Determination of Ash Content in Home-grown Wheat Flour in Japan by Near-infrared Diffuse Reflectance Analysis

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Ash content is one of matters of concern in a viewpoint of breeding programme for Japanese home-grown wheat. A near-infrared diffuse reflectance (NIR) technique was applied to determination of ash in 60% extraction flours from 2 varieties of the wheat. A distinct tendency that flour of a finer particle size has a higher ash content was observed in the NIR spectrum, resulting in an unstable property of diffuse reflectance which was affected by mutual interaction of moisture in the flour with its flour particle. As a result of calibrations performed, the most accurate calibration was obtained with a multiple linear regression analysis using second derivative of absorbances at 4 specific wavelengths involving an absorbance at 2344 nm which was assigned to a bran-related substance in the flour. It is possible to determine ash indirectly in connection with an inclusion of the bran-related fraction in the flour. However, prediction of ash in "unknown" wheat flours from different varieties from those used for performing the calibration resulted in serious errors on account of a bias effect between the varieties.

Production of Japanese home-grown wheat had been decreasing until around 1975, however, it has been again increased as a substitutional crop for surplus rice production in these 5 years and is expected to reach 1.2 million tons in a near future. Since the home-grown wheat is inferior to imported one in a viewpoint of milling quality, a new variety having a desirable quality is one of the targets of breeding programme. Ash is an important factor affecting flour quality as well as moisture, protein etc. Since the home-grown wheat contains rather higher ash than the imported one, one of efforts in the breeding programme is directed to screen a variety of lower ash content.

The most well-known procedure of ash determination is to analyze oxidized residues remained after incineration of flour at a high temperature\(^1\). This method is not useful for the purpose of breeding programme in which a great number of samples are necessary to be analyzed at a time because of its time-consuming procedure. Thus a more convenient method has been required to be developed.

NIR technique was successfully applied to determinations of moisture and protein in 60% extraction flour from home-grown wheat\(^2\), but it has been a question for a long time whether this technique has applicability to determination of ash. Since ash content in wheat endosperm is quite low as compared to outer bran layer in general, a part of ash in flour is assumed to be derived from an inclusion of bran fraction\(^3\).

This study was carried out in order to assure a possibility of the NIR technique for determination of ash in 60% extraction flours from 2 varieties of Japanese home-grown wheat in connection with the inclusion of bran-related material into flour.

Experiments

Materials

Wheat samples used were 2 varieties of Japanese home-grown wheat, "Asakaze" (ASA)
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and “Norin No.61” (N61). Each variety was cultivated at 8 different producing areas. All the samples were milled with a Bühler Test Mill into 6 streams consisting of fractions of 3 breaks (1B, 2B and 3B) and 3 middlings (1M, 2M and 3M). Totally 96 samples of which ash contents ranged from 0.27% to 1.03%, depending on the stream where the samples were collected, were used for “calibrations” samples.

To test a performance of calibrations obtained, other 41 samples of 60% extraction flour were used for “unknown” samples. Seventeen samples of them were consisted of flours from different varieties from ASA and N61. The 60% extraction flours were prepared by blending practice of flours from each stream in order of (1B+1M), (2B+2M) and (3B+3M) to the extent of 60% yielding.

Defatted flour samples as well as defatted bran and short bran samples were prepared with a procedure as follows. Samples were defatted in 85% methanol at 80-85°C for 10 hours. After the solvent was removed by filtration, the samples were washed out with methanol. The washing was repeated twice with ethanol and also twice with acetone. The samples were finally dried at a room temperature for 2 days.

**Instrument**

Near-infrared reflectance measurement was made by using a Neotec Research Composition Analyzer (Model 6350) in the wavelength region from 1100 nm to 2500 nm with a single-beam mode. Reflected light from the surface of sample filled in the cell was detected by 4 PbS diodes which were fixed at an angle of 45° against a light beam incident upon the sample.

A reflectance (R) was calculated by comparing a near-infrared energy reflected from the sample with that from a standard reference specially made of a ceramic material. A transformed reflectance (log(1/R)) or OD was recorded in a floppy disk with data of ash content determined by the conventional incinerating method1). The recording was carried out every 2 nm through the scanning region. All the data processing were performed by using a Data General Computer (NOVA III) fixed to the instrument.

**Regression Analysis**

In order to make calibrations, a stepwise multiple linear regression analysis was performed to correlate the data of NIR spectrum to ash content in the same way as reported by KAFFKA et al.4) In addition to ordinary OD, math using second derivative of OD (described hereafter by dOD) was also involved in independent parameters of the regression analysis.

**Results and Discussion**

**Relationship between ash content and particle size of flour**

As shown in Fig. 1, OD becomes larger as ash content in flour decreases. A maximum difference in OD between 2 samples of which ash contents are 0.29% and 1.01%, respec-
tively, was 0.0411 of OD obtained at 1930 nm. This corresponds to 11.8% of OD at 1930 nm for flour containing 1.01% of ash.

As for the absorption at 1930 nm which is assigned to moisture, near-infrared spectrum has the most appreciable influence of the size of flour particle. Because absorption coefficient due to moisture is exceedingly large in comparison to other components, an interaction of flour particle with moisture affects the spectrum most strongly at this wavelength. As mentioned later in detail, correlation coefficients between ash content and OD value at 1930 nm for ASA and N61 were negatively significant. This shows a fact that OD becomes smaller in the samples containing more ash. In other words, flour containing more ash is assumed to have a finer particle size.

Fig. 2 shows distributions of particle size measured by a Coulter Counter method with ASA flour collected from 1B and 3M streams. The particle size can be classified into 3 groups, that is, the fine (6~8μm), the medium (17~22μm) and the coarse (50~60μm), respectively. It is interesting that the former 2 groups are consistently observed in both flours collected from breaks and middlings streams, while the last one is remarkable only in the flour from middlings stream. The fine and the medium particles are supposed to be associated with a fraction of wheat starch without doubt, but not the coarse one5).

According to an observation with a scanning electron microscopy, only the flour from middlings stream contains many pieces of inclusions as shown in Fig. 3. In addition, the flour has a tendency to form a lump easily.

The inclusions are rather larger in length than the size of largest starch granule. They are assumed to contribute to the coarse particle in Fig. 2. From this point of view, it is supposed that a tendency that flour containing a higher ash content has a finer particle size can be also observed even in flours obtained by blending each stream.

In order to corroborate this assumption, correlation coefficients between ash content and OD value at 1930 nm were calculated with blended flours from 3 varieties of imported wheats such as First Canadian White (1CW), Western White (WW) and Northern Spring (NS). The correlation coefficients were −0.36 for 1CW, −0.53 for WW and −0.87 for NS. It is clear that negative correlation coefficients still remain, though the former 2 values are not so high as those of 60% extraction flours as described later. Then, it is necessary to develop a suitable method in order to compensate for a bias in the spectrum which is affected by the specific characteristic concerning the flour particle.

Consideration of absorption at 2345 nm

It was reported that an improvement of accuracy in the ash determination was made by addition of 2345 nm to a series of wavelengths in a filter type of NIR instrument3). As shown in Fig. 4, second derivative spectra demonstrate that OD values around 2345 nm and 1728 nm seem to be related to ash content. According to a spectra-structure correlation reported6), absorptions at these wavelengths can

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Fig. 3 Scanning electron microscopy of homegrown wheat flours from 1B (left) and 3M (right) streams in Bühler Test Mill

Fig. 4 Second derivative NIR spectra of homegrown wheat flours containing different ash contents
be assigned to \(-\text{CH}_2\). It was reported that an absorber at 2345 nm was associated with cellulose in bran included in flour\(^3\). The absorption at 2345 nm clearly disappeared in the spectrum of solvent-extracted or defatted flour as shown in Fig. 5. This absorption is more distinguishable in the spectra of bran and short bran than that of flour itself, and drastically disappeared in the defatted samples as well.

As a result, the absorber at 2345 nm is assumed to be a bran-related material without doubt. However, this is not associated with cellulose but a solvent extractable substance. We assume that it is associated with oil in bran included in flour. This assumption can be supported by a general tendency that there is a highly positive correlation coefficient between ash and oil contents in flour\(^3\).

**Calibrations**

Calibrations performed are summarized in Table 1. The calibrators are classified into 3 groups, depending on sample sets used. The first 2 kinds of calibrators involve inde-

<table>
<thead>
<tr>
<th>Flours</th>
<th>Number of samples used</th>
<th>Math(^{1}) used</th>
<th>Wavelength selected (nm)(^{2})</th>
<th>SEE (%)(^{3})</th>
<th>R(^{4})</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>W 1</td>
<td>W 2</td>
<td>W 3</td>
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<td></td>
<td>1928(^\d)</td>
<td>1910(^\d)</td>
<td>1950(^\d)</td>
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<td>1724(^\m)</td>
<td>1528(^\m)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2306(^\d)</td>
<td>2244(^\d)</td>
<td>1860(^\d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2344(^\m)</td>
<td>2246(^\m)</td>
<td>2306(^\m)</td>
</tr>
<tr>
<td>Norin No. 61</td>
<td>48</td>
<td>OD</td>
<td>1928(^\d)</td>
<td>1722(^\d)</td>
<td>1526(^\d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1928(^\d)</td>
<td>2344(^\m)</td>
<td>2410(^\d)</td>
</tr>
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<td></td>
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<td></td>
<td>1092(^\d)</td>
<td>2308(^\d)</td>
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<td></td>
<td></td>
<td></td>
<td>2344(^\m)</td>
<td>2246(^\m)</td>
<td>2306(^\m)</td>
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<tr>
<td>Combined</td>
<td>95</td>
<td>OD</td>
<td>1930(^\d)</td>
<td>1726(^\d)</td>
<td>1512(^\d)</td>
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<td></td>
<td></td>
<td>1930(^\d)</td>
<td>2345(^\m)</td>
<td>2062(^\d)</td>
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<td></td>
<td></td>
<td></td>
<td>1784(^\d)</td>
<td>2310(^\e)</td>
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<td>2344(^\m)</td>
<td>2246(^\m)</td>
<td>2306(^\m)</td>
</tr>
</tbody>
</table>

1) Math used is the form of independent variables in the regression analysis for calibration; dOD refers to second derivative of OD
2) Wavelengths with "c" and "m" were selected enumeratively by computer and manually, respectively
3) Standard error of estimation
4) Multiple correlation coefficient
pendent sample sets obtained from each variety of ASA or N61, respectively. As for another calibration, all flours were used into a single-combined set of samples. One sample in the 48 ASA samples was deleted from the set of samples when regression analysis was carried out, because a big noise in the spectrum was found.

As the result of a stepwise multiple regression analysis using ordinary OD math for independent parameters, the computer selected 1928 nm or 1930 nm enumeratively as the first important wavelength. This wavelength is well-known as an absorption due to water. Correlation coefficients between OD value at this wavelength and ash content were as high as \(-0.881\) to \(-0.921\). These negatively high correlation coefficients indicate that near-infrared diffuse reflectance becomes less as ash content increases on account of an interaction of flour particle, which is related to ash content, with moisture in the flour.

The wavelength of 1722 nm or 1726 nm selected as the second band is assigned to \(-\text{CH}_2\). The absorption at this wavelength disappeared in defatted flour sample in the same manner as mentioned on 2345 nm. Wavelength of 1512 nm or 1526 nm which is close to \(-\text{NH}\) absorption band\(^7\) was selected as the third wavelength. The intensity of this absorption is weak in the spectrum of flour as well as bran and short bran. Wavelength of 2202 nm or 2204 nm which may be assigned to \(-\text{OH}\) was selected as the fourth wavelength. Standard error of estimation in the multiple regression analysis was gradually reduced with addition of a new wavelength, however, as a result of statistical analysis, it was not significant to add a new wavelength to the fourth one.

Calibrations using \(d^2\text{OD}\) math for independent parameters in the multiple regression analysis showed a fairly improvement. The first wavelengths selected numeratively by the computer were 1782 nm, 2306 nm and 1784 nm, for calibrations with ASA and N61 samples, and with the single-combined samples, respectively. Absolute values of correlation coefficient between \(d^2\text{OD}\) at the respective wavelengths and ash content were as large as 0.948 to 0.957, depending on the calibrations. The correlation coefficient showed positive values at 1782 nm and 1784 nm, and negative one at 2306 nm. According to a characteristic of the second derivative spectrum, the positive correlation coefficient indicates a contrary relationship between \(d^2\text{OD}\) value and ash content and vice versa. The intensity of absorption at 1782 nm is medium in the spectrum. This absorption seems to be more distinguishable for defatted samples. The 2306 nm was assigned to \(-\text{CH}_2\), and its absorption intensity was strong in the spectrum. Wavelengths next to the first one were automatically selected for each calibration, but some wavelengths were common in plural calibrations.

In addition, calibrations were performed analytically by using 2344 nm, which is essentially equivalent to 2345 nm, as the first wavelength in the regression analysis. As discussed above, an absorber causing this absorption is verified to be a bran-related material in the flour. Though standard error of estimation (SEE) slightly increased in the calibrations obtained analytically, the prediction for “unknown” samples was more accurate than that in case of using the calibration obtained enumeratively.

**Prediction**

Table 2 shows results of prediction for ash content in 60% extraction “unknown” flours.

<table>
<thead>
<tr>
<th>Flours</th>
<th>Number of samples used</th>
<th>SEP(%) (^{1})</th>
<th>Bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asakaze</td>
<td>6</td>
<td>0.071</td>
<td>-0.181</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.038</td>
<td>-0.065</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.051</td>
<td>-0.077</td>
</tr>
<tr>
<td>Norin No. 61</td>
<td>18</td>
<td>0.076</td>
<td>-0.207</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.050</td>
<td>-0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.093</td>
<td>-0.067</td>
</tr>
<tr>
<td>Others</td>
<td>17</td>
<td>0.127</td>
<td>-0.191</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.075</td>
<td>-0.097</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.105</td>
<td>-0.118</td>
</tr>
</tbody>
</table>

\(^{1}\) Standard error of prediction
with various calibrations using d^2OD math. The results with calibrations using ordinary DO math are not shown, because bias calculated as a mean difference between predicted and laboratory ash contents was found to be too big.

An improvement in both of standard error of prediction (SEP) and bias was obtained with the calibrations using d^2OD math, particularly, with the calibrations using the d^2OD value at 2344 nm as the first wavelength in place of one obtained enumeratively in the regression analysis. This verified that an addition of the 2344 nm took an important role for improving the accuracy of prediction.

Prediction of ash in wheat flour from different varieties from those used in the calibration resulted in a serious bias. This means that calibrations have less interchangeability in spite of use of the second derivative math.

With a single-combined calibration, the SEP became more noticeable than that with individual calibrations. As shown in Table 2, all the standard errors of prediction which was carried out by means of individual calibrations are almost similar to those reported with calibrations in which the wavelength (2345 nm) was not involved, but they are rather bigger than those with calibrations involving this wavelength5). In conclusion, this study indicates that the NIR technique has possibility of being applied to determination of ash in 60% extraction flour from Japanese home-grown wheat, though further study is still necessary to improve the accuracy.

A preliminary study suggested that we should consider more carefully on the problem of multiple scattering effect due to an interaction of flour particle with moisture in flour. It is assumed that scattering coefficients among the samples used exceeded the limit of a constant value since they had a wide range of ash content. Because diffuse reflectance is quite sensitive to flour particle size which is significantly related to ash content, even a little difference as much as 1% to 2% in a mean value of moisture content between calibrating and predicting samples may strongly interfere with the NIR spectrum at the absorption band due to water especially, causing a serious error for the prediction.

REFERENCES


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