Spectral Features Characterizing Rice Wine “Sake” Variety Using Mid-Infrared Spectroscopy

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We developed an infrared spectroscopic evaluation method of Sake (rice wine), whose quality and taste highly depend on the chemical contents, the interactions between the components, and the pH value, using a Fourier transform infrared (FT-IR) spectrometer equipped with an attenuated total reflection (ATR) accessory. The objective of this study is to understand the spectral features of Sake and the main components because the component balances of the amino and organic acids originating from white rice as the raw material and being produced during brewing processes could closely relate to the Sake characteristics. The spectral features characterizing the Sake variety were mainly observed in four regions on the mid-infrared (MIR) spectra. The Sake varieties was able to be distinguished as a difference in the spectrum pattern by considering the pH dependence of the amino and other organic acids based on the ionic dissociation equilibrium theory. Furthermore, the Sake, which has almost a similar characteristic, such as the polishing ratio of the rice as the raw material is different by only 5% or the Sake product before and after it matured, could be identified on the spectral data. Consequently, the infrared spectral information analysis would be acceptable as a new method to evaluate a profile of Sake for the quality evaluation relating to the taste and the non-destructive on-line monitoring of the brewing process.

Keywords: Sake, Mid infrared spectrum, FT-IR/ATR method, ionic dissociative component, ionic dissociation equilibrium theory

1. Introduction

Recently, the quality evaluation and control has been important for almost all systems, especially for the food manufacturing process. However, it is difficult to construct a quality model for the quality evaluation of food. On the other hand, exporting the high-quality agricultural products and its processing foodstuffs is one of the important tools for the Japan’s growth strategy. Rice wine or “Sake” is one of the traditional fermentation foods in Japan and has characteristic flavors and tastes. The manufacturing process has many steps and the process control requires the experience of experts [1]. Especially, the brewing process of Sake simultaneously involves two enzymatic reactions, i.e., the dissolution/saccharification of starch in the rice as the raw material and ethanol fermentation of the glucose. As a result, many variations of Sake are manufactured. The quality and taste of Sake highly depend on the chemical components such as alcohol, sugar and organic acids. Therefore, various wet chemical methods, such as high-performance liquid chromatography (HPLC), are used for the qualitative or quantitative determination of the chemical components of alcoholic beverages [2, 3]. However, the wet chemical methods are in time consuming, waste generating, and expensive procedures even if these wet chemical methods have a high accuracy. In addition, the Sake quality may not be evaluated only with the quantitative value of the components measured by the wet chemical analysis because the characteristic of Sake is formed under the condition of mixture with various components [4]. Hence, the development of rapid and pretreatment-free methods is required when the quality evaluation during the processing and high numbers of samples have to be checked.

The spectroscopic methods, such as Near-infrared (NIR) and Mid-infrared (MIR) spectroscopy techniques, have a great potential for the quality evaluation in the food and agriculture industries in recent years because
they are rapid, not expensive and "as-it-is" methods [5–12]. In addition, the information about all the organic components in a food is theoretically contained in the absorption of the infrared spectrum. The quantitative evaluation of the main components and the interaction of the organic components in the food could be analyzed using the spectroscopic information. However, the spectral analysis regarding the food quality is very difficult because the spectral feature is a complicated change based on the component balance of the organic components and on their conditions such as the pH value. Therefore, some chemometric techniques, such as the principal component analysis (PCA) and partial least squares (PLS), are used to extract the spectral features and to develop the calibration equation for the quantitative analysis of the target components [5–9]. However, information which is explained by the calculated principal components (PCs) using the chemometrics models is not clear because the PCA is used to reduce the dimensionality and because the calculated PC, which is the maximized distribution of the data, is not always based on the target component. If the PC can be better calculated based on the target component information, the chemometric technique is one of the powerful tools to extract useful information from the complex spectral features [13]. Therefore, a theoretical analysis of the spectral feature changes is very important to create a useful chemometrics method for evaluations of the food quality. In addition, the spectral pattern of each food would be applicable as the spectroscopic fingerprint data for identification of the kind of the food [6].

The objective of this study is to understand the spectral features of Sake and the main components because the component balances of the amino and organic acids originating from white rice as the raw material and being produced during brewing processes could closely relate to the Sake characteristics. At first, we investigated the spectral feature of thirty brands of Sake which have various qualities and tastes. To determine the spectrum region suitable for spectroscopically understanding of the Sake variety, the Sake samples were classified based on the differences in the polishing ratio of the rice as the raw material, specific classes of Sake, Sake meter value (SMV) called "Nihonsyudo" in Japanese, or the amino acid values (AAV). Next, to confirm and explain the variety of the spectral features of the Sake, we focused on the sugar, alcohol, and several acids that determine the quality and taste of the Sake. The spectral pattern of the amino and organic acids has a pH dependence, therefore, we extracted the molar spectra of the ionic dissociation species of amino acids (glutaminic acid and glycine) and the other organic acids (citric, acetic, lactic, succinic and malic acids) based on the ionic dissociation equilibrium theory [14–16]. After then, we reconstructed the spectrum of the Sake model by a calculation in pH range of actual Sake products. Finally, we tried the identification based on the Sake spectra when their characteristics are very similar by considering the distribution range of the spectrum which depended on pH values of the Sake model.

2. Materials and Method

2.1 Samples

Thirty brands of Sakes manufactured and sold by 13 manufacturers in the Mie prefecture, Japan, were used as the commercial products of the Sake samples. The specific classes of the purchased Sakes were "Junmai Daiginjo (n=4)", "Junmai Ginyo (n=12)", "Junmai (n=9)", "Special Junmai (n=2)", and "Honjozo (n=3)”. The basic properties of the Sakes are shown in Table 1. The average, and the minimum and maximum pH values were 4.44 (SD: 0.22), 3.90 and 4.80, respectively.

2.2 FT-IR spectroscopy

The MIR spectra of the samples were measured using a FT-IR spectrometer (Nicolet Magna-IR 750, Thermo Fisher Scientific Inc., USA) equipped with an attenuated total reflectance (ATR) accessory (DuraSampleIR; SensIR Technologies, USA). The instrument was allowed to purge for several minutes prior to acquisition of the spectra in order to minimize the spectral contribution due to atmospheric water vapor. Sixty–four scans of symmetrical interferograms at a 4 cm⁻¹ resolution were added up for each spectrum. To eliminate the baseline shift in the spectra and to separate the overlapping peaks and shoulders, the second derivatives of the spectra were calculated using the Savitzky–Goray method with seventeen points. In order to obtain the spectral characteristics of the Sake component, the ethanol and water spectra were subtracted from the Sake ones.

2.3 Extraction of the molar spectra of the ionic dissociation species

We assumed that the spectrum of the ionic species could not be affected by the hydrogen ion concentration and that the spectral additivity for the ionic species was satisfied. The second derivative spectrum could be
decomposed by the second derivative spectra of the ionic species [14–16].

\[
\frac{d^2 Abs(\nu, pH)}{d\nu^2} = \sum \frac{d^2 Abs_i(\nu)}{d\nu^2} C_i (\text{pH, pK}_i)
\]

(1)

where \( d^2 Abs(\nu, pH)/d\nu^2 \) is the second derivation of the absorbance of the ionic dissociative material such as the glutaminic acid. \( \nu \) and \( i \), respectively, represent the wavenumber and ionic charge. \( d^2 Abs_i(\nu)/d\nu^2 \) denotes the second derivative of the molar absorbance of the ionic species, \( C_i (\text{pH, pK}_i) \) is the molar concentration of the ionic species, and \( \text{pK} \) is the ionic dissociation constant. According to the ionic dissociation equilibrium, \( C_i (\text{pH, pK}_i) \) can then be expressed as a function of the pH, and the concentration of the dissociative material with the parameters of the dissociation constants. In order to extract the molar second derivative spectra of the ionic dissociation species for each reagent from those of the dissociative materials in the aqueous solutions, we performed multiple linear regression (MLR) analysis between the second derivative spectrum of the absorbance \( d^2 Abs (\nu, pH)/d\nu^2 \) at each wavenumber and the concentration of the dissociative species, \( C_i (\text{pH, pK}_i) \) [14–16].

3. Results and Discussion

3.1 Spectral variation of Sake variety observed on MIR spectra

Figure 1 shows the raw, second derivative, and subtracted second derivative MIR spectra of the thirty brands of Sake which were obtained after spectral subtraction of the ethanol aqueous solution. Average spectra of each specific type of the Sake were displayed in the Fig.1. The two large peaks observed at around 1600 and 3300 cm\(^{-1}\) on the raw spectrum are due to the absorption of water. The two peaks observed at around 1045 cm\(^{-1}\) are due to the absorption of ethanol. In the fingerprint region from 1300 to 900 cm\(^{-1}\), many peaks were observed, such as the CO and C–OH stretching modes, which overlapped each other [17–19]. To eliminate the baseline shift in the spectra and to separate the overlap-

| ID NO. | Specific class  | Polishing ratio [%] | Ethanol Content [g/dm\(^3\)] | SMV value [-] | Acidity | Amino Acid Value | pH | Brix% | Manufacturer |
|--------|-----------------|---------------------|-------------------------------|--------------|--------|-----------------|----|-------|--------------|
| 1 ”Junmai Ginjo” | 60 | 118.0 | -5 | - | - | - | 4.70 | 11.2 | A |
| 2 ”Junmai Ginjo” | 60 | 121.0 | -5 | 1.5 | 1.8 | 4.54 | 10.4 | A |
| 3 ”Junmai Ginjo” | 60 | 118.8 | -5 | 1.5 | 1.7 | 4.67 | 10.6 | A |
| 4 ”Junmai” | 60 | 124.1 | -5 | 1.6 | 1.3 | 4.49 | 11.0 | B |
| 5 ”Honjozo” | 60 | 123.1 | -2 | 1.4 | 1.5 | 4.60 | 11.0 | C |
| 6 ”Honjozo” | 60 | 128.2 | 0 | 1.4 | 1.4 | 4.46 | 11.5 | C |
| 7 ”Junmai Daiginjo” | 50 | 124.1 | 0 | 1.6 | 0.9 | 4.34 | 11.0 | B |
| 8 ”Special Junmai” | 60 | 130.5 | 1 | 1.7 | 1.4 | 4.55 | 11.9 | D |
| 9 ”Honjozo” | 60–65 | 121.1 | 1 | 1.3 | - | 4.61 | 10.9 | E |
| 10 ”Junmai” | 60 | 123.7 | 1 | 1.4 | 1.1 | 4.38 | 10.7 | F |
| 11 ”Junmai Ginjo” | 60 | 139.7 | 2 | 1.6 | 1.1 | 4.53 | 12.3 | G |
| 12 ”Junmai Ginjo” | 60 | 125.1 | 2 | 1.6 | 1.1 | 4.34 | 11.1 | G |
| 13 ”Junmai” | 60 | 125.1 | 2 | 1.4 | - | 4.49 | 11.0 | H |
| 14 ”Junmai” | 60 | 131.5 | 2 | 1.8 | 1.0 | 4.17 | 11.4 | I |
| 15 ”Junmai Ginjo” | 60 | 139.9 | 2 | 1.6 | 1.5 | 4.40 | 12.5 | G |
| 16 ”Junmai” | 60 | 118.9 | 2 | 1.4 | 0.8 | 4.26 | 10.4 | F |
| 17 ”Junmai” | 60 | 142.9 | 3 | 1.3 | 1.6 | 4.80 | 12.5 | J |
| 18 ”Junmai” | 60 | 138.6 | 3 | 1.3 | 1.4 | 4.67 | 12.4 | J |
| 19 ”Junmai” | 60 | 127.9 | 3 | 1.5 | 1.4 | 4.56 | 11.3 | H |
| 20 ”Junmai” | 60 | 124.4 | 3 | 1.6 | 1.2 | 4.07 | 10.9 | I |
| 21 ”Junmai Daiginjo” | 50 | 123.8 | 3.5 | 1.6 | 1.1 | 4.45 | 10.3 | K |
| 22 ”Junmai Daiginjo” | 50 | 124.0 | 4 | 1.5 | - | 4.68 | 10.3 | K |
| 23 ”Junmai Ginjo” | 55 | 138.2 | 4 | 1.4 | 1.2 | 4.53 | 11.9 | L |
| 24 ”Special Junmai” | 60 | 126.9 | 5 | 1.9 | 2.4 | 4.51 | 11.0 | E |
| 25 ”Junmai Ginjo” | 65 | 123.7 | 5 | 1.8 | 1.2 | 4.60 | 10.5 | K |
| 26 ”Junmai Ginjo” | 60 | 123.0 | 5 | 1.8 | - | 4.19 | 10.1 | M |
| 27 ”Junmai Ginjo” | 60 | 139.8 | 5 | 2.0 | 1.5 | 4.37 | 11.4 | M |
| 28 ”Junmai Ginjo” | 55 | 121.8 | - | 1.4 | 0.8 | 4.16 | 10.4 | F |
| 29 ”Junmai Ginjo” | 55 | 124.9 | - | - | - | 4.03 | 11.0 | D |
| 30 ”Junmai Daiginjo” | 50 | 117.8 | - | - | - | 3.90 | 10.4 | D |
ping peaks and shoulders, the second derivatives of the spectra were calculated (Fig. 1b). The Sake spectrum seems to be overlapped by the spectrum of the ethanol aqueous solution because ethanol is the major component of Sake. Therefore, we subtracted the ethanol aqueous information from the Sake spectra (Fig. 1c). As a result, the spectral features characterizing the Sake variety were mainly observed in four regions ranging from 950 to 1200, from 1300 to 1450, from 1500 to 1750, and from 2850 to 3000 cm\(^{-1}\) on the MIR spectrum. To confirm this, the Sake variety was classified based on the differences in the polishing ratio of the rice as the raw material, specific classes of Sake, SMV, or AAV. The outer layer of the brown rice is removed and only the internal part (mainly starch) is used for the brewing of Sake. The white rice with the polishing ratio of 55% means that the rice had 45% of the outer layer removed. The specific class of Sake means a high-quality in the order of "Junmai Daiginjo", "Ginjo" "Honjozo" [1, 2]. "Junmai Daiginjo", a specific type of "Junmai Ginjo" is positioned as the premium brands. It is brewed using white rice and more than 50% polishing as the raw material. SMV is the international standard that was established by Japanese Sake manufacturers and is calculated based on the sugar and ethanol concentrations in the Sake products. The lower/negative SMV numbers indicate an increasing sweetness, and the higher/positive numbers indicate a drier Sake. AAV is one of the important factors for the quality evaluation of the Sake because a specific amount of amino acids can play an important role in the reconciliation of the Sake taste. In all cases, different spectral patterns of the four regions were more clearly observed (Fig. 2). The peaks observed in the region ranging from 1200 to 950 cm\(^{-1}\) may be mainly due to the absorption of the sugars [17-19]. The peaks observed in the regions ranging from 1450 to 1300 and ranging from 1750 to 1500 cm\(^{-1}\) may be attributable to the absorption of various amino and other organic acids [16].

The intensity of most of the observed spectral peaks on the MIR spectrum is theoretically caused by the amount of each organic contents of Sake. However, the order of the intensity of the spectral peak may differ partially even if the Sake variety classified based on the differences in the polishing ratio, specific classes, SMV, and AAV. The spectral pattern may vary according to the changes based on the organic component balance and also the interaction conditions that include the pH value. Therefore, we considered the spectrum feature as the spectral pattern characterizing Sake variety. In fact, the quantitative information about the main components and the interactions of the organic components in the Sake could be observed using the spectroscopic analysis [16]. However, the peaks of the amino and other organic acids which determine the taste of Sake were overlapped and the observed peak has a pH dependence on the spectra. Therefore, we focused on the spectral behavior (pattern) of the amino and other organic acids which determined the Sake taste.

### 3.2 Influences of pH on spectral characteristics of the main components of Sake

Table 2 shows the composition of the typical main components of the Sake [20]. In these components, the spectral characteristics of the amino and other organic acids change due to the pH value of the solution because these are ionic dissociative materials. In a previous study, the pH dependence of the spectral characteristics of ethanol and sugar aqueous solutions were not observed [15]. The glycerol pH dependence of the spectral characteristics of glycerol aqueous solutions was also not observed. Therefore, we extracted the molar spectra of the ionic dissociation species of the amino acids (glycine [21] and glutaminic acid) and other organic acids (citric, 

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Fig. 1 Raw (a), second derivative (b), and subtracted second derivative MIR spectra (c) of the thirty brands of Sake.
MIR-spectral features of Sake variety 283

Acetic, lactic, succinic and malic acids) based on the ionic dissociation equilibrium theory [14-16]. As shown in Figures 3a-g, the spectral pattern of each ionic dissociation species of the main components is significantly different. This result suggested that the spectral features of the ionic dissociative constituent drastically changes depending on the pH values. Using the extracted second derivative spectrum of the ionic species, we could reconstruct the spectra of each ionic dissociative material in the Sake with the pH values of 3.90 (minimum), 4.44

(a) Polishing ratio: 50%, 55%, 60%

(b) Specific class: "Junmai Daiginjo", "Junmai Ginjo", "Junmai", "Honjozo"

(c) SMV ("Nihonshyudo"): 5, 3, 0, -5

(d) Amino Acid Value: 1.8, 1.4, 1.1, 0.8

Table 2  Composition of Sake model [20].

| Components       | Concentration [mg/100 ml] |
|------------------|---------------------------|
| Alcohols         |                           |
| Ethanol          | 127.000                   |
| Glycerol         | 7.250                     |
| Sugars           |                           |
| Glucose          | 13.600                    |
| Maltose          | 1.350                     |
| Amino acids      |                           |
| Glycine          | 81.8                      |
| Glutaminic acid  | 120                       |
| Organic acids    |                           |
| Lactic acid      | 544                       |
| Succinic acid    | 439                       |
| Malic acid       | 414                       |
| Acetic acid      | 52.0                      |
| Citric acid      | 49.0                      |
(average) and 4.80 (maximum). The pK values and the calculated results of the concentration of the dissociative material of the each ionic dissociative material were summarized in Tables 3 and 4. The molar absorbance spectra of the glutaminic, citric, acetic, succinic, and malic acids were changed depending on the pH values of the Sake changes ranging from only 3.90 to 4.80 (Figs. 3h–n). On the other hand, the molar absorbance spectra of the glycine and lactic acid were almost not changed in this pH range. The spectral variation of the commercial Sake observed in Figs. 1 and 2 might depend not only on the composition of the main components, but also the spectral behavior change depending on the pH change. To confirm the pH dependence of the Sake spectra, we also
calculated the Sake model spectra for the pH values of 3.90, 4.22 (subtract the standard deviation from the average), 4.44, 4.66 (add standard deviation to the average), and 4.80 (Fig. 4a). The absorption of the calculated Sake model spectra is lower than the commercial Sake spectra (Fig. 2) because the calculated spectrum was reconstructed by adding only the main acids component absorptions. However, the spectral feature of the variations in the actual Sake could be expressed because the main peaks and the spectral pattern almost corresponded to the commercial Sake spectra. These results suggested that the spectral pattern of the Sake model expressed the characteristics of the actual Sake and is based on the quantitative information of the main components and interaction of the organic components in the Sake. In addition, the theoretical analysis of the spectral pattern differences would be possible based on the ionic dissociation equilibrium theory. To make the chemometrics model more stable, a theoretical analysis of the spectrum pattern changes will be important. As shown in Fig. 4a, the pH dependence on the spectrum pattern was clarified based on the pH variation observed with commercial Sake. The spectral variation of Sake would be evaluated including the pH dependency of the actual commercial Sake, except at around 1720, 1550, and 1400 cm$^{-1}$.

### 3.3 Identification of the spectra of Sake with very similar characteristic

As was stated previously, pH dependency of the Sake spectrum was clarified based on the ionic dissociation equilibrium theory. However, the composition of the organic contents of the actual Sake showed slight differences even if the product was made by the same manufacturer. The difference in the composition of the organic contents might be due to the brewing period and used tank that usually differ for the production of the same brand of Sake. Although the difference between the same brands of Sake is very interesting, however, we measured the Sake samples which could be neglected the difference as much as possible as the first step of this study.

Figures 4b and c show the spectral feature difference of Sake when their characteristic is very similar. In the Fig.4, the standard deviation (SD) of the second derivative spectrum was displayed on each of the spectra using gray area, and the peak positions with different spectral patterns were displayed using arrow. In Fig. 4b, the spectral pattern differences were observed when the polishing ratio of the rice as the raw material is different by only 5% for “Junmai Ginjo” which was made by the same manufacturer. Although the difference between the same brands of Sake is very interesting, however, we measured the Sake samples which could be neglected the difference as much as possible as the first step of this study.

#### Table 3 Dissociation constants $pK_i$ of main components of Sake.

| Components          | $pK_1$ | $pK_2$ | $pK_3$ |
|---------------------|--------|--------|--------|
| Glycine [21]        | 2.35   | 9.78   | -      |
| Glutaminic acid     | 2.18   | 4.20   | 9.59   |
| Citric acid [16]    | 2.87   | 4.35   | 5.69   |
| Acetic acid [22]    | 4.56   | -      | -      |
| Lactic acid [22]    | 3.61   | -      | -      |
| Succinic acid [16]  | 4.00   | 5.24   | -      |
| Malic acid [22]     | 3.24   | 4.71   | -      |

#### Table 4 Concentrations of the ionic species of the seven components calculated with different pH values.

| Components     | pH values   |
|----------------|-------------|
|                | 3.90 (minimum) | 4.44 (average) | 4.80 (maximum) |
| Glycine        |             |              |               |
| Cation         | 0.02741     | 0.008063     | 0.003536      |
| Zwitterion     | 0.9726      | 0.9919       | 0.9965        |
| Anion          | 0.000001282 | 0.000004534  | 0.00001043    |
| Glutaminic acid|             |              |               |
| GA             | 0.01253     | 0.002003     | 0.0004814     |
| GA$^-$         | 0.6578      | 0.3646       | 0.2097        |
| GA$^{2-}$      | 0.3297      | 0.6335       | 0.7989        |
| GA$^{3-}$      | 0.000000673 | 0.000004485  | 0.00001296    |
| Citric acid    |             |              |               |
| CA             | 0.06419     | 0.011157     | 0.002802      |
| CA$^-$         | 0.6878      | 0.4299       | 0.2385        |
| CA$^{2-}$      | 0.2440      | 0.5288       | 0.6721        |
| CA$^{3-}$      | 0.003958    | 0.02974      | 0.08659       |
| Acetic acid    |             |              |               |
| AA             | 0.8205      | 0.5686       | 0.3653        |
| AA$^-$         | 0.1795      | 0.4314       | 0.6347        |
| Lactic acid    |             |              |               |
| LA             | 0.3390      | 0.1289       | 0.06065       |
| LA$^-$         | 0.6610      | 0.8711       | 0.9394        |
| Succinic acid  |             |              |               |
| SA             | 0.5463      | 0.2386       | 0.1042        |
| SA$^-$         | 0.4339      | 0.6572       | 0.6572        |
| SA$^{2-}$      | 0.01983     | 0.1042       | 0.2386        |
| Malic acid     |             |              |               |
| MA             | 0.1593      | 0.03943      | 0.01220       |
| MA$^-$         | 0.7280      | 0.6250       | 0.4429        |
| MA$^{2-}$      | 0.1128      | 0.3356       | 0.5449        |
that the difference in both the products is only due to the polishing ratio of the rice because the rice as the raw materials is also cultivated at the affiliated Farm Station of faculty of Bioresources, Mie University. In the region from 1200 to 950 cm\(^{-1}\), which mainly observed the sugar absorption, the spectral pattern and the intensity of the main peaks were almost the same. The content of the sugar in both Sakes will be almost the same because the brewing processes were simultaneously carried out and the rice as the raw material is exactly the same. However, the difference in the polishing ratio of the rice is reflected in the spectral pattern of the other regions ranging from 1450 to 1300, from 1750 to 1500, and from 3000 to 2850 cm\(^{-1}\). The spectral patterns of these regions are slightly different. The spectral feature of these regions might correspond to the absorption of the amino and organic acids which will be traced to the remaining outer layer of the polished rice.

Figure 4c shows the spectral pattern differences between the “Junmai Ginjo” product and the matured one which is aged and sold by the same manufacturer. We directly purchased these two brands of Sake from the brewery of the manufacturer. In the region from 1200 to 950, the absorptions of mainly sugars were reduced after the aging. Sugars containing the Sake might be decomposed during the aging. In contrast, the absorption in the other regions was increased for the peaks because the composition of the amino and organic acid might be changed by aging. As a result, the spectral pattern changed after aging.

These results suggested that the spectral features indicated the characteristic information of the Sake variety even if the Sake variety is only slightly different. The IR spectroscopic method has a great potential for the quality evaluation of the Sake products. The differences in the spectral pattern among the Sake varieties would be applicable to the spectroscopic fingerprint data for the evidence of the food qualities based on the actual sensing
data. In addition, the spectral difference would be comprehensively evaluated in the created chemometrics models based on the spectrum information. A more stable PLS model would be obtained using the clear information of the spectral absorption bands caused by the balance and the interaction of the organic components. The theoretical analysis of the spectral information as shown in this study is a significant and important step for the quality evaluation and the brewing process control of Sake and other fermented foods.

4. Conclusions

The characteristics of the Sake variety were able to be identified as a difference in the MIR spectral pattern based on the amino and organic acids and on the pH dependence. Although the spectral behavior of commercial Sake is complicated because the spectral features depend not only on the composition, but also on the pH dependence of the main components. Furthermore, identification of the spectra of Sake with similar characteristics was successful. The spectroscopic fingerprint data can be applied in evidence of the high-quality products when Sake is exported as a Japanese specialty product. In addition, more stable chemometrics models would be constructed because the spectral features of the commercial Sake could be more theoretically expressed. Thus, a significant potential of the infrared spectroscopic method for the evaluation of the food quality was provided in this study. The infrared spectral information analysis would be acceptable as a new method to evaluate a profile of Sake and the non-destructive on-line monitoring of the brewing process.

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中赤外分光法を援用した銘柄が異なる
日本酒のスペクトル特性把握

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食品の品質を客観的に評価することは、食品加工プロセスの制御はもとより、日本の特産物として高品質な農産物や加工食品を輸出する際の品質保証データとしても重要である。本研究では、日本の伝統食品であり戦略的輸出品として重要度を増している日本酒に着目し、銘柄、すなわち味が異なる日本酒の赤外分光特性の把握を行った。FT-IR/ATR 法にて日本酒30銘柄の中赤外スペクトルを取得した。主要成分であるエタノールの情報を差し引いて、銘柄の違い、とくに原料米の精米歩合、特定名称分類、日本酒度、アミノ酸度の違いに注目して日本酒を特徴づける4つの波数領域（950〜1200, 1300〜1450, 1500〜1700, および2850〜3000 cm⁻¹）を抽出した。これらの領域のスペクトルパターンの違いについて、味覚関連物質としての糖、アルコール、アミノ酸とその他の有機酸に着目して解析を行った。日本酒の味や品質は成分バランスの違いによって生じると考えられるが、一方で解離成分であり味覚関連物質である酸類のスペクトルパターンはpHに依存する。そこで、解離平衡理論に基づいてアミノ酸（グリシンとグルタミン酸）とその他の有機酸（クエン酸、酢酸、乳酸、コハク酸、およびリンゴ酸）について、pH変化に伴うスペクトル変化挙動を把握した。具体的には、それぞれの酸の水溶液についてpHを段階的に変えてスペクトルを測定し、pHとスペクトルパターンの変化について重回帰分析により解離成分のモル吸収スペクトルを抽出した。抽出した各成分の解離成分スペクトルを用いて、日本酒のpHの平均値、最小値および最大値におけるそれぞれの酸のモル吸収スペクトルを合成することで、特定した領域のスペクトルパターンがこれらの酸に由来することを確認した。さらに、日本酒モデルの主要な酸の成分組成に基づいてスペクトルを合成した結果、ピーク位置やスペクトルの変化が観察される波数帯は実際の日本酒と良く一致し、さらに日本酒がもつpHにおけるスペクトル変化幅が把握できた。すなわち、日本酒モデルスペクトルパターンのpH依存性を考慮して日本酒そのものに由来するスペクトルパターンの違いが観察できると考えられた。そこで、極めて特徴が似ている銘柄の日本酒について、その違いがスペクトル上で把握できるかを確かめた。その結果、精米歩合が50および55%の同一酒蔵の日本酒、および同じ日本酒でも熟成の有無が異なるのみの極めて特徴が似ている日本酒について、特定したスペクトル領域において特徴的差異が観察できた。赤外分光法は日本酒の品質評価や醸造プロセス管理への適用に有用である。本研究の成果は、光指紋情報に基づいた食品の品質保証システムの構築や安定したケモメトリックスモデル構築のためのスペクトル解析、スペクトル情報に基づいた加工プロセス管理手法を開発する上で重要な意味をもつであろう。

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