

# Two-Dimensional Vibration Spectroscopy of Rice Quality and Cooking

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## ABSTRACT

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Rice samples were taken from a study of rice milling properties that affect quality. The spectra of milled and cooked samples were taken in the near-infrared, mid-infrared, and Raman region. These spectra, two regions at a time, were regressed by a two-dimensional technique to develop contour maps that indicated the correlation of two spectral regions. These relationships demonstrate that it is possible to recognize the hydration effects caused by gelatinization (cooked samples vs. milled rice). Three water (O-H stretch) spectral bands (960, 1445, 1,930 nm) in the near-infrared (NIR) show marked differences between milled and cooked rice.

The difference spectra indicated that there were additional phenomena occurring besides the addition of water. These differences are apparent in both C-O-H and N-H bands, which indicate that water is interacting with both starch and protein. The two-dimensional technique developed in this laboratory was used to get a better interpretation of what occurs during cooking. The Raman spectrum, which is relatively insensitive to water (O-H stretch), revealed only changes in protein that could be associated with denaturation.

Over the past several years we have studied the spectral properties of rice by near-infrared (NIR), mid-infrared (MIR), and Raman spectroscopy in an effort to develop predictive models of rice composition and quality. These studies include the effect of postharvest processing conditions and drying regime on taste and texture (Champagne et al 1997, 1998; Windham et al 1997) as well as compositional models to determine quality from laboratory assays (Barton et al 1997; 1998a,b). Rice quality can be measured in the cooked product, however, assays are traditionally conducted on precooked samples. Himmelsbach and Gamble (1996) examined the dynamic cooking of rice by proton magnetic imaging. This was a very costly and time-consuming experiment. It would be useful to acquire similar information from the vibration spectrum that can be acquired in a more timely, cost-effective manner.

Ten years ago, two-dimensional (2D) spectroscopy techniques were developed that allowed the comparison of different spectral regions and correlated the responses (Barton et al 1992). The technique has been used to describe the process of digestion (Barton et al 1992), lignin (Barton and Himmelsbach 1993), carbohydrates (Barton et al 1995), and the difference between hard red winter and hard red spring wheats (Barton et al 1996). The 1st International Symposium on 2D Correlation Spectroscopy was held in Sanda, Japan, in August 1999. The meeting showcased some new techniques for 2D correlation as well as provided a forum for historical perspectives. The technique of cooking rice is steeped in tradition as much as it is in the application of the science of rheological properties. This study looked at the process of gelatinization and water uptake in milled rice using vibrational spectroscopy techniques and correlated the NIR, MIR and Raman regions. Other studies, such as Kawamura et al (1996) and references cited therein, have looked at the quality of cooked rice, but usually from the pasting and rheological properties. This study examines the extent to which vibrational spectra can provide information on cooked rice that can be correlated to compositional parameters.

## MATERIALS AND METHODS

### Rice Samples

Four rice cultivars from the 1994 season including M401 and Koshikahari grown in Arkansas and California, Bengal grown in Arkansas, and Calrose grown in California were harvested and dried as in Champagne et al (1997). All of the samples (120 total) were shelled using a rice machine (model SB, Satake) and then immediately milled. Regular (light) milling was accomplished using a laboratory pearler (model SKD, Satake one pass mill). The first pass was with a 50-g weight in the 5th position; the second pass was with a 50-g weight in the 3rd position. Deep milling was performed on 250-g portions of the regular milled rice using a laboratory grain testing mill (model TM05, Satake). Milling conditions were 1 min at 1,250 rpm using a fine mesh abrasive wheel. Brokeners were removed with appropriate laboratory sizing devices using standard indented plates and cylinders as described in Champagne et al (1996). Apparent amylose content was determined by the method of Juliano (1971) at the Rice Research Unit. Protein (N × 5.95) was determined by the combustion method in New Orleans, LA, and Athens, GA (AOAC 1990).

### Spectroscopic Analyses

Visible/near-infrared spectra were obtained on a scanning monochromator (model 6500, Foss NIRSystems, Silver Spring, MD) in reflectance mode over a wavelength range of 400–2,498 nm. The instrument was operated by a software package (NIRS3, v.3.11, Infrasoft International, Port Matilda, PA) that includes modules for acquisition and processing of spectra. Whole grain milled rice (100 g) and cooked rice samples were scanned in a transport cell as described by Delwiche et al (1996) and Barton et al (1998).

The MIR spectra were obtained on a spectrometer (model 850, Nicolet Analytical Instruments, Madison, WI) interfaced to a PC running Nicolet OMNIC software. The sample's spectra, a total of 128 scans, were taken using a Globar source, a KBr beamsplitter, and an MCT-B detector at 4 cm<sup>-1</sup> resolution. Diffuse reflectance spectra were obtained with a Spectra Tech-Nicolet advanced diffuse reflectance accessory.

Raman spectra were obtained from two subsamples on a spectrometer (model 950 FT-Raman, Nicolet) interfaced to a PC running Nicolet OMNIC (v 4.1) software. A laser light source of 1.064 μm at 500 mW of power was used with a CaF<sub>2</sub> beamsplitter and a Ge detector. The data obtained were the result of 128 scans at a resolution of 16 cm<sup>-1</sup>. Interferograms were processed into the frequency domain with Happ-Genzel apodization. The spectra of subsamples were averaged to give a single spectral file for each sample. No attempt was made to remove fluorescence from the spectra, and only the Stokes region of the spectrum (4,000–200 cm<sup>-1</sup>) was utilized.

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Two-dimensional correlations used two methods 1) the static classical least squares method developed by Barton et al (1992, 1995) and Barton and Himmelsbach (1993, 1996) and 2) the modified dynamic methods of Noda et al (2000) to examine the synchronous and asynchronous behavior of rice gelatinization.

## RESULTS

To better see the differences in the spectral correlations, small files were constructed with a gradient of compositional differences. The correlations are governed by a simple set of two rules. First, the file must contain a gradient of difference and it is best if it is a single gradient to reduce the complexity. Second, the signal to noise (S/N) in both regions is the most important factor. Files were selected from the spectra of the 120 samples to represent one cultivar (Calrose) deep and light milled and from two drying regimes; all four cultivars deep and light milled; constant protein content; and constant amylose content. The range of data in the total file was fairly broad as detailed in Table I. Table II contains the standard error of cross validation (SECV) for NIR and Raman models for protein and amylose. These models yield consistent results over a broad range of optical geometries and two spectral regions. The difference in the magnitude of the SECV values is the precision of the reference data and the S/N difference of the NIR versus Raman spectrometers. The limited data model was obtained by taking only every 16th data point, to leave only 53 data points in the PLS1 model. This model has less spectral data but also less noise, as was shown in the work of Archibald et al (1998) where a technique was used to eliminate areas of the spectra that hurt the model's precision.

The cooking of rice is not just the addition of water. If it were, the difference spectrum of milled minus cooked rice would show two large water bands in the NIR at 1,445 nm (1st overtone O-H stretch) and 1,940–1,960 nm (combination band). Figure 1A contains the spectra of milled and cooked rice, as well as the difference spectrum (cooked minus uncooked milled rice). Figure 1B is analogous to Fig. 1A but it depicts the corresponding Raman spectra. These figures show the real difference between NIR and Raman spectroscopy: that the Raman is very insensitive to water and water effects. The NIR is very sensitive and shows the added water as well as hydration effects of the starch protein complex. The difference spectrum in Fig. 1A displays a separation into at least two bands in the water of the 1st overtone band at 1,445 nm. The 1st overtone of the combination band at 960 and the combination band at 1,930 nm do not show any separation, but the 936 nm band is

shifted to a lower wavelength. These separations and shifts at shorter wavelengths indicate a hydrogen-bonded O-H stretch. In Fig. 1B, the amplitude of the difference spectrum is multiplied by 100 and shows no apparent differences in spectral content to that of either of the rice spectra. The Raman insensitivity to water is clearly evident. However, the Raman is very sensitive to changes in the protein bands at 1,453 and 1,650  $\text{cm}^{-1}$ .

Figure 2 is a contour map of the coefficient of determination ( $r^2$ ) versus the near-infrared spectrum of the set of milled rice samples and the same cooked rice samples. There are many places that show O-H to O-H correlations as one looks across and up the 960 nm line on the map. If water alone accounted for the differences, then only the two major bands (1,930 nm and 1,445 nm) would correlate. However, looking across the 1,930 band on the vertical wavelength axis one sees at least six regions of high correlation, including the 1,445 nm and 1,930 nm bands. There are a number of other ways to view these correlations, notably the method of Barton et al (1992, 1993, 1995, 1996) and the synchronous and asynchronous techniques of Noda et al (2000). The Noda et al (2000) correlation technique employs the Hilbert mathematical transform because it was developed for dynamic cases in which a polymer was deformed over time or temperature. As such, the correlation mag-

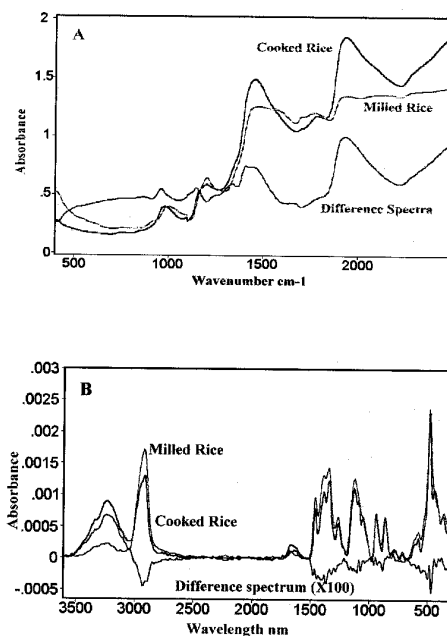


Fig. 1. Near-infrared spectra of milled and cooked rice and the difference spectrum (milled rice – cooked rice) (A). Raman spectra of milled and cooked rice and the difference spectrum (milled rice – cooked rice  $\times$  100) (B).

TABLE I  
Range of Data for Rice Samples<sup>a,b</sup>

Constituent	Range	Mean	Standard Deviation
Amylose	16.40–23.50	19.42	1.51
Protein	3.90–8.30	5.98	0.88

<sup>a</sup> Percentage on a dry matter basis.

<sup>b</sup> Barton et al (1998).

TABLE II  
Standard Error of Cross Validation for Amylose and Protein by Optical Geometry and Spectral Region<sup>a,b</sup>

Optical Geometry and Region	Amylose	Protein
6500 Reflectance, full range	0.52	0.22
6500 Reflectance 1,100–1,700	0.37	0.22
6500 Reflectance limited range	0.11	0.09
6500 Spinning cup, full range	0.55	0.25
6500 Spinning cup, 1,100–1,700	0.43	0.22
6500 Spinning cup, limited range	0.54	0.19
Raman	1.19	0.18

<sup>a</sup> Percentage on a dry matter basis.

<sup>b</sup> Barton et al (1997, 1998).

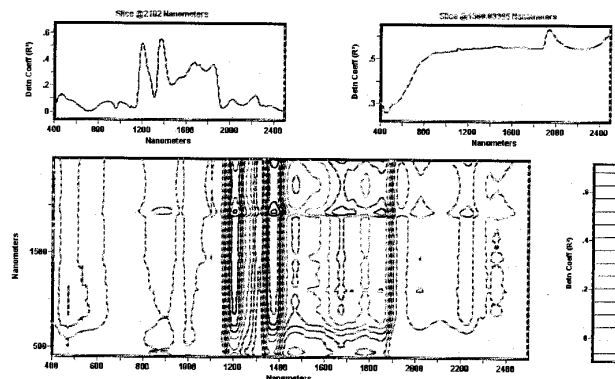


Fig. 2. Near-infrared correlation map of milled rice and cooked rice at constant protein content.

nitudes are quantitative in the same sense as those of Barton et al (1992, 1993, 1995, 1996). A synchronous correlation map was made on the rice cultivar Calrose. There are several places of high correlation in the Raman spectra, notably the amide A and B at 3,200  $\text{cm}^{-1}$ , aliphatic C-H stretch at  $\approx 2,900 \text{ cm}^{-1}$  and the 1,583  $\text{cm}^{-1}$  band for the aromatic ring in aromatic amides. The correlation between C-N stretch at 1,841 nm in the NIR and aromatic ring of aromatic amide at 1,583  $\text{cm}^{-1}$  in the Raman is evident. The Raman is very sensitive to aromatics. Ordinarily one would not expect a correlation at 1,583  $\text{cm}^{-1}$  in the Raman because this is also the usual location for Amide II, which is infrared active but not a Raman active band. The asynchronous correlation map shows something quite different. The 1,841 nm C-N stretch is most highly correlated to aliphatic asymmetrical C-H stretch at 2,917  $\text{cm}^{-1}$  and to C-H-O stretch at 1,142  $\text{cm}^{-1}$ . In both cases, synchronous and asynchronous, the major correlations reflect the removal of the protein coat from the starch by deeper milling.

When a file of six samples is generated with the protein content constant at 6.7% and amylose content at 19.6–23.2%, some different correlations become significant. In Fig. 2, the highest correlations of the cooked rice to milled rice is at 1,370 and 2,102 nm. This is a correlation of C-H-O (amylose) to first overtone aliphatic C-H (lipid) stretch combination and the 1,202 second overtone aliphatic C-H stretch. The next three highest correlations in the milled rice are 1,666 nm first overtone aromatic C-H stretch, the 1,730 nm first overtone C-H stretch, and the 1,960 nm combination O-H (water) band. In the cooked rice, there is a high correlation to water O-H all across the cooked rice (960, 1,445, and 1,960 nm). The Raman correlation map of milled and cooked rice at constant protein content in Fig. 3 has many bands of very high correlation ( $r^2 > 0.8$ ). The highest correlation is between amide A and B at 3,200  $\text{cm}^{-1}$  to the symmetric aliphatic C-H stretch at 2820  $\text{cm}^{-1}$  and a corresponding correlation to aromatic C-H stretch at 3,068  $\text{cm}^{-1}$ .

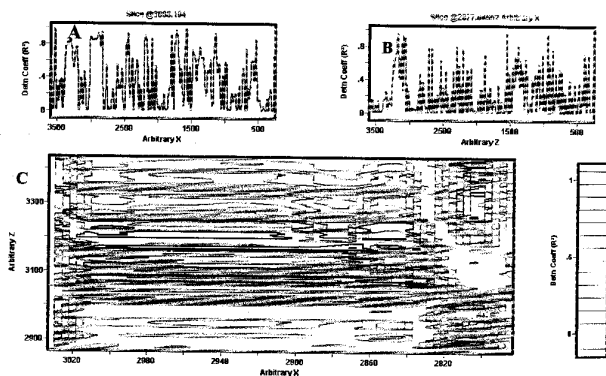


Fig. 3. Raman correlation map of milled rice and cooked rice at constant protein content.

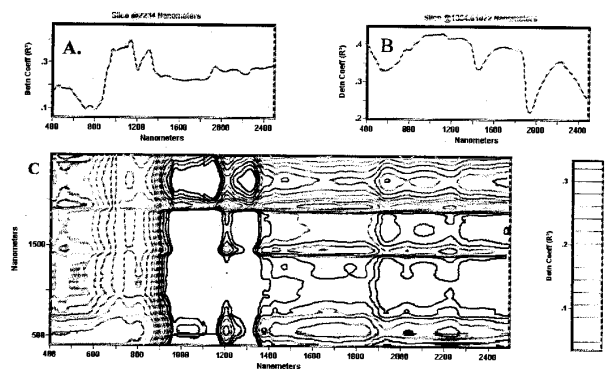


Fig. 4. Near-infrared correlation map of milled rice and cooked rice at constant amylose content.

When a file of six samples at constant amylose content at 21.6% and protein content at 5.7–7.6% is generated, other correlations are significant. The correlations are not as high but do indicate some different properties of the cooked rice (Fig. 4C contour map, ordinate axis). The contour map slice that goes across the milled rice spectrum from 1,445 nm has its highest correlations at 1,202 nm, second overtone C-H stretch (methyl-); 1,666 nm aromatic C-H stretch; 1,715 nm aliphatic C-H stretch; 1,839 nm C-N stretch, and 2,234 nm C-H-O stretch with little or no correlation to O-H stretch. In fact, the slice in Fig. 4B shows a minimum at the water absorption band 1,960. It appears that the absorption of water during the cooking process is more related to lipid content than to carbohydrate.

The contour map of the correlation of NIR to Raman for milled rice is shown in Fig. 5. The slices depicted are for 1,690 nm aliphatic C-H stretch in the NIR to amide A 3,284  $\text{cm}^{-1}$  in the Raman. In addition, the 960 O-H stretch band is also correlated to the Raman amide A. The Raman band's correlations are very narrow compared with the NIR correlations that give the appearance of small dark lines (high  $R^2$ ) among the lower  $R^2$  gray lines. Figure 6 shows Raman correlations of milled and cooked rice. There is only one correlation that stands out in the synchronous map (Fig. 6C), that of amide A and B (3,184 and 3,210  $\text{cm}^{-1}$ ) (Fig. 6A) in milled rice to symmetrical C-H stretch (2,925  $\text{cm}^{-1}$ ) in cooked rice. The synchronous NIR milled and cooked rice in Fig. 7 is one of free water in cooked rice (1,928 nm) correlated to aliphatic C-H, C-H-O and C=O in amide in the milled rice.

The contour map that resulted from a separate set of six samples with a constant protein (4.8%) and a constant amylose content (18.2%) was almost identical to that of the constant amylose alone (Fig. 4). The principal correlation in cooked rice was to some color component at 602 nm in the milled samples. There was a high correlation to each water O-H stretch (740, 960, 1,445, 1,930 nm) in the

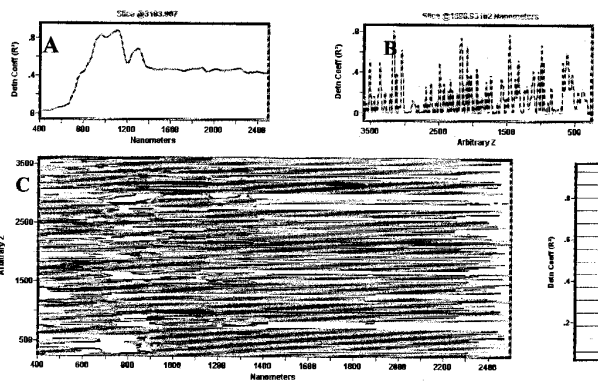


Fig. 5. Near-infrared and Raman correlation contour map of milled rice.

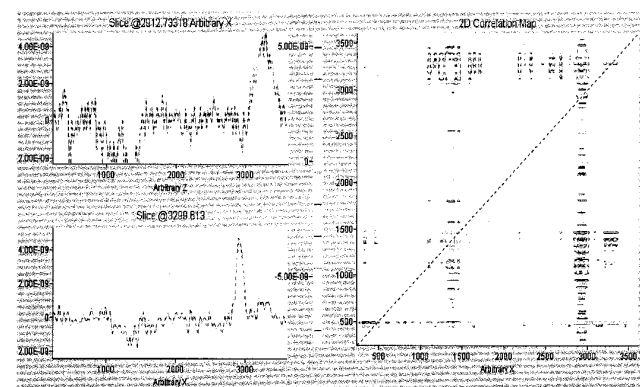


Fig. 6. Synchronous two-dimensional map of Raman spectra of milled and cooked rice.

cooked rice to 602 nm in the milled rice. Milled rice samples are white and there are differences in whiteness. Raman is a scattering technique, and the correlation could be just a change in scattering properties because of gelatinization and not a factor of composition.

The Raman contour map and correlation slices for milled and cooked rice has many correlations that are very high ( $r^2 > 0.9$ ) as in Fig. 3. However, there are more areas of the narrow dark contours with high correlation when the protein content is constant. The principle bands are those for amide A and B at 3,184 and 3,210  $\text{cm}^{-1}$  to C-H stretch (2,850 and 2,925  $\text{cm}^{-1}$ ).

If a file of samples is used in which both protein and amylose are held constant at 4.7 and 18.0 %, respectively, the most obvious correlation is aliphatic C-H stretch (lipid) across both milled and cooked rice and the depression of the correlation to O-H water in the cooked rice at 960, 1,445, and 1,930 nm (Fig. 8). Figure 9 is a contour map and correlation slices with a file of only Koshihikari. Compared with Fig. 2 for all four cultivars, it shows a predominant correlation of C-H-O at 2,274 nm to aliphatic C-H stretch and very few other correlations.

### DISCUSSION

The concept of cooking rice seems very straightforward. It is, however, a very complex process that involves more than just the addition of water and heat. The bands that appear in the difference spectra of Fig. 1 can be found throughout the contour maps and correlation slices of the other figures. The bands are principally water O-H stretch (960, 1,445, 1,930 nm) with some difference in C-H and C-H-O stretch as well. It is very difficult from the parent spectra to discern these minor differences. The 2D techniques highlight and make possible an interpretation of what occurs during

the process. The contour maps and X variable (A) correlation slices in Figs. 2 and 9 are similar for the milled rice and cooked rice file and the Calrose and Koshihikari files. These maps have significant C-H stretch correlation to the O-H water bands, which supports the findings of Chung et al (1975) that the minor lipid component in grains have a large role in rheological properties. The correlations to the protein fraction are different for each file but there are similarities at 2,054 and 1,863 nm.

Figures 6 and 7 point out the differences in the Noda dynamic 2D correlation (2000) and the Barton et al static classical least squares method (1992, 1993, 1995, 1996). The dynamic 2D correlation is designed for a single sample scanned during a perturbation over time. The regression calculations are performed with either a Fourier or Hilbert transform. The synchronous correlation will show autocorrelations on the diagonal and a positive correlation for anything changing the same direction. Conversely, those bands changing in the opposite correlation direction are denoted by a negative correlation. The asynchronous correlation plots shows order of events in that positive correlation is an event that precedes and negative correlation is an event that must follow. The synchronous correlations in Fig. 6 show the amide A and B in the Raman correlated to the aliphatic stretch in the cooked rice (3,299 vs. 2912  $\text{cm}^{-1}$ ). A similar asynchronous plot of Raman versus NIR shows the C-N (1,843 nm) correlation in the NIR to aliphatic asymmetrical stretch in the Raman (2,917  $\text{cm}^{-1}$ ) for cooked rice. The denaturation of protein is occurring with time such that a positive correlation is revealed in both regions, while the interaction of lipid with protein has a definite order, thus one is positive and the other negative.

The classical least squares two dimensional (CLS-2D) works best when there is a clear gradient of difference in the sample set. To simplify the contour maps, small files were prepared that attempted to hold at least one parameter constant. When protein was held constant at  $\approx 6.7\%$ , the predominant features of the contour maps are correlations between amide (N-H and C-N) and aliphatic C-H stretch as seen in Figs. 2 and 3. When the amylose content was held constant, the correlations to water were apparent in the NIR contour map (Fig. 4) of milled and cooked rice. A contour map of Raman and NIR for milled rice with constant amylose content (Fig. 5) indicates water correlation for NIR, only protein and C-H stretch for the Raman. This same trend is present in the dynamic 2D of Noda (2000) maps (Figs. 6 and 7) where the Raman is all protein and C-H stretch and the NIR contains water correlations.

In Fig. 9, the Koshihikari cultivar exhibits correlations that most resemble those in Fig. 4 where amylose content is held constant. In Koshihikari, the amylose content only varies 18–22% and the protein content is as broad as in any of the files. The major point here is that Calrose is an American cultivar that should approximate Koshihikari and its correlations in the NIR for milled and cooked rice are quite different.

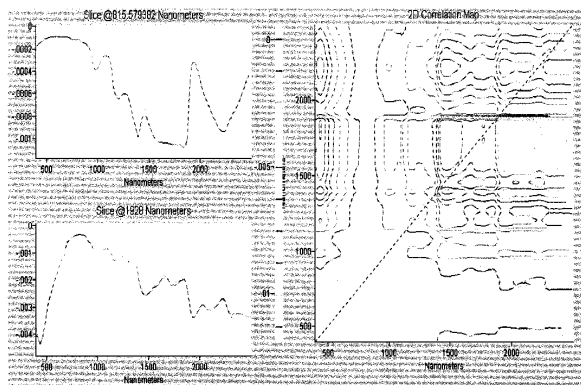


Fig. 7. Synchronous two-dimensional map of near-infrared spectra of milled and cooked rice.

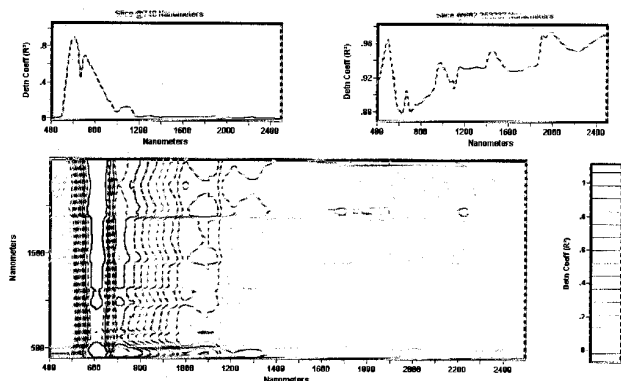


Fig. 8. Near-infrared correlation map of milled rice and cooked rice at constant protein and amylose content.

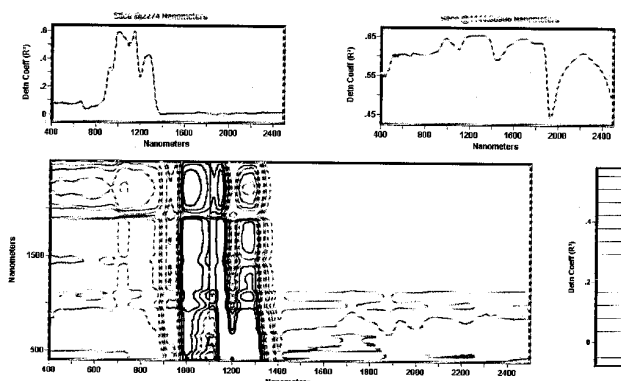


Fig. 9. Near-infrared correlation map of milled and cooked rice for the cultivar Koshihikari.

## CONCLUSIONS

The results of this study show that cooking rice is a complex process that involves protein, amylose (starch), and the very minor lipid content of the rice. Near-infrared spectroscopy is sensitive to the water interactions and Raman spectroscopy is sensitive to protein and starch interactions. The static CLS-2D is a good technique for eliciting the various interactions observed in evaluating rice cultivars and composition parameters. The dynamic 2D process of Noda (2000) is useful in showing the order of the various interactions. Neither the band assignments nor the correlations yield any surprise information. The major conclusion is that, through the use of 2D techniques, we can get a more complete interpretation from the spectra and come closer to being able to determine end use quality than from simple models alone.

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