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Feasibility study on estimation of rice weevil quantity in rice stock using near-infrared spectroscopy technique

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Thai rice is favored by large numbers of consumers of all continents because of its excellent taste, fragrant aroma and fine texture. Among all Thai rice varieties, Thai *Hommali* rice is the most preferred. Classification of rice as premium quality requires that almost all grain kernels of the rice be perfectly whole with only a small quantity of foreign particles. Of all the foreign particles found in rice, rice weevils can wreck severest havoc on the quality and quantity of rice such that premium grade rice is transformed into low grade rice. It is widely known that rice millers adopt the "overdose" fumigation practice to control the birth and propagation of rice weevils, the practice of which inevitably gives rise to pesticide residues on rice which end up in the body of consumers. However, if population concentration of rice weevils could be approximated, right amounts of chemicals for fumigation would be applied and thereby no overdose is required. The objective of this study is thus to estimate the quantity of rice weevils in both milled rice and brown rice of Thai *Hommali* rice variety using the near infrared spectroscopy (NIRS) technique. Fourier transforms near infrared (FT-NIR) spectrometer was used in this research and the near-infrared wavelength range was 780–2500 nm. A total of 20 levels of rice weevil infestation with an increment of 10 from 10 to 200 mature rice weevils were applied to 1680 rice samples. The spectral data and quantity of weevils are analyzed by partial least square regression (PLSR) to establish the model for prediction. The results show that the model is able to estimate the quantity of weevils in milled *Hommali* rice and brown *Hommali* rice with high R^2_{val} of 0.96 and 0.90, high RPD of 6.07 and 3.26 and small bias of 2.93 and 2.94, respectively.

Keywords: Rice weevil; Thai *Hommali* rice; near infrared spectroscopy.

1. Introduction

Apart from being a political crop, rice is one of Thailand's important economic crops and a major export income earner of the kingdom for decades. In 2011, Thailand exported 10.71 million tons of rice and generated a profit of approximately USD 6.3 billion.[1] Among the exported rice, Thai *Hommali* rice is classified as premium grade rice and is favored worldwide by consumers because of its unique aroma. The characteristics of Thai *Hommali* rice are long grain kernel, low amylase content (14–18%) and with gelatinization temperatures of 70–74°C.[2] As such, its texture becomes soft and sticky when cooked.

The milling process of Thai *Hommali* rice in the rice mills begins with dehusking or hulling of paddy rice to obtain brown rice, and then the bran is polished to obtain white rice or milled rice. However, most rice mills store both milled rice and brown rice to meet the demands of different customer groups.

During storage, rice is subjected to damage induced by either physical factors or biological factors or both. Examples of damage caused by physical and biological factors have large variations in storage temperature and insect attacks.[3] The biological factors, especially insect attacks, are reported to induce far greater damage to rice quality than the physical factors. FAO reported that in Thailand, insects could cause the damage up to 10% of rice in storage.[4] The damaged grains not only result in an economic loss but could also be turned into a political issue. Buyer countries would reject the delivery of rice if a large proportion of rice grains is damaged or if the rice is infested with insects or both. Of all the insects, the one found most in the rice stock is rice weevils (*Sitophilus Oryzae L.*).[5] The cycle of rice weevils consists of four stages: egg, pupa, larva and adult (as shown in Fig. 1). As seen in Fig. 2, in the adult stage, rice weevils become noticeably black in appearance, thereby allowing easy visual detection if the rice stock is infested.

Rice weevils propagate by laying eggs inside rice kernel. From the egg to pupa stages, rice weevils grow inside the rice kernel and feed on starch until they reach the adult stage before emerging from the kernel.[6] Additionally, the mature rice weevilsgnaw other rice kernels and excrete on the damaged kernels. The waste, then, causes reduction in rice quality.

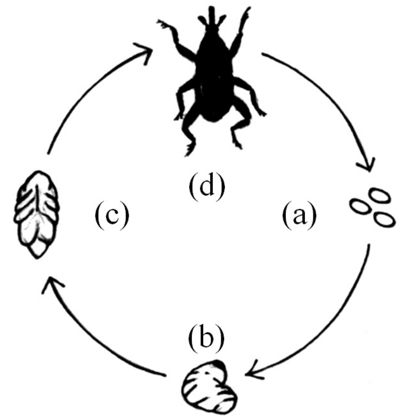

Fig. 1. The cycle of rice weevil (a) egg stage (b) pupa stage (c) larva stage (d) adult stage.

Fig. 2. A snapshot of rice stock infested with rice weevils.

Generally, rice exporters fumigate rice produce with phosphine (PH_3) to disinfest rice weevils. PH_3 is a gas produced by the reaction of aluminium phosphide or zinc phosphide with moisture in the ambient air.[7] The toxic PH_3 gas can kill and control the birth of rice weevils. However, its residues adversely affect human respiratory system, particularly lungs, brain, heart, liver and kidneys. The toxicity of PH_3 prevents the electron transportation in mitochondria and subsequently causes death.[6,8] The appropriate recommended dose must be strictly followed for effective disposal of rice weevils, for the safety of the handlers of the chemical, and for reduction of the toxic intake by humans.[6]

Even though rice weevils are easily visually detectable under normal light, those spotted are the rice weevils that inhabit on the surface whereas the rice weevils underneath are undetected. To estimate the quantity of rice weevils with the proposed technique, the NIR waveband is transmitted through rice to detect rice weevils by means of different chemical compounds between rice weevils and *Hommali* rice. The NIRS technique measures the chemical composition of biological materials using the diffuse reflectance or transmittance of the samples at several wavelengths.[9,10] The NIRS technology can also measure the concentration of components with different molecular structures, such as protein, water, starch and hydrogen bonding.[10,11] The NIR spectral region of 780–2500 nm lies between the visible and mid-infrared regions of the electromagnetic spectrum. In addition, NIRS have been widely used with several other food products including grains, cereals and oilseed.[10,12] It is also used in breeding programs for quality improvement of cereals, such as wheat flour yield, barley malting quality, durum semolina yield, rice milling yield and oat groat percentage. In addition, the technology is applicable to crop management, receivable testing and online process control.[10,13,14] In later years, the NIRS technique has been steadily refined for evaluation of rice kernel components and detection of foreign particles with faster speed, greater reliability, higher accuracy and nondestruction.[15] The reflectance frequencies from NIRS, which are varied depending on materials being scanned and different absorption spectra of the materials chemically, are used in material composition analysis. NIRS have been, then, applied to distinguish the interested objects from combined material, such as; detecting the insect infested chestnut, identifying stored-grain insects, detecting the insect fragments in wheat flour and so on.[16–18] Apart from that, NIRS have been used to evaluate the nonorganic materials as well, for instance, contaminating sand and soil in fuel.[19] It is proven that NIRs technique is able to discriminate the organic and nonorganic objects from materials by using the difference of chemical components detection. Therefore, reflectance spectra of other similar noticeable objects with rice weevil are unable to influence the rice weevil detection because both objects have different chemical compositions. Protein and carbohydrate (mainly starch) are the main constituents of Thai rice[2] whereas protein, chitin and lipids are the main constituents found in rice weevils.[18] So, the difference in the chemical compounds of rice and rice weevils contributes to differences in the NIR absorption spectra. The application of the NIRS technology to estimate the quantity of rice weevils in rice stocks is not only appropriate for determining the number of insects but also helps avoid the overdose of the chemical in fumigation, thus reducing toxic residues on rice. The use of the technology can be further extended to the rice quality control division, especially to rice destined for export. Thus, the objective of this research is to estimate the rice weevil quantity in rice stock with the NIRS technique.

2. Research Methodology

2.1. *Preparation of rice and rice weevil*

The rice samples used throughout the experiment were 100% premium grade Hommali rice which were milled into Hommali rice (MHR) and brown Hommali rice (BHR). The average dimensions of MHR were 2.03, 7.10 and 1.65 mm ($W \times L \times T$) and of BHR were 2.10, 7.44 and 1.70 mm ($W \times L \times T$). The average weights of one grain of MHR and BHR were about 0.019 and 0.021, respectively.

Mature rice weevils had been raised and fed with 13.5% moisture content of Hommali rice in the controlled room with temperature of 28–30°C and with the relative humidity of 55–60% for 45 days. The dimensions of one rice weevil were 1.03, 3.29 and 0.74 mm ($W \times L \times T$) on average and the average weight of one rice weevil was 1.85 mg.

2.2. *Preparation of samples*

The experiments were divided in two parts to estimate the amount of rice weevil in both MHR and BHR categories. In each category experiment, 800 samples were infested with rice weevils for 20 levels, varying from 10 to 200 rice weevils, and the 40 samples were free from infestation, this made 840 samples for measuring in each category. Then rice sample was filled in each sample to make 100 g and the sample was gently shaken 20 times for uniform mixing and each sample was performed for 10 replications on NIRS measuring.

2.3. *Collection of the sample spectra*

The MHR and BHR samples (i.e., 1680 samples) were each divided into two sets by ratio of 4:1 (i.e., calibration set: validation set). As such, the calibration and validation sets of the MHR and BHR samples were each 672 and 168 samples. Multi Purpose Analyzer Fourier transforms near infrared (FT-NIR) Spectrometer (Bruker Corporation, Germany) was then employed to collect the NIR spectra from the samples. The spectra were the NIR absorbance within the wavelength range of 780–2500 nm with 0.5-nm resolution, which is expressed as log $1/R$, where R is the diffused reflectance. A cylinder-shaped rice holder of 8.7 cm in diameter, 9.0 cm in height and 0.5 cm in thickness was used to hold the rice samples for scanning. The base of the container through which light passed was of transparent crystal quartz and the side was of tin-plated metal. The top of the container was an opening to fill and empty the rice samples. A total of 64 spectra of NIR absorbance measured with the spectrometer of each replication were collected and averaged, and the results were then reported on a computer screen. The 10 replications of each sample were averaged again to get the representative spectrum of one sample.

2.4. *Statistical data analysis*

The best suited mathematical spectral treatment method from all methods (i.e., Constant offset elimination, Straight line subtraction, Vector normalization (SNV), Min-Max normalization, Multiplicative scattering correction (MSC), First derivative, Second derivative, First derivative + Straight line subtraction, First derivative + SNV, First derivative + MSC) was selected for pretreatment the NIR raw absorbent spectra. Then, the pretreated spectra and the number of weevils were analyzed with the partial least-squares (PLS) regression of the OPUS 7.0 (Bruker Corporation, Germany) program to establish the prediction model. The optimal number of PLS factors was determined based on the least residual sum of square by the OPUS 7.0 program whereby suitable ranges of wavelengths were automatically identified. The regression coefficients indicate NIR absorption values of protein and carbohydrate in rice; and protein, lipids and chitin in rice weevils which affect the determination of quantity of weevils by means of molecular vibration.

The predictive capacity of the model in regard to the calibration sets of MHR and BHR was expressed in coefficient of determination (R^2_{cal}) and root mean square error of calibration (RMSEC), while that of the model in regard to the validation sets of both rice groups was in R^2_{val}, root mean square error of prediction (RMSEP), ratio of standard deviation of reference data in validation set to standard error of prediction (RPD of 3.0 or higher is required for classification and quality control,[20]), and average of differences between actual value and NIR value (i.e., bias).

3. Results and Discussions

The experiments indicated the absorbent NIR spectra of the noninfested and infested rice with weevils. Figures 3 and 4 show the raw absorbent spectra between the 100% MHR and BHR compared with 200 rice weevils infestation and with the 100% rice weevil respectively. However, significant overlap in individual spectra across all wavelengths indicates that absorbance at one specific wavelength could not be used to estimate rice weevils. The important wavelengths are determined from the regression coefficient plots.

Table 1 shows that in the MHR case the suitable ranges of wavelength for the calibration model for detection of rice weevils in the rice samples are 1060–1840 nm and 2170–2360 nm. The best suited

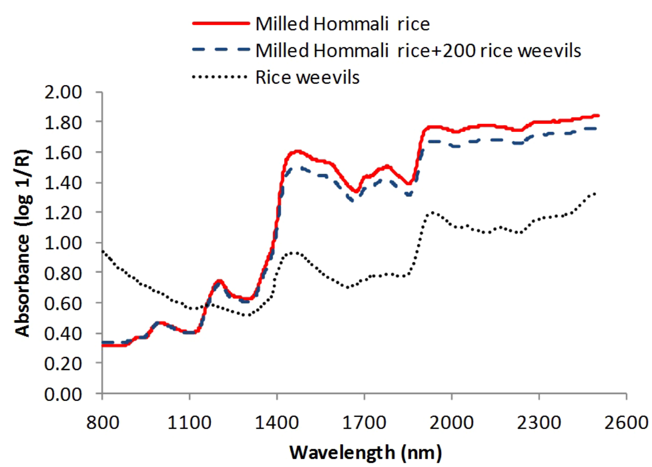

Fig. 3. Raw absorbent spectra of 100% milled Hommali rice, 200 rice weevils infested and 100% rice weevil.

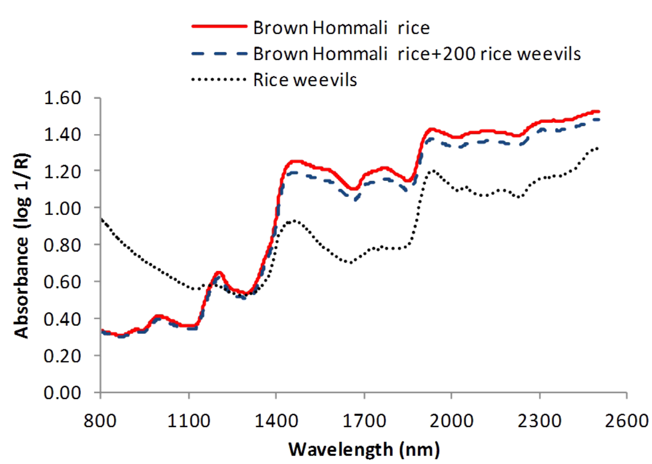

Fig. 4. Raw absorbent spectra of 100% brown Hommali rice, 200 rice weevils infested and 100% rice weevil.

Table 1. The results of PLSR models in estimation of rice weevil populations in milled *Hommali* rice and brown *Hommali* rice.

Type of rice	Wavelength ranges	Pretreatment	PC	R^2_{cal}	R^2_{val}	RMSEC	RMSEP	RPD	Bias
Milled rice	1060–1840 nm 2170–2360 nm	Min-Max normalization	9	0.97	0.96	9.66	10.4	6.07	2.93
Brown rice	1060–1640 nm 1830–2360 nm	Straight line subtraction	7	0.90	0.90	19.6	18.7	3.26	2.94

Note: PC – The optimal number of principal components.
R^2_{cal} – The coefficient of determination of calibration set.
R^2_{val} – The coefficient of determination of validation set.
RMSEC – Root mean square error of calibration.
RMSEP – Root mean square error of prediction.
Bias – The average of difference between actual value and NIR value.
RPD–Ratio of standard deviation of reference data in validation set to standard error of prediction.

mathematical spectral treatment method was Min-Max normalization and the optimal number of PLS factors was 9, which yielded R^2_{cal} and RMSEC of 0.97 and 9.66, respectively. Meanwhile, the results of the validation set showed R^2_{val}, RMSEP, RPD and bias of 0.96, 10.4, 6.07 and 2.93, respectively.

In the case of BHR, the same table shows that the appropriate wavelength ranges for the calibration model to detect rice weevils in the rice samples were 1060–1640 nm and 1830–2360 nm. The suitable number of PLS factors was 7, which yield R^2_{cal} and RMSEC of 0.90 and 19.6, respectively. The results of the validation set showed R^2_{val}, RMSEP, RPD and bias of 0.90, 18.7, 3.26 and 2.94, respectively. The best mathematical spectral pretreatment method for this case was Straight line subtraction.

The correlations between the actual number of rice weevils and the predicted number of rice weevils in the MHR and BHR rice samples of the validation set in PLS models are respectively depicted in Figs. 5 and 6. The results show a significantly positive correlation between the actual number and predicted quantity of rice weevils in both MHR and BHR cases as indicated by high R^2 of 0.96 and 0.90 and slopes of 0.9874 and 0.8863, respectively.

The regression coefficient plots as shown in Figs. 7 and 8 indicate that the important wavelengths for detection of rice weevils in MHR are 1070, 1120, 1210, 1320, 1375, 1410, 1430, 1560, 1730, 1770 and 2310 nm while those in BHR are 1065, 1170, 1220, 1320, 1390, 1440, 1495, 1560, 1640, 1895, 2050, 2230 and 2310 nm. These wavelengths are representations of the chemical bonds of hydrocarbon (C–H), i.e., 1070, 1120, 1170, 1210,

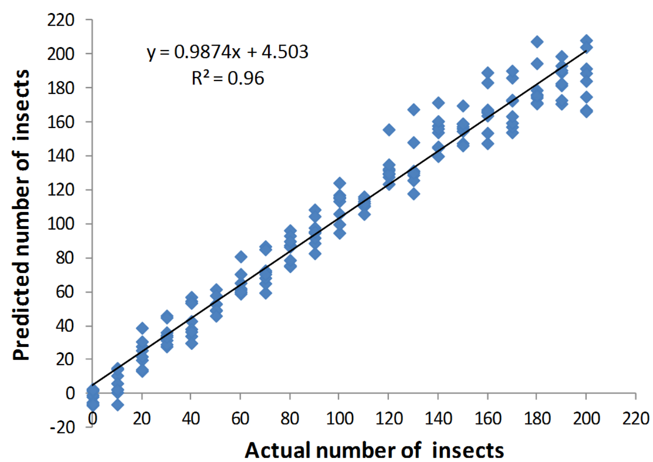

Fig. 5. Scatter plot of actual and predicted population of rice weevils in milled *Hommali* rice samples.

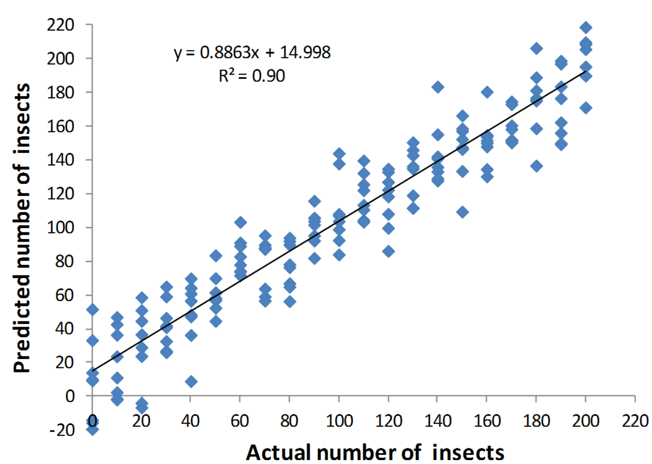

Fig. 6. Scatter plot of actual and predicted population of rice weevils in brown *Hommali* rice samples.

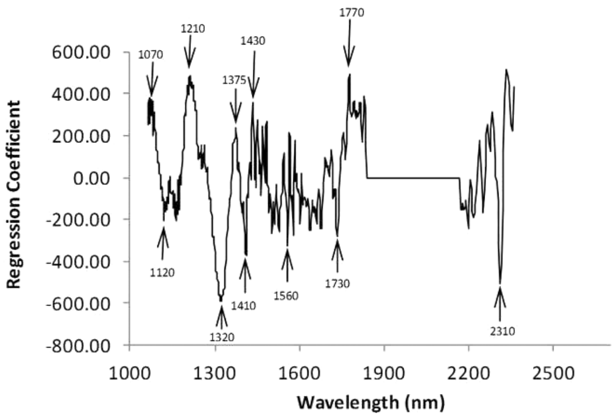

Fig. 7. Regression coefficient plot of milled *Hommali* rice.

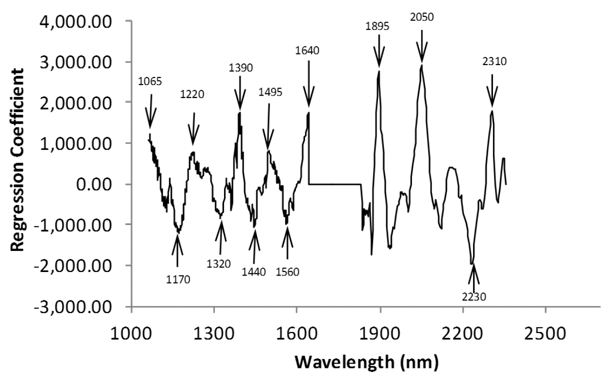

Fig. 8. Regression coefficient plot of brown *Hommali* rice.

1220, 1320, 1375, 1390, 1440, 1495, 1560, 1640, 1770, 1895, 2050 and 2310 nm; of protein and amino acid (N–H), i.e., 1065, 1430, 1495, 1560 and 2230 nm; and of starch and glucose (O–H), i.e., 1410, 1440 and 1895 nm.

A number of researchers[17,18,21–23] applied the NIRS technique to the separation of noninfested wheat kernels from contaminated wheat kernels and inhabiting insects; and also to isolation of insect fragments in wheat flour from the wheat flour. Their research works concluded that the absorbent spectra varied according to the amount of cuticle of insects. Cuticles consist of high amounts of chitin and lipid, both of which are main components of C–H structure.

Chitin, mainly composed of β(1-4) linked units of amino sugar N-acetyl-D-glucosamine, is a polysaccharide found mostly in the carapace of, for instance, insects, crabs, prawns and lobsters. Previous research reported that crab chitin (β(1-4) linked hexasaccharide of 2-acetamido-2-deoxy-D-glucopyranoside) had very high NIR absorption at 1178 and 1500 nm[18,21] both of which were the wavelengths employed in this proposed model.

The cuticular lipids of insects are composed of hydrocarbons, esters, ketones, epoxides, alcohols, fatty acids, sterols and triacylglycerols.[18,24,25] The cuticular lipids of a live rice weevil account for approximately 0.08% of its weight and contain n-alkanes and n-alkadienes.[18,26] The CH_3 and CH_2 groups are common chemical constituents of most lipid classes, particularly the long-chain hydrocarbons. Therefore, the spectra of cuticular lipids of rice weevils are most absorbed in the wavelengths of 1130 and 1670 nm.[17,18] Both wavelengths are also NIR bands of the model in this research work for approximation of rice weevils in the rice samples. The NIRS technique, then, has the potential to be used for estimation of the rice weevil quantity in MHR and BHR stocks.

The established model provides the amount of rice weevil in 100 g of a sample which can calculate the concentration of rice weevil in terms of either mass or volume in rice stock from the physical properties of rice and rice weevil, reported in Sec. 2.1. The obtained concentration, then, can be applied for determining the suitable chemical fumigation in rice stock for reducing the use of toxicity.

4. Conclusion

The application of the NIRS technique to estimate the quantity of rice weevils inhabiting MHR and BHR rice stocks is a novel technique that yields $R^2 \geq 0.90$ and RPD > 3.0 for both rice types. In addition, the NIRS technology offers a faster and more accurate result without destruction of rice products. Although this approach is unable to differentiate between live and dead rice weevils, it could still be employed to reasonably estimate and then control with an appropriate dose of PH_3 the quantity of rice weevils in rice stock. The reduction in PH_3 used not only translates into more savings for the rice millers but also means lowered pesticide residues on rice products and thereby less harm to rice consumers.

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