Original paper

Measurement of Residual Bran Distribution on Milled Rice Using Fluorescence Fingerprint-derived Imaging

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Rice is typically consumed after milling, a process of removing the husk and bran layers on rice surface. The degree of bran residue remaining on milled rice directly affects the rice quality. This work proposed to detect the bran residue on a single rice grain using fluorescence fingerprint-derived imaging nondestructively. In the experiment, combinations of fluorescence excitation and emission wavelengths that could effectively distinguish the bran and endosperm pixels were identified through fluorescence fingerprint (FF) spectroscopy. Fluorescence images of milled rice samples at these wavelengths were acquired. A support vector machine classifier was then developed to predict the bran residue on rice grains using the FF-derived images as the inputs. It was demonstrated that the proposed method could observe the distribution of bran residue and could predict the percentage of bran residue on milled rice grains with an error of 3.54%.

Keywords: excitation emission matrix (EEM), fluorescence fingerprint (FF), sparse linear discriminant analysis (SLDA), support vector machine (SVM), surface lipid content (SLC), degree of milling (DOM)

Introduction

Rice (Oryza sativa L.) is a critical staple food for human population. It is typically consumed after milling, a process that removes the husk and bran layers from rice surface. The degree of milling (DOM) determines the taste and quality of rice. It is crucial to rapidly and nondestructively evaluate the amount of bran residue on rice surface in the field. This work developed an approach to detect the bran residue of a single rice grain using fluorescence fingerprint-derived imaging (FFDI).

Nondestructive approaches have been proposed to rapidly assess the bran residue. Chen et al. (1997) determined the DOM on 3 rice cultivars by using visible and near-infrared spectroscopy. Liu et al. (1998) quantified the percentage of the bran layer residue on a single rice grain based on machine vision and image analysis. Gangidi et al. (2002) evaluated DOM variation by using Fourier transform infrared spectroscopy. Shiddiq et al. (2011) estimated rice bran residue from regular digital images using an adaptive-network-based fuzzy inference system. Chen and Kuo (2014a) demonstrated the use of hyperspectral imaging to measure the bran distribution on milled rice surface.

Fluorescence fingerprint (FF) spectroscopy, also known as excitation-emission matrix spectroscopy, is a technique that simultaneously collects a range of excitation spectra at various emission wavelengths (Bieroza et al., 2012). Combining all the emission and excitation information as an FF makes it possible to observe subtle discrepancies among organic matters (Chen et al., 2003; Zhou et al., 2013). As a highly sensitive and selective inspection approach, FF spectroscopy was applied to discriminate wines of different varieties (Yin et al., 2008), predict the buckwheat flour ratio in noodles (Shibata et al., 2011), determine the chemical changes in olive oil (Tena et al., 2012), and detect the aflatoxin in corn grains (Fujita et al., 2013; Hruska et al., 2014).

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In recent years, FF spectroscopy has been extended to FF imaging, an approach that acquires an FF for each pixel in an image. FF imaging has been applied in the field of food and agriculture to access the starch distribution in dough (Kokawa et al., 2013), visualize the structural changes in soybean seeds during germination (Tsuta et al., 2007), and identify the viable bacteria distribution on pork surface (Nishino et al., 2013).

FF imaging acquires dozens or hundreds of contiguous narrow-band fluorescence images. This could be a drawback for on-line applications because the time and cost for acquiring the abundant images can be considerable. Studies in the past performed principal component analysis (PCA) for wavelength selection (Tsuta et al., 2007) or reduced the wavebands for image acquisition (Kokawa et al., 2011) to limit the number of wavelengths. However, PCA is a post-process technique and does not eliminate the images needed to be acquired. In the other hand, reducing the waveband range blindly may cause the loss of essential information and reduce the estimation accuracy.

In this study, sparse linear discriminant analysis (SLDA; Clemmensen et al., 2011) was applied to select a set of key wavelengths for FF imaging. The FF images at the selected wavelengths, also referred to as FF-derived images, were then acquired and applied to discriminate bran and endosperm pixels on milled rice surface. Hence, the accuracy of the FF imaging can be retained, while the images to be acquired can be reduced.

The specific objectives of this research were to (1) collect FFs on bran and endosperm powders of rice grains, (2) determine a few optimal combinations of excitation wavelengths ($\lambda_{\text{ex}}$) and emission wavelengths ($\lambda_{\text{em}}$) to be used in FFDI, (3) develop an FFDI system and a classifier to identify the bran residue on milled rice surface, (4) and evaluate the performance of the proposed approach by correlating the results with a chemical analysis method.

**Materials and Methods**

**Rice sample preparation** Rice samples of a Japanese cultivar, Tai Keng Number 9, were dried to moisture content of approximately 10% using the procedure and equipment described in the work by Chen (1993). The rice samples were sealed in thick plastic bags and then were stored in an oven at 25°C to maintain their moisture content before further treatment. The dried rice seeds were hulled in a commercial paddy separator (THM-1, Long-Good; Kaohsiung, Taiwan) to remove the husker and to obtain brown rice. The brown rice was further treated to obtain grain samples using a laboratory mill (McGill No. 2, Rapsco; Brookshire, TX, USA). The mill consists of a cutter bar, a 2-kg milling weight, and a lever arm. The weight was placed on the lever arm approximately 20 cm away from the saddle center (Andrews et al., 1992; Pan et al., 2007). The brown rice was milled for 40 s. Approximate 50 g of grains was milled in a batch. Broken kernels were removed from head rice after milling.

**Excitation and emission wavelength selection** Optimal combinations of $\lambda_{\text{ex}}$ and $\lambda_{\text{em}}$ for distinguishing bran and endosperm were determined. In the process, 10 powdered bran and endosperm samples, respectively, were prepared. See Chen and Kuo (2014b) for the details of powdered sample preparation. The FFs of the powdered samples were acquired by using a fluorimeter (FlexStation3, Molecular Device; Sunnyvale, CA, USA). The $\lambda_{\text{ex}}$ ranged from 250 to 450 nm in an increment of 10 nm, and the $\lambda_{\text{em}}$ ranged from 300 to 550 nm in an increment of 10 nm. The ranges of $\lambda_{\text{ex}}$ and $\lambda_{\text{em}}$ were selected because the FFDI system (with a built-in UV mirror module) developed in this study only possessed the capability to provide UV excitation. Note that expanding the ranges of $\lambda_{\text{ex}}$ and $\lambda_{\text{em}}$ to visible light may help in identifying more key wavelengths for bran and endosperm discrimination.

SLDA was applied to select combinations of $\lambda_{\text{ex}}$ and $\lambda_{\text{em}}$ based on the FFs. SLDA is a shrinkage technique that imposes constraints in linear discriminant analysis to achieve attribute sparseness. In the beginning of the SLDA process, all the combinations of $\lambda_{\text{ex}}$ and $\lambda_{\text{em}}$ in the FFs were included in the attribute set. Constraints were then applied to remove the combinations from the attribute set until there was only one combination retained. SLDA was practiced because it reaches sparseness effectively and is computationally efficient. The calculation of SLDA was performed by using MATLAB (The MathWorks; Boston, MA, USA). The selected $\lambda_{\text{ex}}$ and $\lambda_{\text{em}}$ were applied to determine the central wavelengths for the band-pass filters in an FFDI system.

![Fig. 1](image_url). Fluorescence fingerprint-derived imaging system.
**Fluorescence fingerprint-derived imaging system** An FFDI system was developed (Fig. 1). The system mainly comprised a xenon lamp (MAX-303 with built-in UV mirror module, Asahi Spectra; Tokyo, Japan), a quartz light guide, a collimator, a microscope (BXFM, Olympus; Tokyo, Japan), a 2X objective lens (PLN UIS2, Olympus; Tokyo, Japan), a monochrome digital camera (ORCA-Flash2.8, Hamamatsu Photonics; Shizuoka, Japan), 2 sets of filter wheels, narrow-band band-pass filters (XBPA series, Asahi Spectra, Tokyo, Japan), and a mirror module. The FFDI system provided excitation light at specific $\lambda_{\text{ex}}$ and collected images at specific $\lambda_{\text{em}}$ by using the band-pass filters. The mirror module was composed of 3 mirrors (3 x 3 cm each), and was utilized to provide more uniform illumination to the objects being studied (Chen and Kuo, 2014b). The FFDI system was enclosed in a dark box to prevent stray light.

**Rice bran-staining and micrograph image acquisition** A dye-staining procedure was applied to rice grains to enable the bran residue to be observed by using optical microscopy. The bran residue forms a covalent bond with Sudan black B (CAS number 4197-25-5; MP Biomedicals, Aurora, OH, USA) and appears dark blue (Ogawa et al., 2002; Sun, 2008; Wood et al., 2012). In the dye-staining, the milled rice grains were soaked in a solution consisting of 0.3% Sudan black B and 70% ethanol for 10 min. Then the grains were rinsed in 70% ethanol for 30 s. The images of the stained rice grains served as references for evaluating the performance of the bran residue detection by using the FFDI system. The images of the stained rice grains were acquired by using the same microscope of the FFDI system and a digital camera (EOS 450D, Canon; Tokyo, Japan).

Pixels on the rice surface covered with and without bran residue were automatically identified by using image processing algorithms. The process included foreground segmentation by using grabCut algorithm (Rother et al., 2004) and binarization by using Otsu’s thresholding (Otsu, 1979). The details of the algorithm implementation are stated in Chen and Kuo (2013). The outcome binary images were referred to as bran images, in which the pixels covered with bran residue were referred to as bran pixels. By contrast, the pixels that were not covered with bran residue were referred to as endosperm pixels. The bran and endosperm pixels were presented in gray and white, respectively.

**Image registration** An image registration algorithm (Lucas and Kanade, 1981) was applied to transform the FF-derived and bran images of the same rice grain into one coordinate system. This transformation was a necessary preliminary step because the corresponding pixels in the 2 images needed to be identified as the training samples in the subsequent classifier development. During image registration, 6 control points corresponding to common features in the 2 images were manually selected. Spatial transformations were then applied to the bran images to rescale and align them with the FF-derived images.

**Pixel classifier development and performance evaluation** A soft-margin support vector machine (SVM) with a radial basis function kernel (Hsu et al., 2003) was implemented to discriminate bran and endosperm pixels on rice surface. The inputs to the model were fluorescence intensities of the pixels in the FF-derived images. The classifier was developed using LIBSVM (Chang and Lin, 2011). The margin and kernel parameters of the classifier were identified by using grid-search (Chang and Lin, 2011) and 10-fold cross-validation (CV).

The developed classifier was then applied to predict bran pixels on rice surface. The performance of the classifier was evaluated by comparing the predicted bran residue content (BRC) to measured BRC. In this study, the BRC is defined as the percentage of rice surface area covered with bran. The BRC error, defined as the measured BRC subtracted from the predicted BRC, was also calculated.

**Results and Discussion**

**Excitation and emission wavelength selection** Figure 2 display the FFs of the powdered bran and endosperm samples. It can be observed that the fluorescence characteristics of the 2 matters are distinct. The FF of the bran demonstrated 2 fluorescent peaks, whereas the FF of the endosperm presented one peak. The fluorescence intensity of the peak centered at $\lambda_{\text{ex}} = 360$ nm and $\lambda_{\text{em}} = 440$ nm for the bran was stronger than that for the endosperm. The FFs also exhibited scattered lights on the top right corner of Fig. 2. Fluorescence fingerprints of powdered (a) bran and (b) endosperm samples.
the figure (Shibata et al., 2011). The scattered lights were excluded from being used in the subsequent analysis.

SLDA was applied to select optimal combinations of $\lambda_{ex}$ and $\lambda_{em}$ from the FFs. Figure 3 illustrates the SLDA shrinkage process for the 10 most critical excitation and emission conditions. Ten-fold CV was performed to select appropriate number of $\lambda_{ex}/\lambda_{em}$ combinations to be applied to the FFDI system. The results indicated that including 3 combinations in the SLDA model gave no CV error. The 3 most effective $\lambda_{ex}/\lambda_{em}$ combinations for discriminating bran and endosperm were 290/430, 340/430, and 360/440 nm.

**FF-derived image acquisition and image registration** In the FFDI system, a band-pass filter with central wavelength at 436 nm, rather than the 430 and 440 nm derived by SLDA, was utilized to provide emission. The purpose of the replacement was to reduce the cost of the system setup. The full width half magnitude of the band-pass filters is 10 nm; thus, the alternation of the emission filter should affect the experiment result at a minimal level.

Figures 4(a), 4(b), and 4(c) display the FF-derived images of a rice grain collected at $\lambda_{ex}/\lambda_{em}$ of 290/436, 340/436, and 360/436 nm. The FF-derived images were acquired using an exposure time of 1 s and an incident angle of approximately 55°. The images demonstrate that a higher level of fluorescence intensity was observed in the area of bran residue along the lateral surface of the grain. This is consistent to the results of the FF analysis (Fig. 2). Figures 4(d) and 4(e) present the micrograph and bran images of the identical rice grain displayed in Figs. 4(a), 4(b), and 4(c). The bran distribution in the dye-stained image (Fig. 4d) was readily distinguishable. In the subsequent bran image (Fig. 4e), the bran and endosperm pixels were represented in gray and white. It was observed that the pattern of bran distribution on the fluorescence images were similar to the pattern of bran distribution on the bran image. Image registration was performed on the FF-derived and bran images. The red crosses in Fig. 4 indicate selected control points.

**Prediction of rice bran residue** A training dataset composed of 200 bran and endosperm pixels, respectively, was formed for developing the pixel classifier. The training samples were selected evenly from 5 rice grains. The region of interest for the training sample selection was the middle lateral surface of the grains. The superior region (i.e., edge) of the grains was avoided because the excitation light to the region could be nonuniform. During the selection, the bran images were regarded as the references for determining pixel type (e.g., bran or endosperm). The fluorescence intensities in the corresponding FF-derived images were identified. The pixel types and the fluorescence intensities together were collected as the training samples. The developed SVM model was applied to classify pixels on rice surface and to predict the BRC.

Figure 5 illustrates the micrograph, bran, and predicted image of the 5 rice grains. In the predicted images, the bran pixels were presented as black dots and were plotted on the top of the fluorescence image collected at $\lambda_{ex}/\lambda_{em}$ of 290/436 nm. The figure demonstrates that the bran and endosperm pixels can be reasonably distinguished using the proposed approach. However, the model did not effectively identify the bran residue distributed on the superior margin of the grains. Some endosperm pixels in these regions were misclassified as bran pixels. The rice surface is curved. The error may result from the nonuniformity of the excitation illumination provided to the rice surface. Note that the mirror module utilized in this study made an advance in illumination uniformity, as compared with the performance of the FFDI system without the mirror module in a previous study (Chen and Kuo, 2014b). A mirror module design that can achieve better illumination uniformity on curved object surface may further improve the performance of the FFDI system.

Table 1 shows the measured BRC, predicted BRC, and BRC errors of the rice samples. Here the measured BRC of the sample rice grains was calculated based on the bran images in Fig. 5. The BRC ranged from 2.66% to 11.97%. The BRC root mean squared error (RMSE) was 3.54%.
Conclusion

This study proposed a procedure to measure bran residue distributed on the surface of milled rice grains using FFDI. In the work, the FFs of powdered bran and endosperm samples were acquired. Analysis demonstrated that the fluorescence characteristics of the bran and endosperm samples were distinct. There optimal combinations of $\lambda_{ex}/\lambda_{em}$ for distinguishing bran and endosperm were 290/436, 340/436, and 360/436 nm. An SVM classifier was developed to predict bran residue on rice surface using the fluorescence images acquired at the 3 selected wavelength combinations as the inputs. The BRC RMSE was 3.54%. Compared to the traditional dye-staining method, the proposed approach could rapidly and nondestructively identify the bran residue distribution on milled rice surface.

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References


