Beer is an alcoholic beverage that is quite popular, and consumed in large quantities. Furthermore, the market is now bustling with beverages such as non-alcoholic beer and low-malt beer with beer-like flavors. Beer, low-malt beer, and non-alcoholic beer can all be considered types of beer. A great many varieties of these have been produced and marketed with modifications to the ingredients to adjust such characteristics as alcohol and calorie content. From the standpoint of spectrometric analysis—assuming that the absorption spectra should reflect specific characteristics depending on the types and quantities of ingredients in different beers—we were very interested to see the kinds of differences that would occur upon actual examination.

Here, we introduce the results of our investigation into the differences in absorption spectra obtained from measurement of a variety of beers using the UV-3600 Ultraviolet-Visible Near-Infrared Spectrophotometer. Also presented here is our attempt to classify different types of beer using multivariate analysis.

### Samples and Measurement Results

The absorption spectra of 14 types of commercially available beers (4 types of beer, 6 types of low-malt beer, and 4 types of non-alcoholic beer) were measured using the UV-3600. Degassing was conducted using ultrasonic irradiation for 3 minutes, and measurement was conducted using a 2 mm optical path length quartz cell and a blank sample consisting of air. The measurement results are shown in Fig. 1, and the analytical conditions in Table 1. In addition, expanded spectra of the ultraviolet region (230 – 400 nm) and the near-infrared region (1400 – 1500 nm and 1650 – 1750 nm) are shown in Figs. 2 through 4, respectively. The large peak in the vicinity of 1450 nm in Fig. 3 is due mainly to the absorption of water, while the peak in the vicinity of 1695 nm in Fig. 4 is attributed mainly to ethanol absorption. For reference, the absorption spectra of water and ethanol (99.5 %) are shown in Fig. 5. It is clear that the peak indicated by the arrow near 1450 nm corresponds to the peaks in Fig. 3, and that the peak indicated by the arrow near 1695 nm corresponds to the peaks in Fig. 4.

Due to the apparent signal saturation in the ultraviolet region of this data, the absorption spectra were measured in the ultraviolet region (230 – 400 nm) once again after diluting all of the samples 5-fold with distilled water. Those results are shown in Fig. 6. The peaks that can be seen in the 230 – 300 nm region are believed to be due mainly to absorption of the protein contained in beers.

### Table 1  Analytical Conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Shimadzu UV-3600 Ultraviolet-Visible Near-Infrared Spectrophotometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement wavelength range</td>
<td>230 nm – 1900 nm</td>
</tr>
<tr>
<td>Scan speed</td>
<td>Medium</td>
</tr>
<tr>
<td>Sampling pitch</td>
<td>1.0 nm</td>
</tr>
<tr>
<td>Photometric value</td>
<td>Absorbance</td>
</tr>
<tr>
<td>Slit width</td>
<td>3 nm</td>
</tr>
<tr>
<td>Detector switching wavelengths</td>
<td>870 nm, 1650 nm</td>
</tr>
</tbody>
</table>
Fig. 2 Expanded Spectra of Fig. 1 (230 – 400 nm)
(Thick Line: Beers; Thin Line: Low-Malt Beers;
Dotted Line: Non-Alcoholic Beers)

Fig. 3 Expanded Spectra of Fig. 1 (1400 – 1500 nm)
(Thick Line: Beers; Thin Line: Low-Malt Beers;
Dotted Line: Non-Alcoholic Beers)

Fig. 4 Expanded Spectra of Fig. 1 (1650 – 1750 nm)
(Thick Line: Beers; Thin Line: Low-Malt Beers;
Dotted Line: Non-Alcoholic Beers)

Fig. 5 Absorption Spectra of Water and Ethanol
(Blue Line: Water; Red Line: Ethanol)

Fig. 6 Absorption Spectra of Samples Diluted 1:5
(Thick Line: Beers; Thin Line: Low-Malt Beers;
Dotted Line: Non-Alcoholic Beers)
We attempted to classify the beers using multivariate analysis. Using the 5-fold dilution absorbance data (230 – 400 nm) and the undiluted sample absorbance data (401 – 1870 nm), we conducted principal component analysis (PCA)\(^1\). The obtained score plot\(^2\) is shown in Fig. 7. “A” corresponds to beer, “B” to low-malt beer, and “C” to non-alcoholic beer. The samples of A, B, and C are clearly grouped accordingly. The closer the points are to each other on the score plot, the greater the corresponding samples should resemble one another. Accordingly, A1 and A3, and C1 and C2 should be similar to each other, and in fact, as can be seen from their respective ultraviolet spectra shown in Fig. 8, they are similar.

Looking at the loading plot\(^3\) shown in Fig. 9 reveals the characteristics of the various groups. As shown in Fig. 9, loading vector components\(^3\) corresponding to the data components of the ultraviolet region are plotted to the right (or upper right) of the center. This indicates that the further to the right a sample is plotted in Fig. 7, the greater its ultraviolet absorption will be. The beer samples A1 – A4, which are actually distributed in that direction, display high ultraviolet absorbance as shown in Fig. 6. Also, in the loading plot of Fig. 9 there are many loading vector components plotted in the upper left quadrant from 1400 – 1480 nm, which corresponds to the absorption of water. This means that the further to the upper left a sample is plotted on the score plot, the closer that sample is to pure water, and the lower the alcohol content. The non-alcoholic beer samples C1 – C4, which are actually distributed in that direction, display high absorbance in the vicinity of 1450 nm, the wavelength associated with water absorption, as shown in Fig. 3.

From the above, it is clear that the further to the right the samples are plotted on the score plot, the greater their ultraviolet absorbance values will be. Correspondingly, the further to the upper left the samples are plotted, the lower their alcohol content will be. Put another way, the further to the right a sample is plotted on the score plot, the greater the amount of organic matter (e.g., protein) it will contain; the further to the upper left a sample is plotted, the lower its alcohol content will be. As for the low-malt beers B1 – B6, their positions in the lower left region are probably due to the fact that their absorbance values are not that high in the ultraviolet region (comparable to those of non-alcoholic beer), while several of them have alcohol content comparable to that of beer.
Summary
We were able to confirm the possibility in this investigation of determining the differences in alcohol and protein content in beers by examining their absorption spectra. Further, by applying multivariate analysis to the acquired measurement data, we were able to classify the groups according to the type of beer, and thereby gain an understanding of their characteristics. Comparative investigation of many products is required in the research and development of food products, but an understanding of the degree of similarity among samples is possible using principal component analysis (PCA). The results obtained in this study suggest that a combination of spectral analysis and multivariate analysis can be effective in the development of food products, including beer.

1) The Unscrambler®, a multivariate analysis software application, was used for conducting analysis. The Unscrambler is a trademark or registered trademark of CAMO. Regarding the present analysis, principal component analysis was conducted using mean centering of the acquired data.
2) A score plot involves the projection of each sample point expressed in multidimensional space on two loading vectors, expressed as a two-dimensional graph. For a description of “loading vector,” refer to the following Note 3.
3) A loading plot refers to the plotting via two-dimensional coordinates of substances corresponding to the loading vectors of the first principal component and second principal component (or another combination of principal components). Here, the loading vector refers to the vector obtained by performing eigenvalue calculation for data matrix.