g 100 g^{-1} DW and 0.99 for glucose, and 0.17 g 100 g^{-1} DW and 1.00 for sucrose, respectively. Therefore, ChlF combined with chemometrics may be a potential tool to nondestructively evaluate sugar accumulation in not only fig fruits, but also any other chlorophyll-containing fruit during development.

Keywords: Fluorescence parameter, *Ficus carica* L., Maturity, PLSR, Sugar.

INTRODUCTION

Fig (*Ficus carica* L.), which belongs to Moraceae, is a tree native to southwest Asia and the eastern Mediterranean region. The common fig is one of the first plants that were cultivated by humans. Kislev *et al.* (2006) reported that fig trees, which preceded cereal domestication by about a thousand years (11,400 to 11,200 years ago), could have been the first domesticated plant of the Neolithic Revolution. It is also one of the most widely produced fruits in the world, with an estimated annual production of 1,077,211 tons of fruit (FAO, 2003).

Fig fruits play an important role in nutrition due to the rich carbohydrate content (almost 65–70%), which is the major source of energy to maintain life activities. In addition, the concentration of carbohydrates in fruits has been of interest because of their important influence on the organoleptic properties, thus, it is a major criterion used to judge maturity and grading of fruits. Therefore, food researchers and plant physiologists have been interested in changes in the carbohydrates occurring during growth and maturation of fruits because of their impact on the market quality of the food product (Glew *et al.*, 2003). Recently, many technologies have been explored for non-destructive measurement of sugar content in fruits, such as nuclear magnetic resonance (Cho *et al.*, 1993), computer vision (Steinmetz *et al.*, 1999; Kondo *et al.*, 2000), and infrared spectroscopy (Bureau *et al.*, 2009; Liu *et al.*, 2010). When compared with these methods,

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chlorophyll fluorescence (ChlF) is a not only non-destructive technique, but also fast and portable method for field measurement. It has been used as an indirect measurement of the physiological status of several chlorophyll-containing fruits (Song *et al.*, 1997; Agati *et al.*, 2005; Noh and Lu, 2007; Ramin *et al.*, 2008; Zheng *et al.*, 2010) as ChlF parameters decrease with decreased photosynthetic activity and chlorophyll content during fruit development (Smillie *et al.*, 1987; Sanxter *et al.*, 1992). In addition, it has also been successfully applied to evaluate the degree of maturity in mango fruits (Lechaudel *et al.*, 2010), grape berries (Kolb *et al.*, 2006) and papaya fruits (Bron *et al.*, 2004). However, as far as we know, there are no data on nondestructive evaluation of fructose, glucose, and sucrose in fig fruits by ChlF technique.

Therefore, the aims of this study were (i) to prove that ChlF technique is a convenient and potential tool for the evaluation of sugar content in fig fruits, and (ii) to develop partial least-squares regression (PLSR) model to predict fructose, glucose, and sucrose as a function of fluorescence parameters.

MATERIALS AND METHODS

Food Materials

The figs (*Ficus carica* L., cv. Branswick) were randomly harvested from the experimental orchard at Zhejiang Normal University (Jinhua, P. R. China). The blossoms were considered to be in full bloom on 20 March 2011, and five maturity categories were collected by sampling the fruits at 40, 60, 80, 100 and 120 days after full bloom (DAF), which were denoted T1, T2, T3, T4, and T5 in this study, respectively. All fig samples were inspected to ensure that fruits were undamaged and not attacked by pests. The fruits were transported by refrigeration at 8°C for 10 minutes to the laboratory.

Chemicals and Reagent

Fructose (purity≥ 99%), glucose (purity≥ 99%), and sucrose (purity≥ 99%) were purchased from Merck (Darmstadt, Germany). Acetonitrile was purchased from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China).

Fluorescence Parameters

Chlorophyll fluorescence (ChlF) parameters were measured using a MINIPAM (PAM-2000) fluorometer (WALZ, Effeltrich, Germany) as reported by Dai *et al.* (2009). The MINIPAM fluorometer system comprised a fluorescence pulser-receiver (MINIPAM host), an optical fiber (MINIPAM/F) and a microcomputer system for data acquisition and analysis (Figure 1). The optical fiber was mounted with an angle of approximately 60° between its tip and the surface of the sample, enabling a fluorescence signal to be transmitted and received over a short distance (minimum of 5 mm) between its tip and the peel of the fruit. Before measurements, fruit samples were kept in darkness for 30 minutes, ensuring that all their PSII reaction centers were open. Each fruit was marked at five locations on the peel for ChlF nondestructive testing. The original fluorescence (F_0) with all

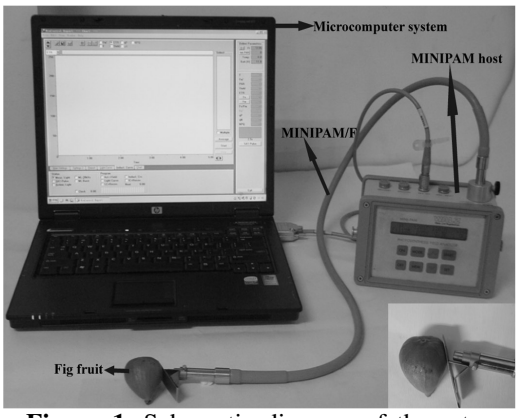

Figure 1. Schematic diagram of the setup for chlorophyll fluorescence testing of fig fruit.

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PSII reaction centers in the open state was determined with a measuring beam at a light intensity of 0.04 μmol m^{-2} s^{-1}, generated by a 650 nm light-emitting diodes. The maximum fluorescence (F_m) with all PSII reaction centers in the closed state was measured under an 18,000 μmol m^{-2} s^{-1} saturation pulse. For obtaining F_s and F_m', the actinic light was turned on and the saturating pulse was applied every 60 seconds until steady-state photosynthesis was reached.

The difference between F_m and F_0 is denominated variable ChlF yield (F_v):

$$F_v = F_m - F_0 \quad (1)$$

F_v/F_m and Yield represent the maximum quantum yield of PSII and the effective quantum yield of photochemical energy conversion in PSII, respectively, which were calculated as follows:

$$F_v / F_m = (F_m - F_0)/ F_m \quad (2)$$

$$Yield = (F_m' - F_s)/ F_m' \quad (3)$$

Where, F_s and F_m' are fluorescence at steady-state photosynthesis and maximum fluorescence in the light, respectively.

Chlorophyll (Chl) Content in Fig Fruit Peel

The peel Chl was extracted with acetone (80%) according to Dai *et al.* (2009). The absorbance of extracts was recorded at 645 nm and 633 nm with a UV–VIS spectrophotometer (Lambda 5, Perkin-Elmer, USA). Chl *a* and *b* concentrations were measured in triplicate and expressed as mg g^{-1} of fig fruit peel.

Glucose, Fructose and Sucrose Content

A HPLC system (LC-10A HPLC Series, Shimadzu, Kyoto, Japan) equipped with a pump system and a refractive index detector (RID-10A) was used for sugar analysis. Fig fruit samples (1 g dry weight, DW) were ground extensively and extracted three times in 5 ml 80% (v/v) ethanol for 30 minutes at 80°C. The extracts were combined and evaporated to dryness in vacuum in a rotary flask in a 40°C water bath. The residues were re-dissolved in 1 ml distilled water and passed through 0.45 μm filter. Ten microliters of sample was then analyzed in a Venusil XBP-NH_2 column (Angela, 250×4.6 mm) and kept at 55°C. The analytical conditions were shown as follows: flow 1.0 ml min^{-1}, eluent twice distilled water with 80% acetonitrile (v/v). The standard curve was prepared for calculation of the content of glucose (y= 0.26x, R^2= 1.00), fructose (y= 0.25x, R^2= 1.00), and sucrose (y= 0.24x, R^2= 1.00). The sugar content was measured in triplicate and expressed as mg g^{-1} dry weight (DW) of fig fruits.

Principal Component Analysis (PCA)

PCA is a chemometric linear and unsupervised technique used for analyzing, classifying, data compression and other aspects of data evaluation. It can reveal hidden structures present in the data set and transform the observed variables (ChlF parameters or sugar content, in our case) into a new set of independent variables known as principal components (PCs). For every PC, each factor in the original data set (ChlF parameters or sugar content) will have a loading that expresses the influence of this factor on the PC. Plots of the so-called PC scores against one another can reveal clustering or structure in the data set and is usually used for studying the classification of the data clusters. In this study, PCA was applied to classify the degree of maturity in fig fruit combined with chlorophyll fluorescence technique. The PCA was performed using the PAST software package (Hammer *et al.*, 2001).

Partial Least Squares Regression (PLSR)

PLSR consists of regression and classification tasks, dimension reduction techniques, and modeling tools. It is a method for comparing two data sets

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(explanatory matrix and dependent matrix) by a linear multivariate model (Zheng and Lu, 2011). Therefore, it is well suited for problems with multicollinear predictor and response variables. In this study, the fluorescence parameters and the sugar contents (fructose, glucose, or sucrose) were used to form the explanatory matrix and dependent matrix, respectively. The development of PLSR prediction models involves two basic steps: training and test phases. Therefore, all data (20 samples in this study) were randomly divided into the training and the test sets. The training set consisted of 70% of the samples, whereas the remaining data (30% of the samples) was used in the test phase. To get a good model, some parameters in PLSR were found by 10-fold cross-validation (10-CV), which avoids overfitting of the model. For PLSR, the optimum number of PCs corresponds to the point at which the *MSE* plot reaches a minimum or begins to level off (Sedman *et al.*, 1997). The PLSR was performed using the software MATLAB (R2010a, the MathWorks Inc., USA) under Windows XP.

Statistical Analysis

All extractions and determinations were carried out at least in triplicate. The statistics used for estimating the performance of PLSR models included coefficient of determination (R^2) and root mean square error (*RMSE*) (Zheng *et al.*, 2011). Correlation analysis and Duncan's test were performed using the statistical analysis systems (SAS, Version 8.1) software package. Differences at $P< 0.05$ were considered significant. The data were analyzed and graphically plotted using OriginLab (OriginPro, Version 7.5).

RESULTS AND DISCUSSION

Changes of Fluorescence Parameters, Chl and Sugar Contents during Development of Fig Fruit

It can be seen from Figures 2 and 3 that the values of F_0, F_m, F_v and Yield and the Chl content were significantly decreased with the maturity stage increase. However,

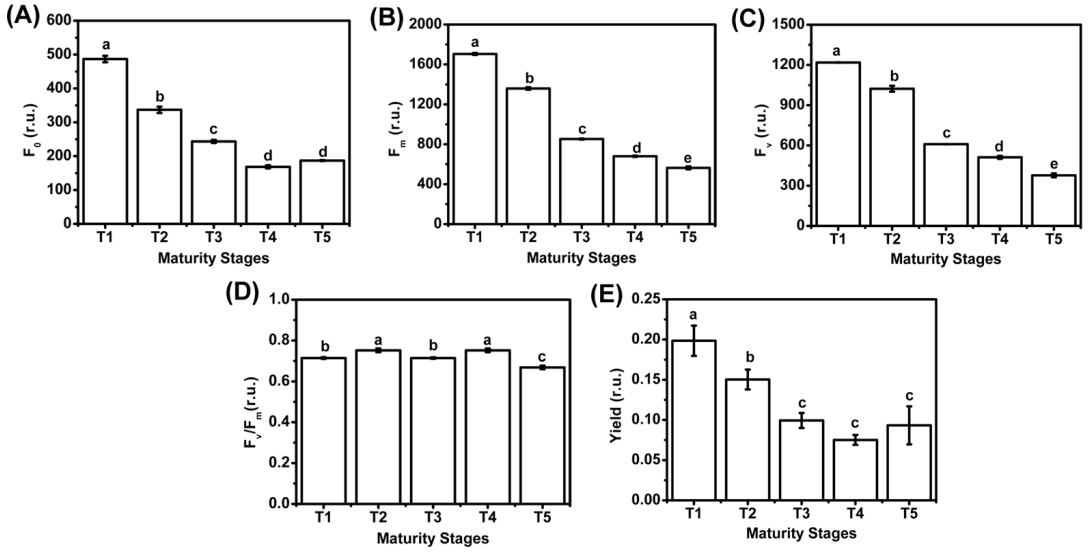

Figure 2. Changes in chlorophyll (Chl) fluorescence parameters (A: F_0; B: F_m; C: F_v; D: F_v/F_m and E: Yield) during development of fig fruit. Different lower case letters indicate significant differences (p <0.05).

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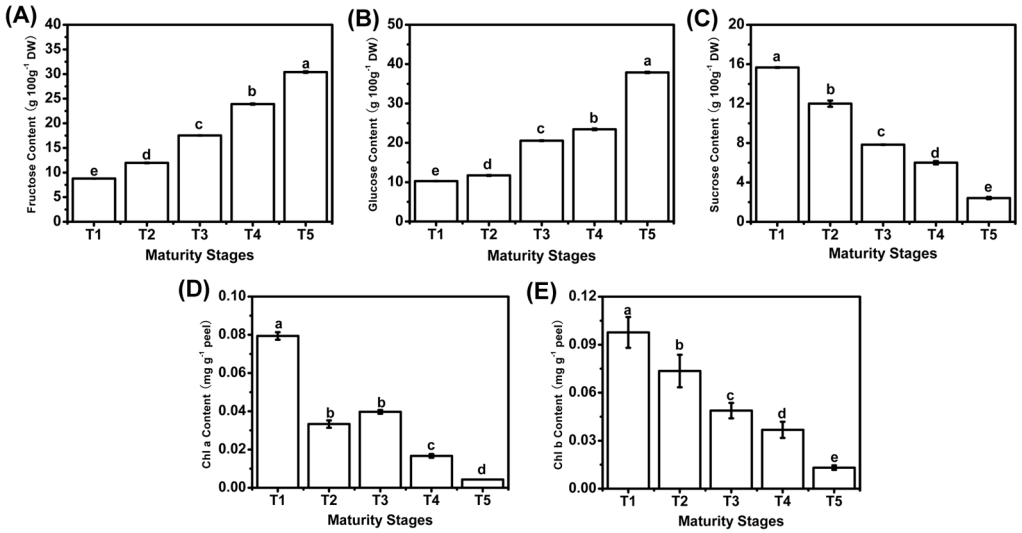

Figure 3. Changes in sugar content (A: fructose; B: glucose and C: sucrose) and Chl content (D: Chl *a* and E: Chl *b*) during development of fig fruit. Different lower case letters indicate significant differences (p <0.05).

an irregular change was observed for F_v/F_m values. Tucker (1993) has reported that fruit ripening processes may affect the ChlF by loss of photosynthetic activity and by decrease in chlorophyll content. In addition, sugar content in fruit increased during chlorophyll decay, these changes being general phenomena of the maturity process. As expected, the contents of fructose and glucose were increased from T_1 to T_5 (P< 0.05), as shown in Figure 3. It is noteworthy, however, that the sucrose content of fig fruit significantly declined during fruit ripening.

The correlations among fluorescence parameters, Chl and sugar contents are given in Table 1. Pearson correlation indicated that Chl content had a significant correlation with F_0, F_m, F_v and Yield (R^2= 0.82-0.96, P< 0.01). However, there was no significant correlation between Chl *a* and Chl *b* with F_v/F_m, as shown in Table 1. This result indicated that variations in F_v/F_m were largely independent of Chl content and resulted mainly from PSII function. Babani and Lichtenthaler (1996) reported that the F_v/F_m provided an estimate of the maximum quantum efficiency of PSII photochemistry. Table 1 also shows that Chl content was statistically significantly correlated to the contents of fructose, glucose, and sucrose

Table 1. Pearson correlation coefficients among chlorophyll fluorescence, chlorophyll content (Chl) and sugar content in fig fruit.

	F_m	F_v	F_v/F_m	Yield	Chl *a*	Chl *b*	Fructose	Glucose	Sucrose
F_0	0.98**	0.96**	0.11	0.95**	0.92**	0.92**	-0.88**	-0.79**	0.94**
F_m		1.00**	0.31	0.94**	0.89**	0.96**	-0.94**	-0.88**	0.98**
F_v			0.37	0.92**	0.87**	0.96**	-0.95**	-0.91**	0.98**
F_v/F_m				0.09	0.18	0.41	-0.48*	-0.66**	0.42
Yield					0.83**	0.82**	-0.81**	-0.72**	0.88**
Chl *a*						0.90**	-0.88**	-0.81**	0.91**
Chl *b*							-0.96**	-0.93**	0.98**
Fructose								0.97**	-0.98**
Glucose									-0.95**

*P< 0.05, and **P< 0.01.

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during fruit development of figs ($P< 0.01$). Kolb *et al.* (2006) also reported that chlorophyll changes correlated well with sugar synthesis for grape. In addition, a strong correlation between ChlF parameters (except F_v/F_m) and sugar concentrations is observed during ripening of fig fruits ($P< 0.01$). The relationships between ChlF and sugar concentrations have also been found in other fruits, such as grape berries (Kolb *et al.*, 2006). Therefore, the correlations among Chl content, ChlF, and sugar content provide a possible method for predicting the content of sugar by ChlF technique.

Classification of the Degree of Maturity in Fig Fruit by PCA-ChlF

The results in Figure 4-A show the distribution of the samples in the scores plot with the first two principal components accounting for 99.65%. The first principal component, PC1, explains 95.79% of the total variation, which was an important variable in discrimination of the five maturity levels. As seen in the PCA scores plot (Figure 4-A), fig fruits were divided into five clusters by the first two PCs based on their sugar content: T_1 (3, 5, 12 and 15), T_2 (7, 9, 11 and 20), T_3 (2, 6, 14 and 18), T_4 (8, 10, 13 and 17), and T_5 (1, 4, 16 and 19). In fact, sugar content of fruits is a major criterion used to judge their maturity and grade. In addition, it can be observed from the loading plot for PC1 (Figure 4-A) that fruit samples from T1 and T2 were characterized by fructose and glucose, and sucrose had main influence on classification of T4 and T5. Thus, the content of sucrose is a chief criterion for judging maturity of fig fruits.

A similar result that a clearly discrimination among the five maturity levels was obtained on the basis of fluorescence parameters (Figure 4-B). The PC1 explains 99.60% of the total variation, and the loading plot for PC1 (Figure 4-B) reveals the fluorescence parameters (F_0, F_m,

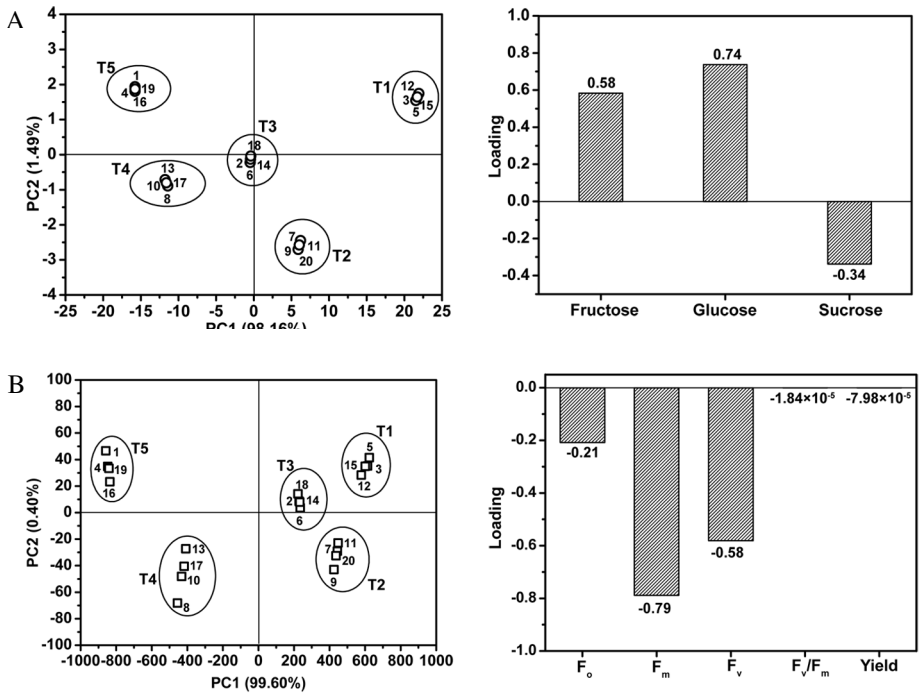

Figure 4. Scores plots of PC1 *vs.* PC2 and loadings plots of PC1 for the classification of the degree of maturity of fig fruits based on sugar content (A) and chlorophyll fluorescence parameters (B). The fruit samples in this study are numbered from 1 to 20.

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and F_v) have great influence on the discrimination of fruit samples from T4 and T5. Therefore, ChlF may be well-suited to evaluate the degree of maturity of fig fruit. The ChlF technique for the determination of the degree of ripeness was also successfully applied by Kolb *et al.* (2006) for grape berries. In our early study, we used this method to sort Chinese jujube based on nutritional constituents (Zheng *et al.*, 2010).

Prediction of Fructose, Glucose and Sucrose Contents by PLSR-ChlF

Figure 5 illustrates the *MSE* plotted as a function of the number of factors in the training phase, which shows that the optimal numbers of PLSR components are 2 for fructose, 3 for glucose, and 3 for sucrose. After training PLSR models, prediction performance must be tested. In this phase, an independent data set (30% of the total data) was utilized. The correlations between the predicted and actual values and the *RMSE* in respect to the training and test sets based on PLSR are also shown in Figure 5. The optimal PLSR could predict fructose, glucose, and sucrose in fig fruits with the *RMSE* of 2.01 g 100 g^{-1} DW, 1.03 g 100 g^{-1}

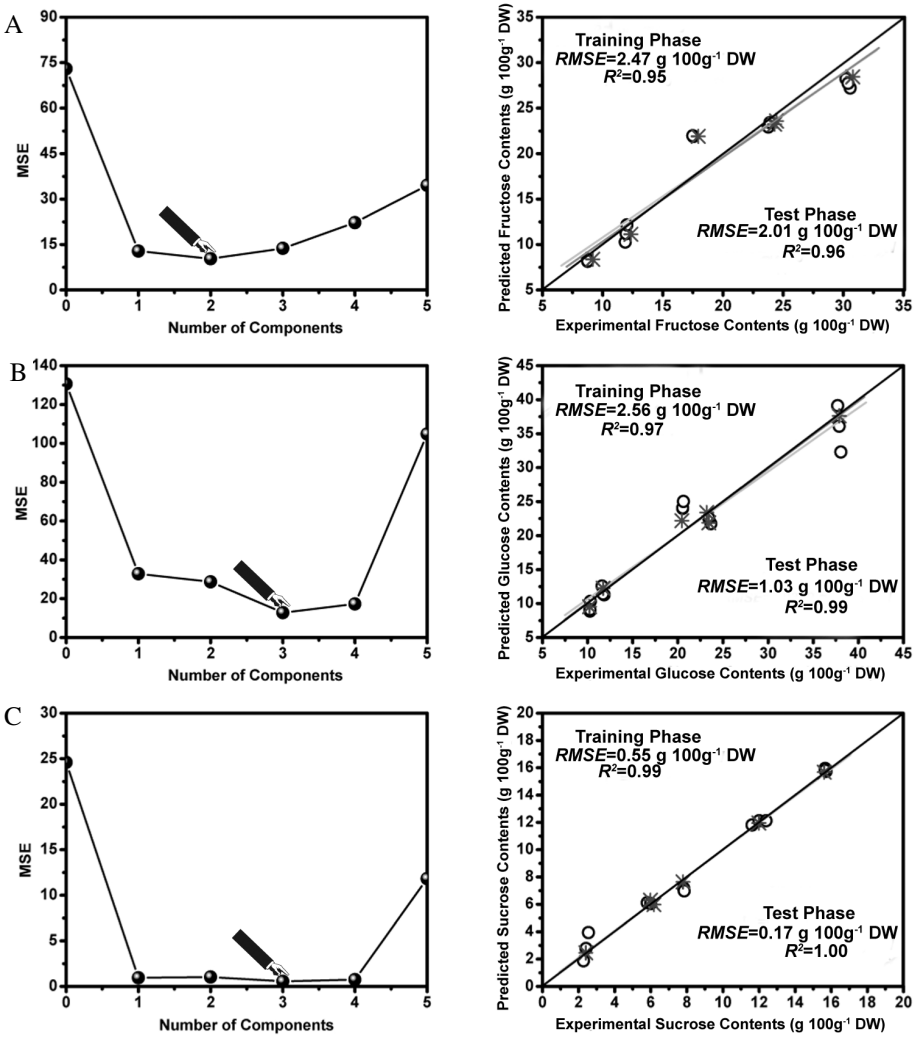

Figure 5. Mean squared error (*MSE*) versus PC factors and correlation of experimental and predicted values at the stages of training (○) and test (*) for the prediction of fructose (A), glucose (B) and sucrose (C) contents in fig fruits by PLSR model.

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DW and 0.17 g 100 g^{-1} DW, and R^2 values of 0.96, 0.99, and 1.00, respectively.

The best parameter for evaluating fruit taste quality is sugar content. Therefore, the development of a reliable, noninvasive method for evaluation of sugar content, before harvest and at the packing site, is critical to the success of the fruit industry. To date, the content of sugar in fruits has been nondestructively measured by many researchers. Kolb *et al.* (2006) applied ChlF for noninvasive evaluation of the concentrations of fructose, glucose, and total sugar in grape (R^2> 0.828). Jarén *et al.* (2001) used NIR technique for evaluation of sugar contents in two grape varieties: Garnacha (R^2=0.89, Standard error of estimate= 1.0508) and Viura (R^2=0.925, Standard error of estimate= 1.0446). Liu *et al.* (2007) also applied FT-NIR technique to predict sugar content of apples with a coefficient determination of 0.8436 and a standard error of 0.773. In addition, ATR-FTIR has been used for the prediction of sugar content ($R^2\geq$ 0.74, RMSE≤ 18%) in apricot fruit by Bureau *et al.* (2009). In this study, PLSR-ChlF is recommended for the prediction of sugar content in fig fruits, due to their lower *RMSE* (0.17-2.01 g 100 g^{-1} DW) and higher R^2 (0.96-1.00).

CONCLUSIONS

The PCA-ChlF was successfully used to evaluate the degree of maturity of fig fruits, and for PLSR-ChlF, the coefficients of determination between experimental and predicted values were greater than 0.96 for the prediction of fructose, glucose, and sucrose in fig fruits. Therefore, the results of this study indicated the possibility of developing a nondestructive technique using the chemometrics based on ChlF for evaluating sugar contents of fig fruits during development.

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ل. جیانگ، ز. شن، ه. ژنگ، و. هی، ج. دنگ، ه. لو

چکیده

در این تحقیق کاربرد روش فلوروسنس کلروفیل (Ch1F) در مورد اندازه گیری غیر تخریبی مقدار قند در طی رشد میوه ارزیابی شد. به منظور درجه بندی و پیش بینی مقدار فروکتوز، گلوکز و سوکروز در میوه انجیر،مدلهای چند متغیره وتجزیه مولفه های اصلی (PCA) و رگرسیون جزیی کمترین نماهای دوم (PLSR) تهیه شد. نتایج این مطالعه رابطه معنی داری بین پارامترهای فلوروسنس و مقدار قند موجود در طی رشد میوه نشان داد.بنا براین PCA-ChlF را می توان به عنوان روشی سریع برای غربال کردن و تشخیص درجه رسیدن میوه بر مبنای مقدار قند استفاده کرد. همچنین،ریشه میانگین مربع خطا (RMSE) و ضریب تبیین (R^2) مربوط بهPLSR-ChlF برای پیش بینی مقدار قند های مختلف چنین به دست آمد: برای فروکتوز (ماده خشک$^{-1}$g 100 g 2.01) و0.96 ، برای سوکروز (ماده خشک $^{-1}$g 100 g 1.03) و0.99 و برای گلوکز (ماده خشک$^{-1}$g 100 g 0.17) و ۱.۰۰. بنا بر این، روش ChlF همراه با شیمی آماری را می توان به عنوان روشی مناسب برای ارزیابی غیر تخریبی تجع قند در طی رشد میوه نه تنها در میوه انجیر که نیز در دیگر میوه های دارای کلروفیل به کار برد.

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