Nondestructive Evaluation of the Nutritional Status of Pearl Oysters (Pinctada fucata martensii) Using a Portable Near-Infrared Spectrophotometer

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Abstract: The aim of the present study was to evaluate the capability of a portable near-infrared spectrophotometer as a simple and nondestructive method to determine the nutritional constituents (glycogen, protein and moisture) closely related to pearl oyster health. The spectra of intact pearl oysters, their soft tissue, and shells were acquired using commercially available equipment with an interactance fiber optics probe. Since the second-derivative spectra of intact samples in the wavelength region of 730 nm to 1000 nm were similar to those of soft tissue, and the shells did not have any particular absorption in the region, the effect of shells on spectra in this region did not appear very strong. Second-derivative spectra in the near-infrared region and multiple regression analysis were used to develop calibration equations for each constituent of soft tissue. Although the protein and moisture determinations using spectra of intact oysters were not as accurate as measurements using spectra of soft tissue, spectra from intact oysters can be used for screening. The calibration equations for glycogen were not accurate, even for the soft tissue measurements. In conclusion, we suggest that portable near-infrared spectrophotometers are suitable for the nondestructive evaluation of pearl oyster health.

Key words: Pearl oyster; Near-infrared spectroscopy; Moisture; Protein

To produce high-quality pearls, it is important to evaluate the nutritional status of pearl oysters (Pinctada fucata martensii) for breeding, culture and selection of mother oysters (the pearl oysters in which the pearl nuclei will be inserted). Glycogen in the adductor muscle is known as an energy reserve of pearl oysters (Numaguchi 1995; Shinomiya et al. 1999). Also, protein and moisture contents in pearl oyster soft tissue have been analyzed to monitor seasonal changes in nutritional state (Suzuki 1957; Sawada and Taniguchi 1961), and to study environmental effects on the nutritional state of oysters (Seki 1972). Although direct measurement of these constituents is an effective method to evaluate pearl oyster health, the analytical procedures require considerable time and sacrifice the oysters. Therefore, a more rapid, easy and nondestructive method to determine the nutritional constituents of pearl oyster is needed.

Near-infrared (NIR) spectroscopy is an analytical method that can be used to determine the constituents of samples easily, rapidly, and nondestructively. Because of these advantages, NIR spectroscopy has been applied to numerous disciplines, such as agriculture, food technology, medical science and the pharmaceutical industry in Japan (Iwamoto et al. 1995). Takimoto (1998) reported the potential for nondestructive measurement of the glycogen content in the adductor muscle of pearl oyster using a research-type NIR spectrophotometer to gather transmittance spectra. In this report, Takimoto suggested that a hand-held spectrophotometer
for evaluating pearl oysters needed to be developed. In recent years, many portable and/or hand-held NIR instruments, costing far less than research-type instruments, were produced in Japan that can measure the spectra of fruits and vegetables (Saranwong and Kawano 2007). Yamauchi et al. (2006) applied one of these commercially available portable NIR instruments to the nondestructive measurement of fat content in fish (horse mackerel). Thus, we investigated the applicability of portable NIR spectrophotometers for determining nutritional constituents, such as glycogen, protein and moisture, in order to estimate the physical condition of pearl oysters.

Materials and Methods

Sample
Pearl oysters were cultured in Ago Bay, Mie Prefecture, Japan. Sixteen to 28 oysters were collected on 8 different days from January 30 to July 1, 2008. A total of 164 oysters (2-years old) were used for the experiment. In this population, the mean whole weight of each oyster was 43.7 ± 4.4 g, the mean shell length was 62.3 ± 3.2 mm, and the mean shell thickness was 62.3 ± 3.2 mm (mean ± standard deviation). Sea water within the shells was removed using a manual shell opener, the shell surface was cleaned, and surface moisture was blotted before NIR spectral acquisition. The soft tissues were freeze-dried and milled with a coffee mill (MK-61N, Matsushita Electric Industrial, Kadoma, Japan) for quantitative analyses.

Reference analysis for soft tissue of oyster samples
Glycogen was determined by the anthrone method (Fukui 1990). The nitrogen content determined by a C/N analyzer (NC-22F, Sumica Corporation, Tokyo, Japan) was converted to protein content by using a conversion factor of 6.25. Moisture was determined by the weight loss after freeze-drying. The glycogen and protein contents of freeze-dried samples were converted to wet weight-based values using the moisture content.

Spectral acquisition
Spectra of pearl oysters were acquired using a portable NIR spectrophotometer (Fig. 1, K-BA100R, Kubota Corporation, Osaka, Japan) that is marketed for measuring sugar and acid content in fruit. The equipment has an interaction fiber optics probe with a concentric outer ring for the illuminator and an inner portion for the receptor (Kawano 2002). The intact oyster was directly placed on the end of the fiber probe, with the right shell valve downwards, and the spectra were captured (Fig. 1a). The shells were then removed, and the soft tissue was placed on a quartz glass covering the fiber optics probe (Fig. 1b) to record spectra. The spectra of the removed shells were obtained in the same manner as for measurement of intact oysters (Fig. 1a). Each sample was covered with a dark bag in order to minimize the effect of outside light. The samples were held at room temperature (17.0 – 24.4 °C) and scanned from 500 nm to 1010 nm with data collected every 2 nm.

Fig. 1. Near-infrared spectrophotometer and spectral acquisition of (a) an intact pearl oyster and (b) soft tissue.
Data analysis

Two sample sets, a calibration set (84 samples) and a prediction set (80 samples), were selected so that the distributions of each constituent were similarly spread (Table 1). For resolution enhancement and baseline correction (Ozaki et al. 2007), second-derivative spectra of each sample were used for calibration and prediction. When producing the derivatives, segment size and gap size (Kawano et al. 1992) were set to 10 and 0 nm, respectively. The calibration equations for glycogen, protein and moisture for intact oysters and soft tissue spectra measurement were developed by multiple linear regression using the second-derivative values of absorbance data from 780 to 1000 nm, which corresponds to the NIR region, and the constituents of the calibration sets.

The accuracy of the calibration equations was determined by the standard error of the prediction (SEP); a low value of SEP indicates high accuracy. The accuracies of different constituents were compared using the ratio of the standard deviation of the prediction set to the SEP (RPD) and the ratio of the prediction set range to the SEP (RER), which were defined to standardize the SEP; high values for RPD and RER indicate reliable results (Williams, 1987).

Data analysis was conducted using the Vision software package (ver. 3.2, FOSS-NIRSystems Inc., Laurel, Md. USA).

Results

Relationships between soft tissue constituents

Figure 2 shows the relationships between moisture and glycogen or protein content in soft tissue of pearl oysters. Moisture and protein content showed a remarkably high negative correlation ($r = -0.98$, Fig. 2a). Also, moisture showed a significant negative correlation with glycogen ($r = -0.58$, Fig. 2b). The protein and glycogen contents showed a significant positive correlation ($r = 0.55$) (data not shown).

NIR-spectra of pearl oysters

Figure 3 shows second-derivative spectra in the wavelength region of 700 nm – 1000 nm for an intact oyster, its soft tissue, and shells. Since the second derivative transfers peak maxima into minima (Heise and Winzen 2002), absorption peaks have been turned into troughs in

**Table 2.** Results of multiple regression and errors between predicted and actual protein, moisture and glycogen contents in pearl oyster soft tissue as determined by near-infrared spectroscopy using second-derivative spectra

<table>
<thead>
<tr>
<th>Spectral measurement</th>
<th>Constituent</th>
<th>Wavelength used (nm)</th>
<th>$R$</th>
<th>SEC (mg/g)</th>
<th>SEP (mg/g)</th>
<th>Bias (mg/g)</th>
<th>RPD</th>
<th>RER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact pearl oyster</td>
<td>Protein</td>
<td>906, 926, 944, 962, 894</td>
<td>0.72</td>
<td>13.6</td>
<td>13.0</td>
<td>1.8</td>
<td>1.3</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>Moisture</td>
<td>970, 928, 904</td>
<td>0.67</td>
<td>18.3</td>
<td>16.8</td>
<td>-2.9</td>
<td>1.3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>952, 792, 932, 916</td>
<td>0.58</td>
<td>1.9</td>
<td>1.4</td>
<td>0.6</td>
<td>1.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>Protein</td>
<td>906, 926, 884, 938, 916</td>
<td>0.92</td>
<td>7.9</td>
<td>7.9</td>
<td>-0.5</td>
<td>2.2</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>Moisture</td>
<td>970, 928, 910, 938</td>
<td>0.91</td>
<td>10.5</td>
<td>9.5</td>
<td>1.3</td>
<td>2.3</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>Glycogen</td>
<td>950, 792, 880</td>
<td>0.56</td>
<td>1.9</td>
<td>1.4</td>
<td>0.6</td>
<td>1.0</td>
<td>5.5</td>
</tr>
</tbody>
</table>

$R$, Multiple correlation coefficient of determination; SEC, Standard error of calibration; SEP, Bias-corrected standard error of prediction; Bias, Mean difference between the analyzed value by the standard method and predicted value by NIRS; RPD, Ratio of the standard deviation of the prediction set to SEP; RER, Ratio of range in the prediction set to SEP. Calibration equations for intact pearl oyster measurement: Protein (mg g$^{-1}$) = 92.5 - 8504 $A_{906}$ + 6519 $A_{926}$ - 2737 $A_{944}$ + 559 $A_{962}$ + 2176 $A_{894}$, Moisture (mg g$^{-1}$) = 890 - 2533 $A_{970}$ - 6396 $A_{852}$ + 5422 $A_{964}$. Glycogen (mg g$^{-1}$) = 1.68 - 428 $A_{932}$ + 133 $A_{928}$ + 553 $A_{932}$ - 543 $A_{1056}$, where $A_{\lambda}$ is $d^{2}\log(1/R)$ at $\lambda$ (nm).

**Fig. 4.** Actual values of constituents of pearl oyster soft tissue versus values predicted by near-infrared spectroscopy by intact oyster measurement using the calibration equation shown in Table 3, in the prediction sample set. Solid line shows $y = x$.

**Fig. 5.** Actual values of constituents of pearl oyster soft tissue versus values predicted by near-infrared spectroscopy by soft tissue measurement using the calibration equation shown in Table 4, in the prediction sample set. Solid line shows $y = x$.

This figure. The intact oyster and soft tissue had strong absorption peaks at 736, 836 and 966 nm, values that approximately correspond to the reported absorption peaks for water (Bonner and Woolsey 1968). The second-derivative spectra of intact samples in the wavelength region of 730 – 1000 nm were similar to those of soft tissue. The shells did not show any particular absorption in the range of 700 – 1000 nm.

**Regression analysis**

Results of multiple regression analyses and predictions derived from the calibration equations are summarized in Table 2 and Figs. 4 and 5. These equations were selected considering a low standard error of prediction (SEP) and a maximum wavelength number without statistical overfitting (Marten and Naes, 1987).

According to the RPD and RER, the calibration equations for protein and moisture contents showed approximately the same accuracy for both intact oyster measurement and soft tissue measurement. The calibration equations for protein and moisture contents of intact oysters were not as accurate as those for soft tissues. The key wavelengths for calibrating protein and...
moisture, 906 and 970 nm, were in the absorption bands for C-H stretching third overtone and O-H stretching second overtone, respectively (Osborne and Fearn 1986). The calibration equations for glycogen were inaccurate for either intact oyster measurement or soft tissue measurement.

**Discussion**

Since the second-derivative spectra of intact pearl oysters in the NIR region were similar to those of soft tissue (Fig. 3), the effect of shells on the spectra in this region was not very strong. Shells did appear to affect the accuracy of the calibration equations for moisture and protein content of intact oysters to some extent, because measurements that included shells were not as accurate as those for soft tissue measurement (Table 2; Figs. 4, 5). Another reason for this result could be the effect of water in the shells on the spectra, because a little water remained in the shells.

Since Takimoto (1998) did not predict the glycogen content in the adductor muscle of pearl oyster using calibration equations, we cannot compare the accuracies of his calibrations to our results. Saranwong et al. (2003) determined the Brix value of intact mango fruit using a similar portable NIR instrument produced by the same company as that used in our study and reported that the RPD was 3.98. Yamauchi et al. (2006) measured the lipid content of horse mackerel nondestructively using another type of portable NIR spectrophotometer and reported that the SEP was 1.6 %. Since the range of lipid content in the report by Yamauchi et al. (2006) was 0.9-23.7 %, the RER is expected to be 14.3. According to the values for RPD and RER, the results of predictions for each constituent of pearl oyster in the present study, even for the soft tissue measurement (Table 2; RPD = 1.0−2.3; RER = 5.5−9.4), were less accurate than the measurements of Brix value of mango (Saranwong et al. 2003; RPD = 3.98) and lipid content of horse mackerel (Yamauchi et al. 2006; RER = 14.3). Although we cannot make definitive conclusions, the reasons for this result may be the reduced uniformity of constituents in the soft tissue of pearl oysters and/or the ranges of the constituent contents of the pearl oysters were smaller than those for the fat content of horse mackerel (9−237 mg g\(^{-1}\), Yamauchi et al. 2006).

Nevertheless, a portable NIR spectrophotometer can be used for the rough assessment of protein and moisture contents of pearl oysters (Figs. 4 and 5). The key wavelengths for calibrating moisture and protein, 906 and 970 nm, were reasonable because the absorptions of protein and water were reported at approximately these wavelengths, respectively (Osborne and Fearn 1986). The similarities of selected wavelengths for calibration equations for protein and moisture (Table 2) appeared to result from the high negative correlation between moisture and protein contents (Fig. 2). The calibration equations for glycogen were not accurate, even for the soft tissue measurements (Table 2; Fig. 5), probably because of low glycogen concentrations (Table 1).

In conclusion, we suggest that a portable NIR spectrophotometer has the potential for nondestructive evaluation of the nutritional status of pearl oysters. Further work is required to show the applicability of this technique for breeding and cultivating pearl oysters to produce high-quality pearls.

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**References**


携帯型近赤外分光計によるアコヤガイの栄養状態の非破壊評価
藤原孝之・青木秀夫・石川 卓・渥美貴史・西川久代・神谷直明・古丸 明

携帯型近赤外分光計により、アコヤガイの栄養状態と密接に関係する成分（グリコーゲン、タンパク質および水分）を簡易かつ非破壊的に定量する可能性を検討した。インタラクタンス方式の同軸光ファイバープレード導入型の近赤外分光計を用いて、殻付きのアコヤガイ、軟体部および貝殻のスペクトルを測定した。730～1000 nm における殻付きの貝と軟体部の 2 次微分スペクトルは類似しており、また貝殻は特に目立った吸収を示さなかったため、貝殻が殻付きの貝のスペクトルに及ぼす影響は小さいと考えられた。2 次微分スペクトルおよび軟体部の各成分定量値を用いて重回帰分析により検量線を作成した。殻付きの貝の測定によるタンパク質および水分の定量精度は軟体部の測定よりも劣ったが、大まかな選別には使用可能と思われた。グリコーゲンの測定精度は極めて低かった。以上のように、携帯型近赤外分光計によりアコヤガイの栄養状態を生きたまま把握できる可能性が示された。