FOURIER TRANSFORM – NEAR INFRARED SPECTROSCOPY FOR RAPID AND NONDESTRUCTIVE MEASUREMENT OF AMYLOSE CONTENT OF PADDY

Ravi Pandiselvam[1*], Venkatachalam Thirupathi[2], Palanisamy Vennila[3]

[1]ICAR –Central Plantation Crops Research Institute,
Physiology, Biochemistry and Post Harvest Technology Division, Kerala, India
[2]Tamil Nadu Agricultural University,
Dryland Agricultural Research Station, Chettinad – 630 102, India
[3]Tamil Nadu Agricultural University,
Post Harvest Technology Centre, Coimbatore , India

Abstract: The quality and quantity of amylose are two important factors that determine the quality of paddy. The feasibility of developing a technique for rapid monitoring of paddy quality by using Fourier Transform - Near Infrared (*FT-NIR*) Spectroscopy combined with chemometrics was investigated. Spectra of 250 paddy samples were collected by using an integrating sphere accessory. The amylose content was analyzed by reference method. It ranged from 13.24 to 27.93%. The spectral data was analyzed by partial least squares (PLS) algorithm and calibration model generated. Correlation coefficient (R^2) for the calibration model was >0.78 with root mean square error of estimation (*RMSEE*) < 1.8. The validation model had R^2> 0.72 and root mean square error of cross-validation (*RMSECV*) < 2. *FT-NIR* spectroscopy identified samples containing amylose at 5184 and 6834 cm^{-1} wave number. A fast, simple and accurate method to quantify the amylose of paddy was developed by using FT-NIR spectroscopy.

Key words: paddy, amylose, chemometrics, FT-NIR spectroscopy, calibration

INTRODUCTION

Paddy is one of the staple food crops in India. *ADT* 43 is the most popular paddy variety grown in all the parts of Tamil Nadu, the reason behind that of *ADT* 43 are resistant to stem borer and gall midge, high tillering and fine rice [1,2]. About 70% of the paddy produced in India is stored at farm level. A small proportion of paddy is used as an ingredient in processed foods and as feed but the bulk is consumed as cooked rice.

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[*] Corresponding author. E-mail: anbupandi1989@yahoo.co.in

This pattern of usage results in the need to store rice over varying periods [3]. Quality maintenance is the main aim of such storage techniques. The economics of the grain which dictate the market value of the grain must be as high as possible during storage and on delivery to the customer [4]. Any attempt to generalize on the quality attributes of rice is accomplished by the diversity in tastes, but the predominant attributes are associated with its starch composition and amylose content.

Amylose is the important component in paddy which affects the parboiling, cooking and eating quality. Changes in the amylose content during storage affects the textural and rheological properties of cooked rice. Amylose content is directly related to water absorption, volume expansion and fluffiness of cooked grains. Thermal and pasting behavior of the aged paddy also depends on the degree of gelatinization. Disruption of the crystalline structure of starch granules during cooking decreases. The structural modifications of starch and protein gels may enhance the hardness of the cooked rice prepared from the aged samples [5].

Ozone fumigation is a novel method used to control the insects in stored grains [6-8]. It highly influences the amylose content changes as compared to other chemical composition. The concentration of ozone optimized based on the amylose changes. Hence, to check the amylose content of paddy grains before and after ozone treatment the study is essential. This study is also useful for *CWC* (central warehousing corporation) at the time of procurement of paddy from farmers and various paddy research institutions for analyzing the quality of paddy [9].

For the determination of amylose content by laboratory method, usually powdered and dry material is needed. The problems in the laboratory method of analysis are length of time required, destructive nature (without removal of husk, it is not possible to quantify the amylose content of rice in laboratory methods) and the requirement of hazardous chemicals as well as their disposal.

NIR spectroscopy is a fast technique that possesses the potential selectivity for screening the products based on qualitative attributes, when coupled with chemometric data analysis techniques [10]. Infrared spectroscopy is a technique that has been proposed as an excellent alternative to traditional methods due to its multiple characteristics such as simplicity, rapidity, reliable, cost effectiveness, potential for routine analysis and non requirement of skilled operator [11]. Monitoring the quality of paddy is a difficult task for farmers, rice researchers and food scientists. The aim of this research was to assess the performance of *NIR* spectroscopy combined with chemometrics in determining the amylose content of paddy.

MATERIAL AND METHODS

Paddy samples. Paddy (*ADT* 43) was obtained from central farm, located in Tamil Nadu Agricultural University and Central Warehousing Corporation, Trichy, India and used for the study. The paddy was cleaned manually to remove all foreign materials such as dust, dirt, chaff and immature paddy.

FT-NIR spectroscopy. FT-NIR spectra were recorded on multipurpose analyzer (Bruker Optics, Germany) equipped with an integrated Michelson interferometer; highly sensitive PbS 12000–4000 cm^{-1} detector, multiple *NIR* measurement accessories for different sampling techniques combined with *OPUS* 7.2 software. For the current study

spectra's were collected in diffuse reflectance mode with sphere macro sample integrating sphere measurement channel. The spectra were acquired in reflectance mode directly on the paddy, over the range 12000–4000 cm^{-1}. Spectra of 250 paddy samples were collected for calibration by using an integrating sphere accessory. For each sampling, 5 g of paddy were analyzed at room temperature and the average spectra were used for further evaluations [9].

Destructive method of estimation of amylose content in paddy. The amylose contents (% w/w) from grains were determined by the spectrophotometric standard method [12]. A total of 100 mg of granules was homogenized with 1 ml of 95% ethanol and 9 ml of 1 M NaOH. The sample was heated for 10 min. in a boiling-water bath to gelatinize the starch. After cooling, it was transferred into a volumetric flask and the volume was made up to 100 ml with water. Then 1 ml of 1 M acetic acid and 2 ml of iodine solution (0.2% I_2, 2% *KI*) were added to a 5-ml aliquot. The solution was made up to 100 ml with water and allowed to stand for 10 min. Spectrophotometric quantification was performed at 620 nm, with a spectrophotometer, using the multipoint working curve method with two repetitions and quartz cells of 10 mm path length. Two determinations were made on separate test portions taken from the same sample in each of the replicates. The apparent amylose content was calculated using an equation obtained from the standard curve.

Preprocessing methods. In this study, three data preprocessing methods were applied comparatively; these were vector normalization, first derivative and first derivative plus vector normalization. Each spectrum is corrected individually by first centering the spectral value. Then, the centered spectrum is scaled by the standard deviation calculated from the individual spectral values. Vector normalization normalizes a spectrum by first calculating the average intensity value and subsequent subtraction of this value from the spectrum. First derivative eliminate baseline drifts and small spectral differences are enhanced [9].

Data analysis. The *OPUS* software 7.2 was used for processing the data and *FT-NIR* models were developed with the full calibration data set. The spectral data were analyzed using PLS regression with various preprocessing techniques.

The performance of final *PLS* model was evaluated in terms of coefficient of determination (R^2) and root mean square error of cross validation (*RMSECV*). The accuracy of the calibration models is obtained according to the highest values of R^2 and lowest *RMSECV* and *RMSEP* values.

$$SSE = \sum \left[\mathrm{Re}\, sidual \right]^2 \tag{1}$$

where:
Residual   [-] - experimental value / predicted value.

The coefficient of determination (R^2) gives the proportion of variability of the property that is described by the model.

$$R^2 = \left(1 - \frac{SSE}{\sum (y_i - y_m)^2} \right) \times 100 \tag{2}$$

where:
$y_i = i^{ih}$ [-] - observation of experimental value,
y_m [-] - mean of the reference results for all samples.

Where, n is the number of samples in the validation set and y_i and $\bar{y}_i$ is the measured and predicted value of the ith observation in the test set, respectively. The number of *PLS* factors included in the model is chosen according to the lowest *RMSECV*.

RESULTS AND DISCUSSION

Spectra investigation. The spectra of original data recorded in the near-infrared region (4000–12500 cm^{-1}) are shown in Fig. 1. From the figure it is seen that almost all the sample is linear within the range of study and thus may give superior results [13]. As the spectra show similar basic *FT-NIR* spectral patterns, mathematical transformations were required to use the *FT-NIR* data for quantitative analysis.
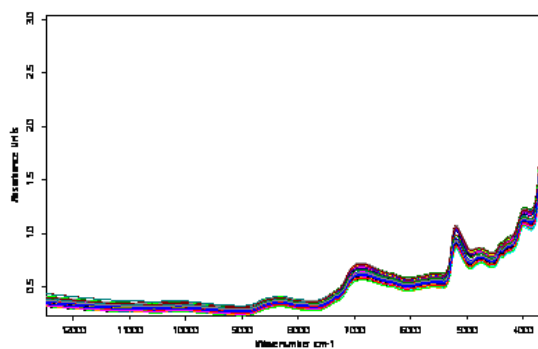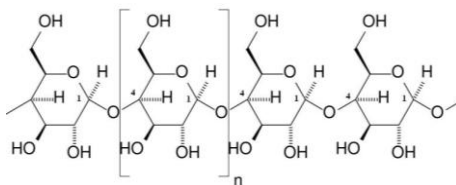

Figure 1. Spectra of paddy samples

Figure 2. Molecular structure of amylose

Fig. 1. also shows the *FT-NIR* spectra of amylose content which has major peaks at absorbance bands (wave numbers) of 3633.42, 3996, 5184, 6834.85 and 8316 cm^{-1}. As seen from Fig. 1, there are water absorption bands around 3633.42 and 3996 cm^{-1} wave number are generally due to O–H stretching of water molecule. These were excluded during analysis. The structure of amylose is shown in Fig. 2. A group of atoms in a molecule may have multiple modes of oscillation caused by stretching and bending motions of the amylose group. The strongest absorption of the spectra was at 5184 cm^{-1} related to the combination of O-H stretching and O-H bending of amylose molecule. Major peaks at absorbance bands or wave numbers of 6834.85 cm^{-1} may be due to the combination of first overtone of O-H anti-symmetric stretching and O-H symmetric stretching of amylose. The weak absorption bands at 8316 cm^{-1} may be due to second overtone of symmetric stretching (–CH bonds) of methyl (–CH$_3$) groups. The OH and –

CH bond vibrations are caused by ingredients such as amylose, protein and water compounds. The frequency that characterizes the stretching vibration of a given bond is proportional to the bond dissociation energy.

Chemometric analysis. The *NIR* spectra were transformed to its first-derivative using PLS function. The transformed spectra were cross-validated to generate calibration models. Cross validation was also done to check the calibrated values are shown in Fig. 3 and 4. It has been reported that the number of samples to develop the calibration model should cover the desired quantitation range for the specific analyses, with a minimum of 20–50 samples depending on the complexity of the problem [14]. Generally large calibration data sets are preferable, because they will provide more accuracy about the variability expected in validation samples. However large number of samples implies that it will take more time and chemical to create the model. Typically open calibration curves are developed, meaning that there is always the possibility to add more data points into the calibration curve to make it more robust [10].
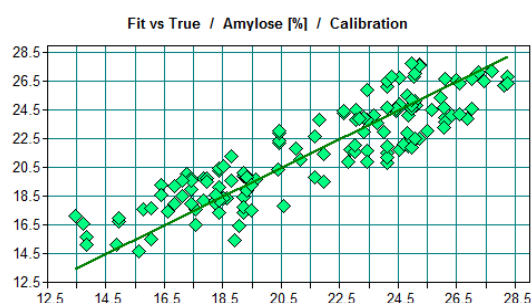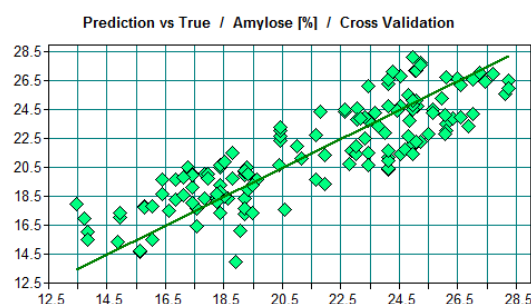

Figure 3. Calibration model of amylose

Fig.4. Validation model of amylose

Models were evaluated in terms of correlation coefficient (R^2), root mean square error of estimation (*RMSEE*) and root mean square error of cross validation (*RMSECV*). Figs. 5, 6 and 7 shows the R^2, *RMSECV* and *RMSEE* values plotted as a function of *PLS* factors for determining amylose content of paddy with first derivative and vector normalization method as the pre-processing technique. Seen from Fig. 5, R^2 value increased and reached an optimum value at *PLS* factor 10. *RMSECV* value decreases sharply up to the *PLS* recording rank of 8 and increased slightly as *PLS* factor increases

from 8 to 10 (Fig. 6). Fig. 7 shows the *RMSEE* value decreases sharply, if *PLS* factor increased from 1 to 10 and reached a minimum value at PLS factor 10. If PLS factor increases it increase the R^2 value and decrease the *RMSECV* and *RMSEE* values. The optimum number of factors is determined by the lowest *RMSECV* and *RMSEE* values and highest values for R^2. From the Figs. 5, 6 and 7, conform that initial *PLS* factor had high impact on R^2, *RMSECV* and *RMSEE* values.
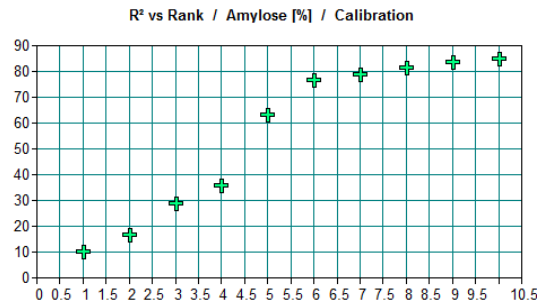
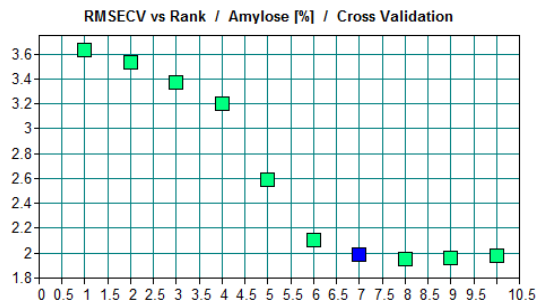*Figure 5. R^2 value as a function of PLS factor*

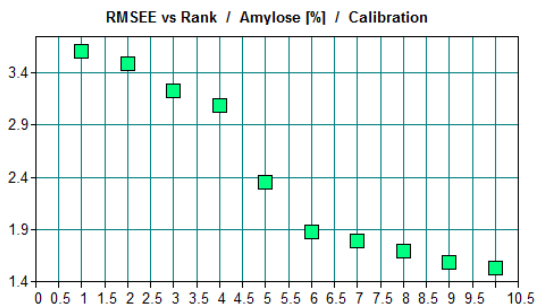*Figure 6. RMSECV value as a function of PLS factor*

Figure 7. RMSEE value as a function of PLS factor

PLS regression method gave R^2 and *RMSECV* values of 78.6 and 1.99, respectively. Correlation coefficient (R^2) indicates the strength and direction of the linear relationship between predicted values and reference values [15]. Ratio of standard deviation to standard error of prediction gives *RPD* (residual predictive deviation) value. *RPD* value

was more than 1.8. If *RPD* value lower than 1.5 is considered insufficient for most applications while *NIR* cross validation models with values greater than two is considered excellent [16]. The results of this study clearly indicate the efficiency of *FT-NIR* for this application.

CONCLUSIONS

An ozone treated paddy grains (*ADT* 43) amylose content was tested using *FT-NIRS. NIR* spectroscopy technique has potential to quantify the amylose from paddy. A fast, simple and accurate method for determination of amylose was demonstrated by using *NIR* spectroscopy at a low cost. It allowed for faster sample preparation and ease of use as compared to laboratory method. The total time required for sample preparation and analysis was less than 2 minutes, compared to 16 h required for amylose content determination by reference method. The overall results demonstrate that *FT-NIR* spectroscopy with *PLS* factor calibration could be successfully applied as a rapid method for quantification of amylose of paddy. It might be an application for paddy quality monitoring in the Central Warehousing Corporation, Food Corporation of India and various rice research stations by using *FT-NIR* spectroscopy.

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FURIJEOVA TRANSFORMACIJA - SPEKTROSKOPIJA U BLISKOJ INFRACRVENOJ OBLASTI ZA BRZO I NEDESTRUKTIVNO MERENJE SADRŽAJA AMILOZE U ZRNU PIRINČA

Ravi Pandiselvam[1], Venkatachalam Thirupathi, Palanisamy Vennila[2]

*[1]ICAR –Centralni institut za istraživanje plantažnih kultura,
Odsek za fiziologiju, biohemiju i postžetvenu tehnologiju, Kerala, India
[2] Poljoprivredni Univerzitet Tamil Nadu,
Istraživačka stanica za sušna zemljišta, Chettinad – 630 102, India
[3] Poljoprivredni Univerzitet Tamil Nadu,
Centar za postžetvenu tehnologiju, Coimbatore , India*

Sažetak: Kvalitet i kvantitet amiloze su dva značajna faktora koja određuju kvalitet pirinča. Ovede je razvijena tehnika brzog merenja kvaliteta pirinča upotrebom Furijeove transformacije – spektroskopije u bliskoj IR oblasti kombinovane sa hemometrijom. Analiziran je spektar 250 uzoraka pirinča integrisanim svernim uređajem. Sadržaj amiloze iznosio je 13.24 do 27.93%. Spektralni podaci su analizirani algoritmom parcijalnih najmanjijh kvadrata i generisan je kalibracioni model. Koeficijent korelacije bio je >0.78 sa srednjim kvadratom odstupanja < 1.8. Validacioni model imao je R^2> 0.72 i srednji kvadrat odstupanja unakrsne procene < 2. *FT-NIR* spektroskopijom su identifikovani uzorci koji sadrže amilozu sa talasnim brojevima 5184 i 6834 cm^{-1}. Tako je razvijen brz, jednostavan i pouzdan metod određivanja sadržaja amiloze.

Ključne reči: *pirinač, amiloza, hemometrija, FT-NIR spektroskopija, kalibracija*