Rapid non-destructive determination of fat content in frozen skipjack using a portable near infrared spectrophotometer

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ABSTRACT: Non-destructive determinations of the fat content in frozen skipjack by the portable and desktop near infrared (NIR) spectrophotometers were performed. In both spectrophotometers, the RPD value, which is an index of the accuracy, was higher at the abdominal part than at the central part of the body. The RPD value obtained by the portable instrument was higher than that of the desktop instrument when measured at the abdominal part. The rapid determination of the fat content in frozen skipjack was possible by determining the NIR spectra at the abdominal part of the fish body using the portable spectrophotometer.

KEY WORDS: fat content, near infrared spectroscopy, non-destructive determination, portable, skipjack.

INTRODUCTION

There are a few reports on the non-destructive near infrared (NIR) spectroscopy analysis of the fat content of marine products, such as skipjack,1 albacore2 and horse mackerel.3 In all cases, it requires about 1 min to analyze each sample including the reference sample. As a mobile diffraction grating was used in the NIR instrument, the NIR analysis speed was too slow for rapid fat content analysis during the distribution channel of marine products, such as onboard, in a market or in a seafood factory. For the practical application of NIR to determine the fat content, it is necessary to minimize the processing time. In case of its application in agriculture, the brix sorting system based on NIR spectroscopy, having a sorting rate of five fruits per second, has been developed. The sorting data can be used for developing a new cultivation technique of high-brix products.

In the present report, the availability of a portable NIR spectrophotometer, which was developed in the agricultural field, was evaluated for the determination of the fat content in frozen skipjack as a rapid and non-destructive analytical method.

MATERIALS AND METHODS

Materials

Sixty skipjack Euthynnus pelamis that were frozen onboard with different fat contents ranging in weight from 1.0 to 3.8 kg and in fork length from 39 to 54 cm were obtained at the Yaizu Fish Market, Shizuoka Prefecture, Japan. The fish were kept at –45°C until the analysis. After NIR spectral analysis, two portions (P1 and P2) from the body as shown in Fig. 1 were subjected to the chemical extraction analysis.

Near infrared spectroscopy and chemical analysis

Two types of NIR spectrophotometer were used in this study for the analysis of the fat content in the fish. One was a portable-type NIR spectrophotometer (FT 20; Fantec Research Institute, Kosai, Japan) and the other was a desktop type (Model 6500; Foss NIRSystems, Silver Spring, WA, USA). The FT 20, which is equipped with a diode array-type sensor in which the silicone sensor is linearly arranged, can carry out high-speed spectral measurements. A fixed-type diffraction grating is used for this spectroscope. The NIRS 6500 equipped with a mobile type diffraction grating is widely used in...
research. The tip of the optical fiber probe attached to each device is shown in Fig. 2. The accumulation time (exposure time) and scanning frequency in the spectral analysis by the FT 20 were set to 80 ms and 16 times at P1 and 50 ms and 16 times at P2, respectively, in order to equalize the output voltage from the sensor. The scanning frequencies during the analysis by the NIRS 6500 were determined 32 times at P1 and P2 after the measurement of a white ceramic board as a reference. It took 4.8 s for each analysis at P1 and 3.0 s at P2 using the FT 20 except for the reference measurement, and it took 22 s using the NIRS 6500. The tip of the optical fiber probe was attached to the frozen fish body (at P1 or P2), and three analyses were carried out at each position. The measurement range of the spectrum was 600–1000 nm by the FT 20 and 400–1100 nm by the NIRS 6500.

The fat content was determined by a previously described method. Briefly, the crude fat content of portions P1 and P2 (50 × 20 × 10 mm) was chemically determined by extracting with diethyl ether using the Soxhlet apparatus.

**Regression analysis**

A partial least squares (PLS) regression was used for the calibrations with the aid of the software UNSCRAMBLER, Ver. 7.5. (COMO AS, Oslo, Norway). PLS was performed by full cross-validation based on the second derivative spectra and chemical analysis value (Table 1). The spectral data in the 700–962 nm region were used for the analysis.

**RESULTS AND DISCUSSION**

**Near infrared spectra**

The 2nd derivative spectra of P1 and P2 of three skipjacks with different fat contents are shown in Fig. 3. In this figure, the bold lines are the measurements derived from the FT 20, and the fine lines from the NIRS 6500. The spectral patterns measured with both instruments were similar, but there was a difference in the degree of absorbance. The absorption near 926 nm that is characteristic of the fish oil was observed in the spectra using both the FT 20 and NIRS 6500 at P1 and P2 from the high fat content sample (P1, 17.6% and P2, 14.0%) and the medium fat content sample (P1, 10.7% and P2, 8.4%). Comparatively stronger absorption was observed in the P2 spectrum compared to P1 when measured by the FT 20. This result contradicts the chemical analysis, which indicated that the fat content of P2 was slightly lower than that of P1. In other words, the larger peaks by fish oil were observed in the spectra of P2 in which the fat content was comparatively low.
Preparation and evaluation of calibration equation for fat content

The results of the PLS calibration determined by a full cross-validation between the second derivative spectrum and the chemical analysis data are shown in Table 2. The correlation coefficients (R) between the NIR spectrum and chemical analysis data are 0.76–0.94. Both bias-corrected standard error of prediction (SEP) and mean difference between the actual value and the NIR predicted value (Bias) obtained by the full cross-validation are also shown in Table 2. From a comparison of the SEP between the NIRS 6500 and the FT 20, the NIRS 6500 analysis was more accurate at P1, but the FT 20 was better at P2.

### Table 1  Analytical data of the sample sets used for determining fat content (%) in skipjack by near infrared spectroscopy

<table>
<thead>
<tr>
<th>Measured portion</th>
<th>Fat content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 60</td>
</tr>
<tr>
<td>P1</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.9–19.7</td>
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<tr>
<td>Average</td>
<td>9.0</td>
</tr>
<tr>
<td>SD</td>
<td>4.4</td>
</tr>
<tr>
<td>P2</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.8–12.6</td>
</tr>
<tr>
<td>Average</td>
<td>5.6</td>
</tr>
<tr>
<td>SD</td>
<td>2.8</td>
</tr>
</tbody>
</table>

P1, P2, Refer to Fig. 1.
SD, standard deviation.

Fig. 3  Second derivative spectra of three representative frozen skipjack having low, medium and high fat content, respectively, analyzed at P1 and P2.
It was difficult to estimate the measurement accuracy between P1 and P2 by the SEP values. This was because the SEP value varied with the standard deviation of the chemical analysis data. For the case in which the standard deviation is the same between the measurement positions, it is then possible to compare directly the measurement accuracies between the measurement positions by the SEP values. But in the case with different standard deviations, the RPD value, which is the ratio of the standard deviation of the reference data in prediction set to SEP, is available for the comparison of the measurement accuracy. The dimensionless RPD value is not affected by the standard deviation of the measured values. The RPD from 2.5 to 3.0 is an adequate value for rough screening, and beyond 3.0 is a sufficient value for normal screening. The RPD value of each calibration equation is shown in Table 2. It was found that the most accurate data were obtained for the analysis at P2 measured by FT 20, then at P2 by the NIRS 6500. As in the former case, this calibration equation could be useful for rough screening as the RPD value is 2.64.

In both instruments, a more accurate data analysis was obtained at P2 than at P1. One of the reasons for this is that the thickness of the skin at P2 is thinner than at P1. The NIR rays irradiated from the tip of the optical fiber probe reached a broader area in the fish meat at P2 than at P1, and the intensity of the NIR spectrum, which was diffused and reflected in the fish meat and returned to the sensor, was also stronger at P2 than at P1. This coincided with the accumulation time that was shorter at P2 than that at P1 in the spectral analysis with the FT 20. By increasing the intensity of the NIR rays and the area of the diffuse reflection passing through the fish meat, the probabilities of the NIR rays being absorbed by the fish meat components and an intense spectrum being obtained are increased. The inaccuracy of the measurement was caused by the skin thickness, so that a stronger characteristic absorption of fish oil in the spectrum measured at P2 was observed than that measured at P1, although the fat contents of P1 were higher than at P2.

In comparison with the measurement accuracy of both instruments at the P2 position where the high accurate analysis data were obtained, more accurate data were obtained by the FT 20 than by the NIRS 6500. As there are many factors such as the intensity and stability of the illuminant, the resolution of the spectrophotometer, the frequency of the scans, and the setting method of the sample to the instruments, it is not easy to clarify the specific cause of the difference in the measurement accuracy. It has been clarified that both models have a similar equivalent measurement accuracy in the sugar content of mango fruit. Therefore, the difference in accuracy between the instruments may be dependent on their suitability for measuring the samples. In the FT 20, illumination and reception were arranged in a line on the head of the optical fiber probe while the head for

### Table 2

<table>
<thead>
<tr>
<th>Measured portion</th>
<th>System</th>
<th>R</th>
<th>SEC (%)</th>
<th>SEP (%)</th>
<th>Bias (%)</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>FT 20</td>
<td>0.79</td>
<td>2.71</td>
<td>3.42</td>
<td>−0.02</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>NIRS 6500</td>
<td>0.81</td>
<td>2.56</td>
<td>2.89</td>
<td>−0.01</td>
<td>1.53</td>
</tr>
<tr>
<td>P2</td>
<td>FT 20</td>
<td>0.94</td>
<td>0.96</td>
<td>1.06</td>
<td>0.00</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td>NIRS 6500</td>
<td>0.87</td>
<td>1.37</td>
<td>1.58</td>
<td>0.01</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Bias, mean difference between the actual value and the near infrared predicted value; R, multiple correlation coefficient; RPD, ratio of standard deviation of reference data in prediction set to SEP; SEC, standard error of calibration; SEP, bias-corrected standard error of prediction.

**Fig. 4** Relationship between the actual values and the near infrared (NIR) predicted values of the fat content of skipjack \((n = 60)\). NIR measurements by the optical sensor were performed at P2 as shown in Fig. 1.
the NIRS 6500 had a concentric outer ring of illumination and an inner portion of receptor (Fig. 2). The surface of the probe could be completely adhered to the mango fruit due to its flexibility, but the surface of the skipjack was curved and frozen skipjack has no flexibility, so that the NIRS 6500 probe could not be fitted to the skipjack surface. For the FT 20, it was considered that this problem did not occur because the shaft of the illuminant sensor was fitted in the body length direction.

The relationship between the chemical analysis data and NIR spectrum at P2 by the FT 20 is shown in Fig. 4. The measured points were linearly scattered for \( y = x \), which passes through the origin. No breakpoint of the gradient of the scattered measuring points or bias was observed. It was made clear that the fat content of frozen skipjack could be accurately measured (SEP = 1.01%) and rapidly (within 5 s) using the portable FT 20.

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