Title: Characterization of carbohydrates, amino acids, viscosity, and antioxidant capacity in rice wines made in Saitama, Japan, with different sake rice

Short title: Characterize sugars and amino Acids in rice wines

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Abstract

We investigated the physicochemical properties Japanese rice wines, including their functional properties and carbohydrate, amino acid content, antioxidant capacity in rice wines in solution and Physicochemical properties in solid state. Three samples were tested. The glucose, allose, and raffinose contents in samples (A, B, C) in g/100 g were: (3.47, 3.45, 7.05), (1.60, 1.63, 1.61), and (2.14, 2.75, 1.49), respectively. The total amino acid in μmol/mL was: (3.1, 3.5, 4.4). Glutamic acid, alanine, and arginine varied in content across the samples. The viscosity (10 °C) and activation energy (ΔE) calculated using the Andrade equation were (2.81±0.03, 2.74±0.06, 2.69±0.03) mPa・s and (22.3±1.1, 22.0±0.2, 21.3±0.5) kJ/mol, respectively. Principal component analysis using FT-IR spectra confirmed the separation of the samples into principal components 2 and 3. The IC_{50} values from the DPPH radical scavenging test were (2364.7±185.3, 3041.9±355.1, 3842.7±228.1) μg/mL. Thus, the three rice wines had different carbohydrate and amino acid contents, viscosities, and antioxidant capacities.

Keyword: rice wine, carbohydrate, amino acid, principal component analysis, antioxidant

INTRODUCTION

Rice wine in Japan and other Asian nations is traditionally produced from the hydrolysis products of starch and polysaccharides present in the rice cultivated in countries, such as China, South Korea, Thailand, the Philippines, and Vietnam by Aidoo et al. (1). One type of rice wine produced in Japan is sake, which has a tradition of over 1,300 years and is popular as a national drink in Japan by Kitagaki et al. (2). Sake manufacturing is a complex process that involves adding koji mold to steamed rice to saccharify the starch, then adding water to form unrefined sake, followed by the addition of yeast for alcoholic fermentation. Sake is rich in nutrients such as sugars, amino acids, organic acids, and aromatic compounds and has a rich taste and flavor by Das et al. (3). In addition, different varieties of sake rice (the raw material), malted rice, and yeast allow sake to be enjoyed in different ways. However, because sake is an alcoholic product, moderate consumption is recommended.

The adage "A little something to drink is the best medicine" is widely known. Appropriate consumption of alcoholic beverages has been reported to reduce cardiovascular risk by promoting insulin-mediated glucose uptake and increasing HDL and apolipoprotein concentrations by Facchini et al. (4) and Emberson et al. (5). Furthermore, alcohol has an appetite-stimulating effect, and drinking sake moderately before or during a meal may help people enjoy the meal and consume the necessary nutrients by Schrieks et al. (6).

Various naturally occurring carbohydrates are present in sake by Tokuoka et al. (7) and Mimura et al. (8). The monosaccharide d-glucose is present in the body as blood sugar and is a source of energy for physical activity by Remesar et al. (9). d-Alose, a rare sugar that acts as the C-3 epimer of d-glucose, is structurally similar to d-glucose. d-Alose competes with glucose for intestinal absorption via SGLT1, which regulates postprandial blood glucose levels by Kishida et al. (10). Therefore, it is not absorbed by GLUT5 and may reduce the increase in blood glucose levels. Raffinose is a sugar composed of galactose, glucose, and fructose, and is widely present in cereals, pulses, vegetables, fruits, and other higher plants as a raffinose family oligosaccharide by Muzquiz et al. (11) and Zartl et al. (12). It improves the balance of the intestinal microflora by promoting the growth of bifidobacteria and lactic acid bacteria in the

human intestine and reducing harmful bacteria by Fernando et al. (13). Furthermore, sake is a fermented food made from rice and has been reported to contain many components, including amino acids, phenolic compounds and kojic acid, which have whitening properties by Okuda et al. (14), Gogami et al. (15) and Bentley et al. (16). Amino acids are components of the body. They include non-essential amino acids synthesized in the body and essential amino acids obtained from the diet. For example, the branched-chain amino acids valine and leucine are essential amino acids that promote muscle protein synthesis, whereas glutamic acid is a non-essential amino acid that acts as a flavor component by Santos et al. (17) and Kurihara et al. (18). Ferulic acid, a phenolic acid, is an antioxidant present in rice by Ito et al. (19).

Ion chromatography is used to analyze carbohydrates by Mitomo et al. (20). Our laboratory previously reported a method for the determination of carbohydrates in fruit wine using a core-shell column and an electrochemical detector (ECD), which can be used in the brewing sector as a simple and effective method for assessing carbohydrate content by Yoshimura et al. (21). Amino acid analysis is used to evaluate the amino acid content of proteins in foodstuffs and herbal medicines by Conde et al. (22). Therefore, it is used for evaluating the amino acid content of rice wine. It can also be conjectured that the unique texture of rice wines, such as viscosity, differs depending on fermentation and production processes.

Recently, several breweries have been locally producing rice wine. These breweries together form a local industry. A facility for easily assessing the characteristics of locally produced rice can help revitalize the local wine industry by providing it with a health-oriented approach. This is expected to allow people to enjoy rice wines in keeping with traditional Japanese culture while promoting the development of regional wine industries amidst growing health consciousness. In this study, carbohydrate, amino acid, viscosity, texture, and antioxidant tests were conducted on three different types of rice wines made from sake rice procured from a brewery in Saitama to examine their characteristics.

MATERIALS AND METHODS

Materials

Three types of rice wines were purchased from a brewery in Moroyama (Saitama, Japan) and used as samples A, B, and C (Table 1). Special-grade glucose was purchased from Fujifilm Wako Pure Chemical Corporation (Tokyo, Japan). Allose and Raffinose were supplied by Matsutani Chemical Industry Co., Ltd. All other reagents (special grade) were purchased from Fujifilm Wako Pure Chemical Corporation (Tokyo, Japan).

(Table 1)

Methods

Preparation of freeze-dried material

Samples A, B, and C were prepared at 40 °C and 25 mbar after solvent evaporation of the alcohol (Rotavapor R-215, Buchi, Switzerland), and the resulting solutions were freeze-dried.

LC measurement

An electrochemical detector (ECD: SU-300, DKK-TOA) was used, with 0.1 mol/L NaOH as mobile phase, a column temperature of 25 °C, flow rate of 0.3 mL/min, an AS8020 autosampler (Tosoh), and a sample injection volume of 20 µL. The columns were ion-exchange columns with a core-shell type filler and reacted with amines (S-30/70=St (styrene)/DVB (divinylbenzene)-5TMDAH (tetramethyldiaminohexane); φ4.6 mm × 150 mm by Yoshimura et al. (21). The theoretical plate numbers (N) of the samples were determined using the internal processing program of the system.

Preparation of standard solutions

Standard solutions were prepared by weighing approximately 20 mg each of glucose, allose, and raffinose and preparing a 200 µg/mL solution using 100 mL of distilled water. Each concentration (1.25 µg/mL, 2.5 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, and 40 µg/mL) was then prepared for calibration curve measurements.

Preparation of rice wines sugar samples in LC

A sample solution of 5 g of each rice wine was weighed and diluted 25, 000-fold with distilled water to determine the carbohydrate content.

2.2.5 Evaluation of the validity of LC measurements

The glucose, allose, and raffinose levels were quantified using standard solutions and evaluated by calculating the linearity as well as the square of the correlation coefficient (R) of the calibration curve for each sample (R^2) (N = 12). The limits of detection (LOD) and quantification (LOQ) were calculated. Reproducibility and precision were assessed using the relative standard deviation (RSD) with known concentrations of glucose, allose, and raffinose, each measured repeatedly for N = 12. The LOD and LOQ were calculated using the following equations:

LOD = 3.3 × (s/a) ･･･eq. 1

LOQ = 10 × (s/a) ･･･eq. 2

s: SD of the intercept for the calibration curve.

a: slope of the calibration curve.

Determination of amino acids

Freeze-dried samples A, B, and C were dissolved in 10 mL of distilled water, filtered through a 0.45 μm filter, and diluted 2-fold with buffer for amino acid analysis as the measurement sample. Amino acids were determined using a JLC-500/V instrument (Japan Information Processing Service Co., Ltd., Tokyo, Japan).

Sugar content determination

The sugar contents of samples A, B, and C were measured using a Master-M (Atago Co., Ltd., Tokyo, Japan) at 25 °C (N = 3).

pH measurement

The pH values of samples A, B, and C were measured using a Horiba pH Meter F-51 (Tokyo, Japan) (N = 3).

Surface tension measurement

The sample solutions were prepared using 5 mL each of Samples A, B, and C, weighed in 3 cm-diameter Petri dishes. Measurements were performed using a DY500 High Performance Surface Tensiometer (Kyowa (Dyne Master, Kyowa Interface Science Co., Ltd., Saitama, Japan)) at 25 °C (N = 3). In addition, a 15% ethanol solution and distilled water were used as reference samples.

Viscosity measurement

Samples A, B, and C (10 mL each) were weighed in a measuring cup to prepare the sample solution. The temperature was gradually increased from 10 °C to 40 °C, and the change in viscosity was measured. The measurements were performed using an SV-10/SV100 (A&D, Tokyo, Japan) (N = 3). The viscosities of a 15% ethanol solution and distilled water were measured as references. The measured viscosity curve was used to calculate the activation energy using the Andrade equation (eq. 3).

$\ln\eta = \ln A + \Delta E/RT$ ・・・eq. 3

η; viscosity (mPa・s)

A; Ordinary number for viscosity

ΔE; apparent activation energy (kJ)

R; gas constant (8.31 J/K・mol)

T; Absolute temperature (K)

Fourier transform infrared (FT-IR) absorption spectrum measurements and principal component analysis.

FT-IR was performed under the ATR method using a Jasco FT/IR-4600 (Jasco Corporation, Tokyo, Japan) under the following measurement conditions: a wave number of 4,000–450 cm^{-1},

scanning time of 16 s, and resolution of 4 cm^{-1}. IR spectra were used for principal component analysis by the PCA program (Jasco Corporation, Tokyo, Japan).

DPPH radical test

A 50 μM solution of 2,2-Diphenyl-2-picry-hydrazyl (DPPH) dissolved in methanol and each sample at different concentrations were added to a microplate at a volume ratio of 1/1 (50 μL DPPH solution and 50 μL distilled water/100 μL sample). The control was 100 μL of water/methanol (1/1) added instead of the sample. Incubation was carried out for 5 min at 37 °C and light-shielded using a Spectra Max microplate reader (Molecular Devices), and absorbance was measured at a wavelength of 517 nm. The inhibition percentage was calculated from the absorbance obtained, and the 50% inhibition concentration was determined. The methanol/DPPH methanol solution mixture (1/1) was considered 0% radical removal (A0), and the water/methanol (1/1) mixture was considered 100% radical removal (blank). The rate of DPPH radical-scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity} = [1- (As-Br) / (A0-Br)] \times 100 \cdots \text{eq. 4}$$

Statistical analysis

Data are expressed as the mean ± standard deviation (S.D.). Comparisons between experimental groups were assessed with the Tukey test, a one-way ANOVA multiple comparison test with $p<0.01$ for significant differences.

RESULTS AND DISCUSSION

Confirmation of conditions for determination of sugars using ECD

Carbohydrate and amino acid content are useful benchmarks for health-conscious consumers when selecting rice wines. Therefore, we measured the glucose, allose, and raffinose levels in samples A, B, and C. Representative chromatographs of glucose, allose, and raffinose

are shown in Fig. 1, with specific peaks at retention times of 13.51 min, 15.58 min, and 19.26 min for glucose, allose, and raffinose, respectively.

(Fig. 1)

Validity of LC measurement

The linearity of the calibration curves for each concentration of glucose, allose, and raffinose and the corresponding correlation coefficient (R^2) were calculated (Table 2). The LOD and LOQ were calculated from the calibration curves. The peak separation was evaluated by calculating the resolution (Rs). For reproducibility and precision evaluation, repeated measurements (N = 12) were performed, and the relative standard deviations (RSD) were calculated. The calibration curves for glucose, allose, and raffinose had R^2 values of 0.998, 0.999, and 0.999, respectively, with good linearity. The LOD (s/a = 3.3) and LOQ (s/a = 10) were: glucose: 0.29 ng/mL and 0.89 ng/mL, respectively; allose: 0.24 ng/mL and 0.72 ng/mL, respectively; and raffinose: 0.68 ng/mL and 2.07 ng/mL, respectively. The resolutions (Rs) of glucose, allose, and raffinose were satisfactory at 2.10, 2.62, and 4.35, respectively. The degrees of separation were satisfactory for all the sugars, with Rs > 1.5. The RSD for glucose, allose, and raffinose were 1.88%, 1.12%, and 1.90%, respectively. The RSD values of less than 5% for all sugars confirmed that the reproducibility of this assay was good and sufficient accuracy was achieved.

(Table 2)

Determination of carbohydrates in each rice wine

The sugar content of each rice wine is listed in Table 3. The glucose contents in Samples A, B, and C were 3.47 g/100 g, 3.45 g/100 g, and 7.05 g/100 g, respectively. Sample C contained approximately twice the amount of glucose compared to Samples A and B. The allose contents in Samples A, B, and C were 1.60 g/100g, 1.63 g/100g, and 1.61 g/100g, respectively. The raffinose contents in Samples A, B, and C were 2.14 g/100g, 2.75 g/100g, and 1.49 g/100g, respectively, indicating that Samples A and B contained approximately 1.5 times more raffinose than Sample C. Allose is generally known for its antioxidant, anti-inflammatory, and anti-tumor

effects by Gao et al. (23), Gao et al. (24) and Tohi et al. (25). Raffinose has been reported to balance the intestinal microflora in humans and inhibit biofilm formation by the Streptococcus species in the human oral cavity by Zartl et al. (12), Fernando et al. (13) and Ham et al. (26). Thus, allose and raffinose have health benefits. Regarding blood glucose levels, although glucose, a monosaccharide, works to raise and maintain blood glucose, allose competes with glucose in SGLT1 in the small intestine, and raffinose is not broken down from trisaccharides because humans do not have the required digestive enzymes for its breakdown by Kishida et al. (10) and Muzquiz et al. (11). Thus, allose and raffinose are less likely to increase blood glucose. Therefore, Samples A and B are less likely to elevate blood glucose compared to Sample C. From the results, samples A and B have higher raffinose contents than Sample C. Thus, we have demonstrated that the difference between rice wines A, B, and C can be evaluated in terms of carbohydrates that contribute to human health and that ECD is a useful and simple device for the determination of carbohydrates.

(Table 3)

Amino acid analysis

Amino acid analysis is a widely used method for evaluating the amino acid content of proteins in crude and processed foods by Tanase et al. (27). Therefore, amino acid measurements were performed for Samples A, B, and C, and the results are shown in Table 4. From this analysis, the total amounts of amino acids in samples A, B, and C were 3.1 μmol/mL, 3.5 μmol/mL, and 4.4 μmol/mL, respectively. Furthermore, different types of amino acids varied in content in Samples A, B, and C. The various amino acid contents in Samples A, B, and C were: glutamic acid (0.25 μmol/mL, 0.28 μmol/mL, and 0.35 μmol/mL); alanine (0.58 μmol/mL, 0.78 μmol/mL, and 0.9 μmol/mL); valine (0.14 μmol/mL, 0.21 μmol/mL, and 0.23 μmol/mL); leucine (0.23 μmol/mL, 0.25 μmol/mL, and 0.32 μmol/mL); histidine (0.01 μmol/mL, 0.04 μmol/mL, and 0.07 μmol/mL); and arginine (0.03 μmol/mL, 0.17 μmol/mL, and 0.47 μmol/mL). Glutamic acid has been reported to contribute to taste and acidity, and alanine to acidity and bitterness by Kurihara et al. (18) and Tanase et al. (27). Valine and leucine are branched-chain amino acids

that comprise muscle proteins and contribute to motor functions. The intake of branched-chain amino acids can help reduce sarcopenia, a disease that causes a decrease in total body muscle mass and muscle strength with aging by Mantuano et al. (28). Histidine is an essential amino acid that works on the sympathetic nervous system to break down fat by working on nerve cells and is converted to histamine in the body by Sakata et al. (29). It is known to exert both hepatoprotective and anti-inflammatory effects by Lee et al. (30). Arginine promotes growth hormone secretion and increases muscle strength by Oh et al. (31). Moreover, it lowers blood pressure through its vasodilating effect by Huynh et al. (32). Thus, in terms of flavor, richness, and benefits to the body, Sample C contained more amino acids that are beneficial to health than Samples A and B.

(Table 4)

Sugar content measurement

The sugar content of the wines was measured as a convenient sweetness index. The sugar contents of Samples A, B, and C were 10.3%, 10.0%, and 9.8%, respectively (Table 5). Regarding the carbohydrate content, Sample C had a higher glucose content than Samples A and B. However, the results of the sugar content measurement were considered to indicate the overall sugar content of each sample.

(Table 5)

pH measurement

The pH measurements were performed to determine the properties of the sample solutions. The pH values of Samples A, B, and C were 4.05, 4.24, and 4.32, respectively (Table 5). Generally, beverages with a pH value of less than 4 can cause acid erosion by Reddy et al. (33). Alcoholic beverages such as plum wine and wine have a pH of less than 4 and may dissolve teeth by Shin et al. (34). All three samples of rice wine, A, B, and C, had a pH of 4 or higher, suggesting that daily consumption of rice wine is unlikely to cause dental caries due to its acid content.

Surface tension measurement

Surface tension measurements were performed to study the variation of viscosity across the sample solutions (Table 5). The surface tensions of the samples were A: 43.20 mN/m; B: 44.08 mN/m; C: 43.90 mN/m; 15% ethanol: 43.35 mN/m; and distilled water: 71.42 mN/m. The surface tension of each sample is lower than that of distilled water due to the weakening of the hydrogen bonds between the water molecules in the aqueous solution. Samples A, B, and C exhibited lower surface tension values than 15% ethanol, and Sample A showed lower values than Samples B and C. This suggests that the composition of the rice wines lowered the surface tension and that the differences among the samples were due to differences in their compositions. Although it was thought that sugar mass affected surface tension, no relationship was observed between sugar mass and surface tension.

Viscosity measurement

The change in the viscosity of each sample solution with temperature was measured (Fig. 2 (X)), and the apparent activation energy (ΔE) was calculated using the Andrade equation. The viscosities of Samples A, B, and C at 10 °C were 2.81±0.03 mPa·s, 2.74±0.06 mPa·s, and 2.69±0.03 mPa·s, respectively and those of 15% ethanol and distilled water were 2.34±0 mPa·s and 1.36±0.03 mPa·s, respectively. The higher viscosity values of the wine samples were due to hydrogen bonding between alcohol and water. The value for Sample A was higher than that for Sample C, probably because of stronger intermolecular interactions in the solution caused by the included components. Thus, the Andrade equation, which expresses the relationship between viscosity and temperature, was used to evaluate the quality of the biofuels. The values of ΔE for the wine samples obtained from the Andrade equation, are useful for quantifying the internal structural changes in solution and evaluating Japanese sake (rice wine) by Tate et al. (35). To calculate ΔE from the Andrade equation, a graph of the logarithm of viscosity as a function of the reciprocal of absolute temperature was plotted (Fig. 2(Y)). The ΔE values for Samples A, B, and C, calculated from the Andrade equation were 22.3±1.1

kJ/mol, 22.0±0.2 kJ/mol, and 21.3±0.5 kJ/mol, respectively. The slope of the straight line corresponding to Sample C is more gradual than that for Samples A and B. Sample C showed a lower ΔE value than A and B even when the temperature changed from 10 to 40 °C. This suggests that wine quality is less likely to change with temperature. Moreover, the differences in the internal structural changes in the rice wine solutions were evident.

(Fig. 2)

^{1}H-NMR measurement

Generally, the spectral area obtained by ^{1}H-NMR measurement is proportional to the number of protons, which is used for qualitative analysis. We measured ^{1}H-NMR to comprehensively analyze the components and contents of each rice wine (Fig. 3). Signals around 1 ppm were attributed to branched-chain amino acids, around 3–5 ppm to carbohydrates, amino acids, and organic acids, and around 7–8 ppm to aromatics. In the 7–8 ppm range, signals were observed at A: 8.03, 8.06, and 8.51 ppm; B: 7.73, 7.95, 8.06, and 8.51 ppm; and C: 7.73, 7.83, and 8.51 ppm, confirming the presence of various aromatic compounds in these samples. The signal area at 2.45 ppm was taken to be 1, and the integral ratios at each chemical shift for A, B, and C around 1 ppm were 75.62, 29.60, and 42.40, respectively; at 3–5 ppm, they were 473.57, 440.2, and 437.92, respectively, and 1.02, 0.69, and 0.83. Although fragmentary, the NMR spectra revealed differences in the contents of carbohydrates, amino acids, organic acids, and aromatic compounds in the samples.

(Fig. 3)

Fourier transform infrared (IR) absorption spectroscopy

FT-IR spectra are useful for evaluating the attributes of functional groups in foods, herbal medicines, or other products and assessing the quality of the products. Therefore, FT-IR measurements of glucose, allose, and raffinose in Samples A, B, and C were conducted using their freeze-dried samples to comprehensively analyze the components contained in each rice wine (Fig. 4). The following peaks were observed from the measurement of sugars alone: glucose:

3394, 3303, 1148, 1111, 1078, 1050, 1023, 997, 916, and 839 cm^{-1}; allose: 3489, 3379, 3338, 1168, 1123, 1094, 1081, 1031, 947, 896, and 885 cm^{-1}; and raffinose: 3943, 3294, 3220, 1148, 1094, 1075, 1049, 1031, 999, 966, 938, 875, 861, and 833 cm^{-1}, respectively (Table 6). The peaks derived from the -OH group in Samples A, B, and C were identified at 3375 cm^{-1}, 3367 cm^{-1}, and 3376 cm^{-1}, respectively. The peaks derived from water in Samples A, B, and C were observed at 1634 cm^{-1}, 1626 cm^{-1}, and 1635 cm^{-1}, respectively. The peaks associated with the C-O-C of sugar for Samples A, D, and C were observed at 1048 cm^{-1}, 1047 cm^{-1}, and 1046 cm^{-1}, respectively. However, because the differences among the samples were difficult to distinguish visually, principal component analysis was performed using the IR spectra of Samples A, B, and C.

(Fig. 4)

Principal component analysis (PCA) using FT-IR spectra.

PCA is used to separate major component spectra from multiple component spectra and to identify components (principal components: PC) that characterize the differences in the spectra. Therefore, a PCA was performed using the IR spectra shown in Fig. 5. In the PC1-PC2 plot A, B, and C were found to cluster at a value of 10 on the PC1 axis. In contrast, for the PC2 axis, a separation of plots was observed around A = 0, B = -0.5, and C = 0.7. In the PC2-PC3 plot, the separation of the A, B, and C plots by PC2 was confirmed near A = 0.2 and B and C = -0.1 against the PC3 axis. In the PC1-PC3 plots, the separation of plots due to PC1 was not observed, but a separation of Samples A, B, and C due to PC3 was observed. The FT-IR spectra of PC1, PC2, and PC3 are shown (Fig. 5) to infer which PC1, PC2, and PC3 were derived from the difference in functional groups. For PC1, a water-derived peak was observed at 3304 cm^{-1}, and sugar-derived peaks were observed at 1048, 1094, 1075, and 1013 cm^{-1}. In the FT-IR results for sugar alone, these peaks were consistent with those of glucose and raffinose. These findings suggest that although PC1 showed peaks derived from glucose and raffinose, the samples were not separated because of the large influence of water, which is common to all samples. PC2 showed peaks at 3363 cm^{-1} derived from the -OH group,

3203 cm^{-1} derived from the -NH group, and 1646 cm^{-1} derived from the -OH due to water content. Thus, the separation of samples by PC2 in the principal component plot may represent differences in the amino acids and aromatic compounds. PC3 peaks derived from the -CH group were observed at 2973 and 2887 cm^{-1}; peaks derived from the C=O group of carboxylic acids were observed at 1739 and 1720 cm^{-1}; and peaks derived from sugars were observed at 1151, 1086, 1040, 937, and 879 cm^{-1}. The sugar chromatogram confirmed the presence of carbohydrates other than glucose, allose, and raffinose, suggesting the presence of carbohydrates and structures other than glucose, allose, and raffinose in PC3.

(Fig. 5)

DPPH radical scavenging test

Rice wine is known to contain antioxidants such as ferulic acid by Ito et al. (19). In addition, ^{1}H NMR measurements showed differences in the chemical shifts and integration ratios in the aromatic region among the samples, which may also affect their antioxidant capacities. Therefore, a DPPH radical scavenging test was conducted to confirm the antioxidant capacities of Samples A, B, and C. Ascorbic acid (ASC) was tested in the same manner for comparison. The IC_{50} inhibitory concentrations were A: 2364.7±185.3 μg/mL, B: 3041.9±355.1 μg/mL, C: 3842.7±228.1 μg/mL, and ASC: 0.98±0.04 μg/mL (Fig. 6). The IC_{50} of Sample A was significantly lower than those of Samples B and C ($p<0.01$), and the IC_{50} of Sample B was significantly lower than that of Sample C ($p<0.01$). Although the IC_{50} values of Samples A, B, and C were very high compared to the IC_{50} value of ASC, differences in the comprehensive antioxidant capacity were observed. Because sake rice is reported to contain ferulic acid, an antioxidant, and a substance related to beauty, this indicates differences in the antioxidant capacities of the samples due to the presence of diverse antioxidants By Ito et al. (19). Hirotsune et al. reported that ethylglucoside suppressed barrier breakdown by promoting keratinocyte differentiation by Hirotsune et al. (36). In this study, substances exhibiting antioxidant activity have not been analyzed in detail. However, the results of the ^{1}H NMR and IR measurements and

DPPH tests were used to verify the comprehensive antioxidant capacity. Further detailed verification of the components responsible for antioxidant activity should be conducted in the future. In this study, samples of rice wine (Japanese sake) from a sake brewery in Saitama Prefecture, Japan, were used as a case study. Sake has a unique flavor owing to the complex combination of rice, yeast, preparation water, and local temperature. We will continue to evaluate the physical properties of sake through instrumental analysis to provide greater information to people worldwide on what components give rice wine its taste.

In this study, the differences in the carbohydrate and amino acid contents of rice wines were measured. The results of the viscosity measurements revealed differences in the internal structural changes in the solution state owing to the differences in ΔE calculated from the Andrade equation. Furthermore, ^{1}H NMR, IR, and antioxidant tests revealed differences in the substances affecting the antioxidant capacity of rice wine (sake). Finally, differences in rice and production methods were shown to affect the characteristics of rice wine, which is drunk as part of traditional Japanese culture. In other words, the differences between products can be identified by examining the physicochemical properties of rice wine (sake). This study is expected to serve as a foundation for understanding the health aspects of rice wines and boosting the local wine-making industry.

(Fig. 6)

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Abbreviations

FT-IR: Fourier Transform Infrared

DPPH: 2,2-Diphenyl-2-picry-hydrazyl

SGLT1: sodium/glucose cotransporter 1

GLUT5: glucose transporter 5

ECD: Electrochemical detector

LOD: Limit of detection

LOQ: Limit of quantification

RSD: Relative standard deviation

ATR: Attenuated Total Reflection

PCA: Principal component analysis

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Figure legends

Table 1 Raw materials of rice wine A, B and C.

Table 2 Validation of the methods (n=12).

Table 3 Contents of Glucose, Allose, and Raffinose in Sample A, B and C.

Table 4 Contents of Amino acid in Sample A, B and C.

Table 5 Brix, pH, Surface tension of sample A, B and C.

Table 6 FT-IR peaks of Glucose, Allose and Raffinose.

Fig. 1 (X) The chromatogram of standard substances peaks. 13.51; Glucose, 15.58; Allose, 19.26; Raffinose. (Y) Calibration curves of sample by ECD. (a) Glucose, (b) Allose, (c) Raffinose.

Fig. 2 Viscosity depend on temperature increase (X). And the logarithmic value of viscosity (Y) on the vertical axis and the temperature on the horizontal axis of sample A, B and C. (a) A, (b) B, (c) C, (d) A, B, C systems.

Fig. 3 ^{1}H-NMR (D_2O) spectra of sample A, B and C. (a) A, (b) B, (c) C.

Fig. 4 FT-IR spectra of (X) (a) Glucose, (b) Allose, (c) Raffinose, and (Y) (d) A, (e) B, (f) C.

Fig. 5 Principal Component Analysis of A, B and C Using FT-IR Spectra score plot of (W) PC1 vs PC2, (X) PC2 vs PC3, (Y) PC1 vs PC3, and FT-IR spectra of (Z) PC1, PC2 and PC3. (a) PC1 (b) PC2 (c) PC3.

Fig. 6 IC_{50} of sample A, B and C in a DPPH radical scavenging test. Values are the mean ±S.D. (n=3) $^{**}P<0.01$ (*Tukey test*)

Table 1 Raw materials of rice wine A, B and C.

Sample	Alcohol content (%)	Rice polishing ratio (%)	Sake degree	Amino acid (mL)	Acidity (mL)	Raw materials
A	16	60	+1	0.8	1.6	Rice (100% Sake musashi produced in Saitama), rice koji (produced in Saitama)
B	15	60	+1	0.8	1.4	Rice (100% Yamada nishiki produced in Saitama), rice koji (produced in Saitama)
C	15	60	+2	1.5	1.4	Rice (produced in Japan), rice koji (produced in Japan)

23) https://www.musashino-asahara.jp.

Table 2 Validation of the methods.

Standard substance	R^2	Rs	RSD (%)	LOD (ng/mL)	LOQ (ng/mL)
				3.3s/a	10s/a
Glucose	0.998	2.10	1.88	0.29	0.89
Allose	0.999	2.62	1.12	0.24	0.72
Raffinose	0.999	4.35	1.90	0.68	2.07

s: SD of absorbance of blank sample (n=12).

a: The slope of the calibration curve near the detection limit

Table 3 Contents of Glucose, Allose, and Raffinose in Sample A, B and C.

Sample	Glucose (g/100g)	Allose (g/100g)	Raffinose (g/100g)
A	3.47±0.03	1.06±0.03	2.14±0.04
B	3.45±0.02	1.63±0.02	2.75±0.05
C	7.05±0.04	1.61±0.01	1.49±0.04

Table 4 Contents of Amino acid in Sample A, B and C.

Amino acid (μmol/mL)	A	B	C	Amino acid (μmol/mL)	A	B	C
PEA	0	0.01	0	Ile	0.09	0.10	0.12
Asp	0.12	0.11	0.15	Leu	0.23	0.25	0.32
Thr	0.07	0.05	0.08	Tyr	0.13	0.17	0.19
Ser	0.15	0.11	0.17	Phe	0.08	0.10	0.13
Asn	0.12	0.08	0.12	GABA	0.04	0.04	0.05
Glu	0.25	0.28	0.35	MEA	0.44	0.36	0.38
Gly	0.5	0.53	0.51	Orn	0.02	0.01	0.04
Ala	0.58	0.78	0.9	His	0.01	0.04	0.07
Val	0.14	0.21	0.23	Lys	0.09	0.08	0.14
Cys	0.02	0.03	0.02	Arg	0.03	0.17	0.47

Table 5 Brix, pH, Surface tension of sample A,B and C.

Sample	Brix (%)	pH	Surface tension (mN/m)
A	10.3 ± 0.1	4.05 ± 0.02	43.20 ± 0.33
B	10.0 ± 0.0	4.24 ± 0.03	44.08 ± 0.23
C	9.8 ± 0.0	4.32 ± 0.03	43.90 ± 0.33

Table 6 FT-IR peaks of Glucose, Allose and Raffinose.

Compound	IR (cm^{-1})
Glucose	3394, 3303, 1148, 1111, 1078, 1050, 1023, 997, 916, 839
Allose	3489, 3379, 3338, 1168, 1123, 1094, 1081, 1031, 947, 896, 885
Raffinose	3943, 3294, 3220, 1148, 1094, 1075, 1049, 1031, 999, 966, 938, 875, 861,833

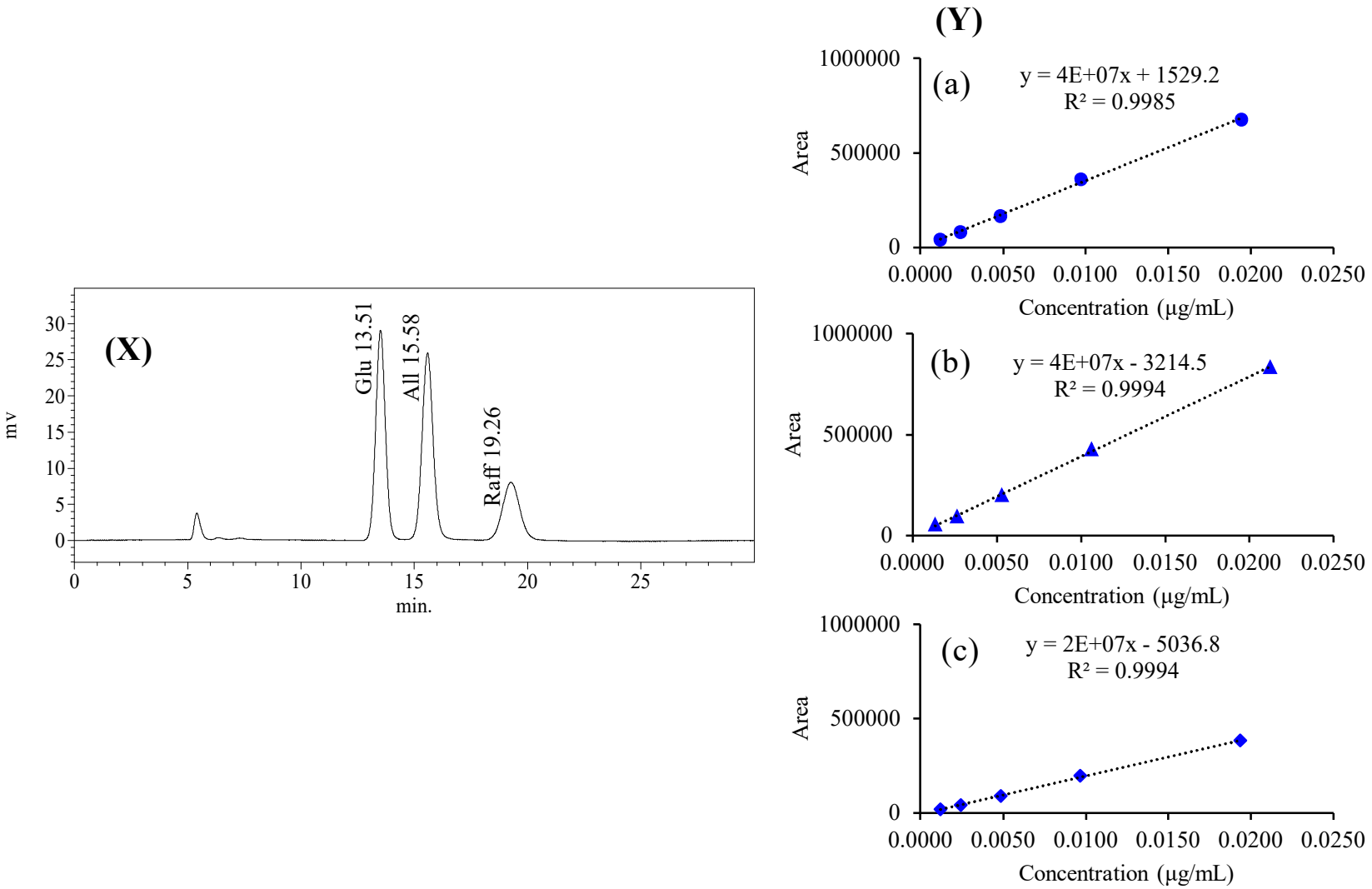

(X)
mv
Glu 13.51
All 15.58
Raff 19.26
min.
(Y)
(a)
y = 4E+07x + 1529.2
R² = 0.9985
(b)
y = 4E+07x - 3214.5
R² = 0.9994
(c)
y = 2E+07x - 5036.8
R² = 0.9994
Area
Concentration (µg/mL)

Figure. 1

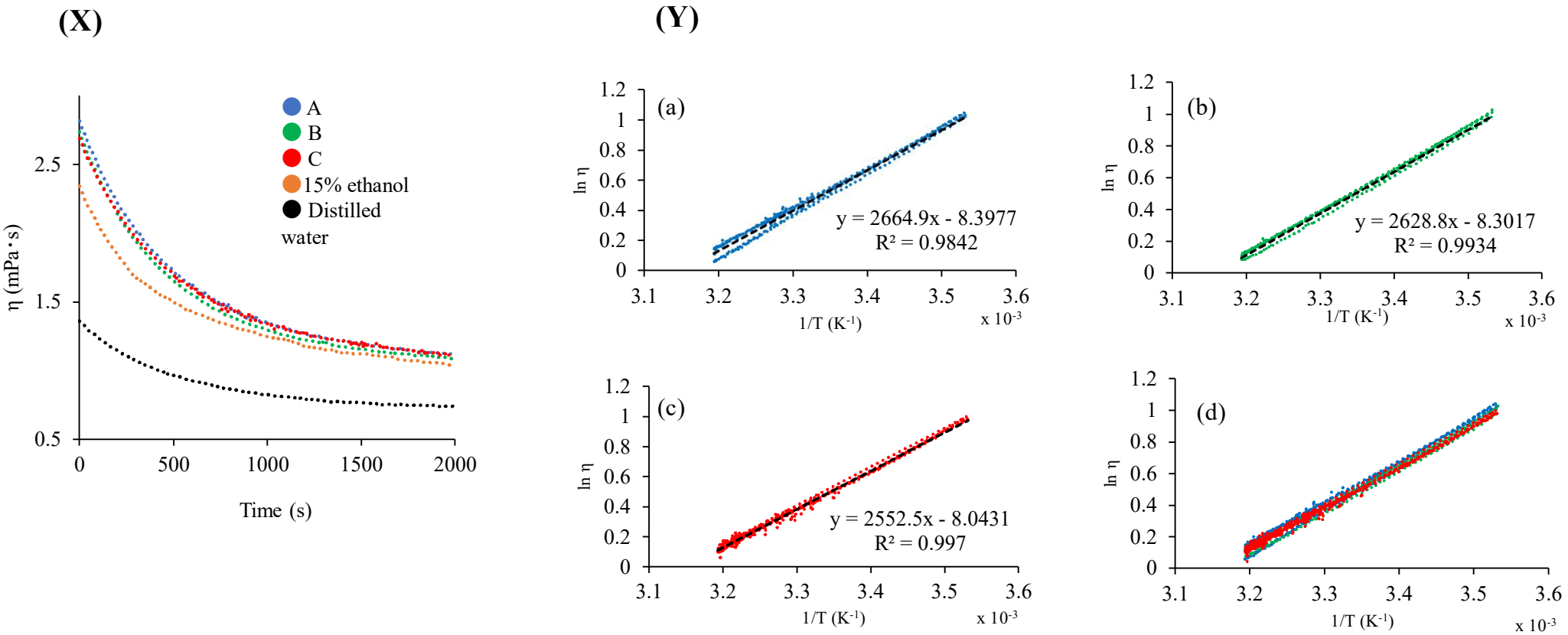

(X)
(Y)
A
B
C
15% ethanol
Distilled water
η (mPa·s)
Time (s)
(a)
(b)
(c)
(d)
ln η
1/T (K-1)
x 10-3
y = 2664.9x - 8.3977
R² = 0.9842
y = 2628.8x - 8.3017
R² = 0.9934
y = 2552.5x - 8.0431
R² = 0.997

Figure.2

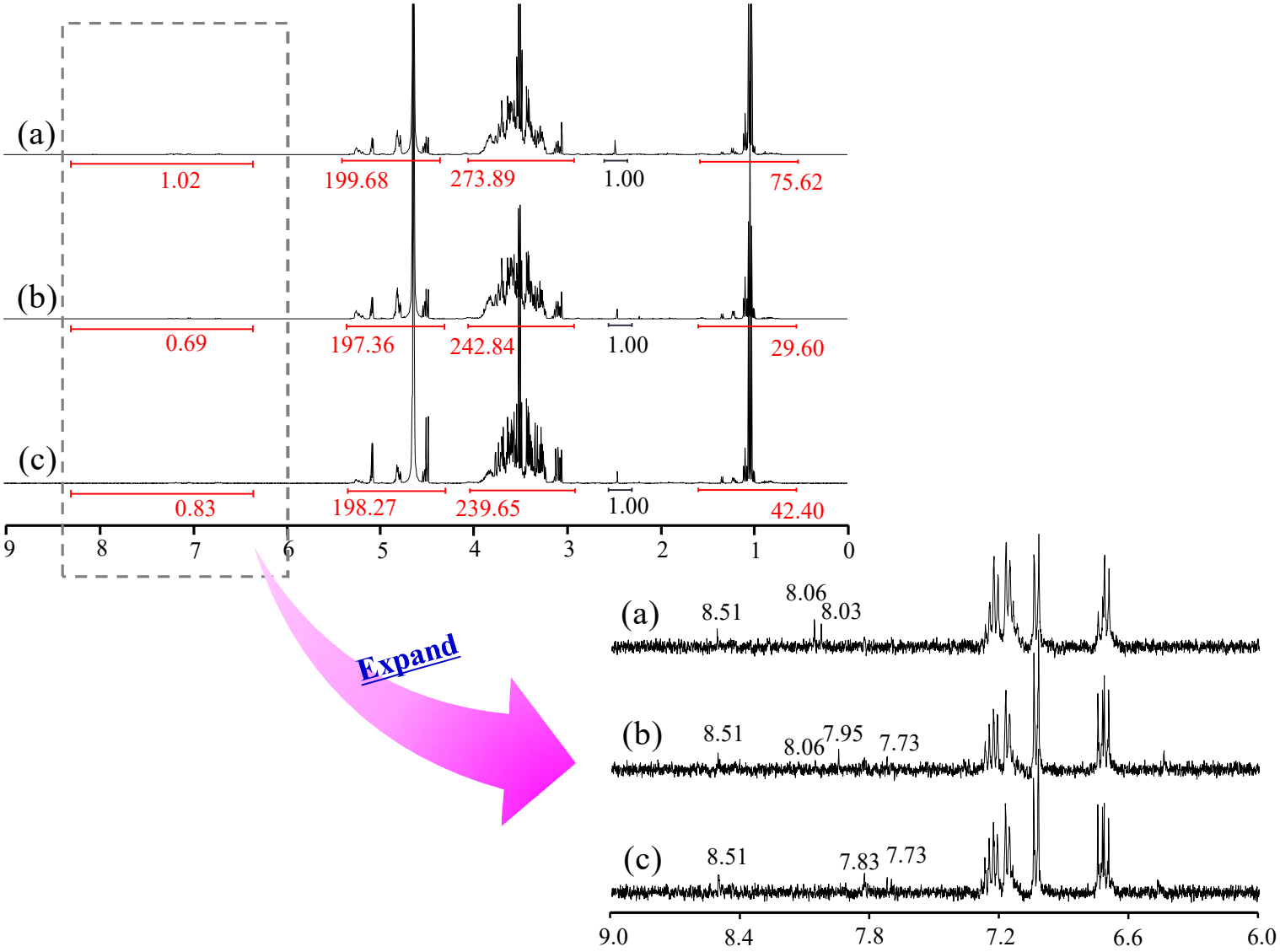

(a)
1.02
199.68
273.89
1.00
75.62
(b)
0.69
197.36
242.84
1.00
29.60
(c)
0.83
198.27
239.65
1.00
42.40
9
8
7
6
5
4
3
2
1
0
Expand
(a)
8.51
8.06
8.03
(b)
8.51
8.06
7.95
7.73
(c)
8.51
7.83
7.73
9.0
8.4
7.8
7.2
6.6
6.0

Figure. 3

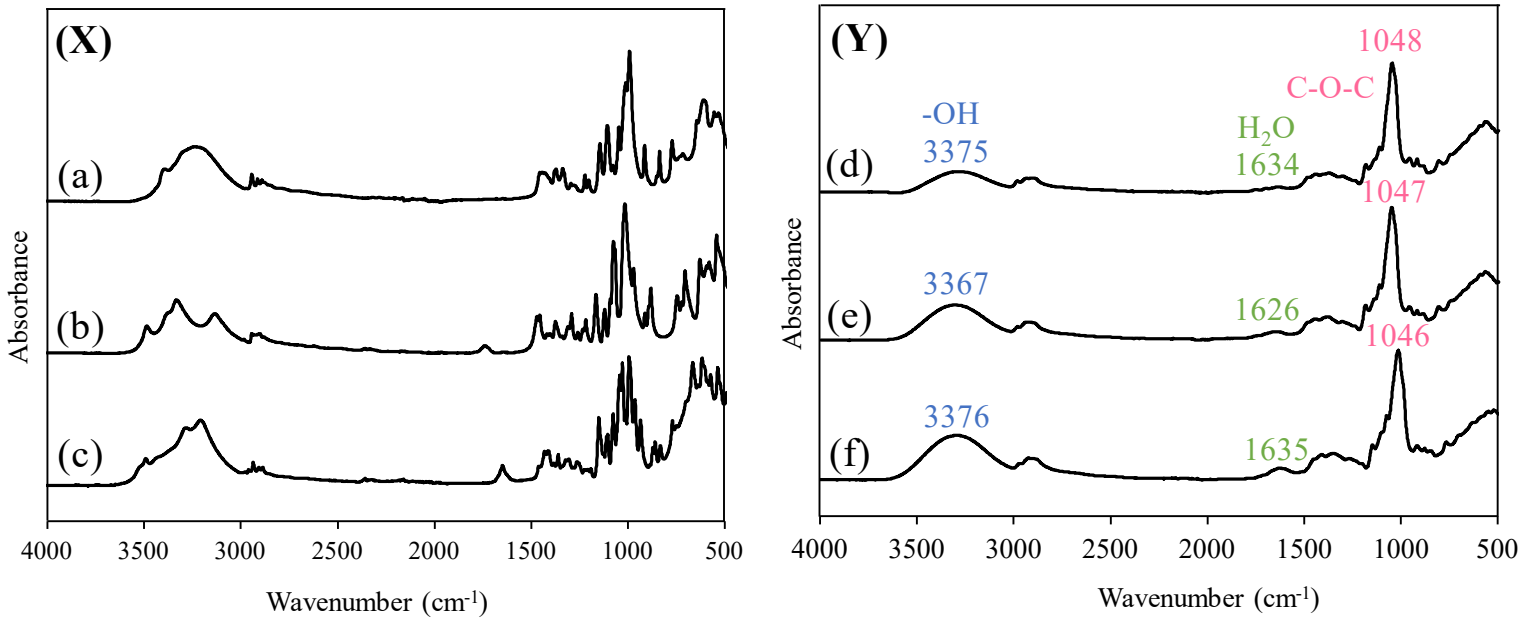
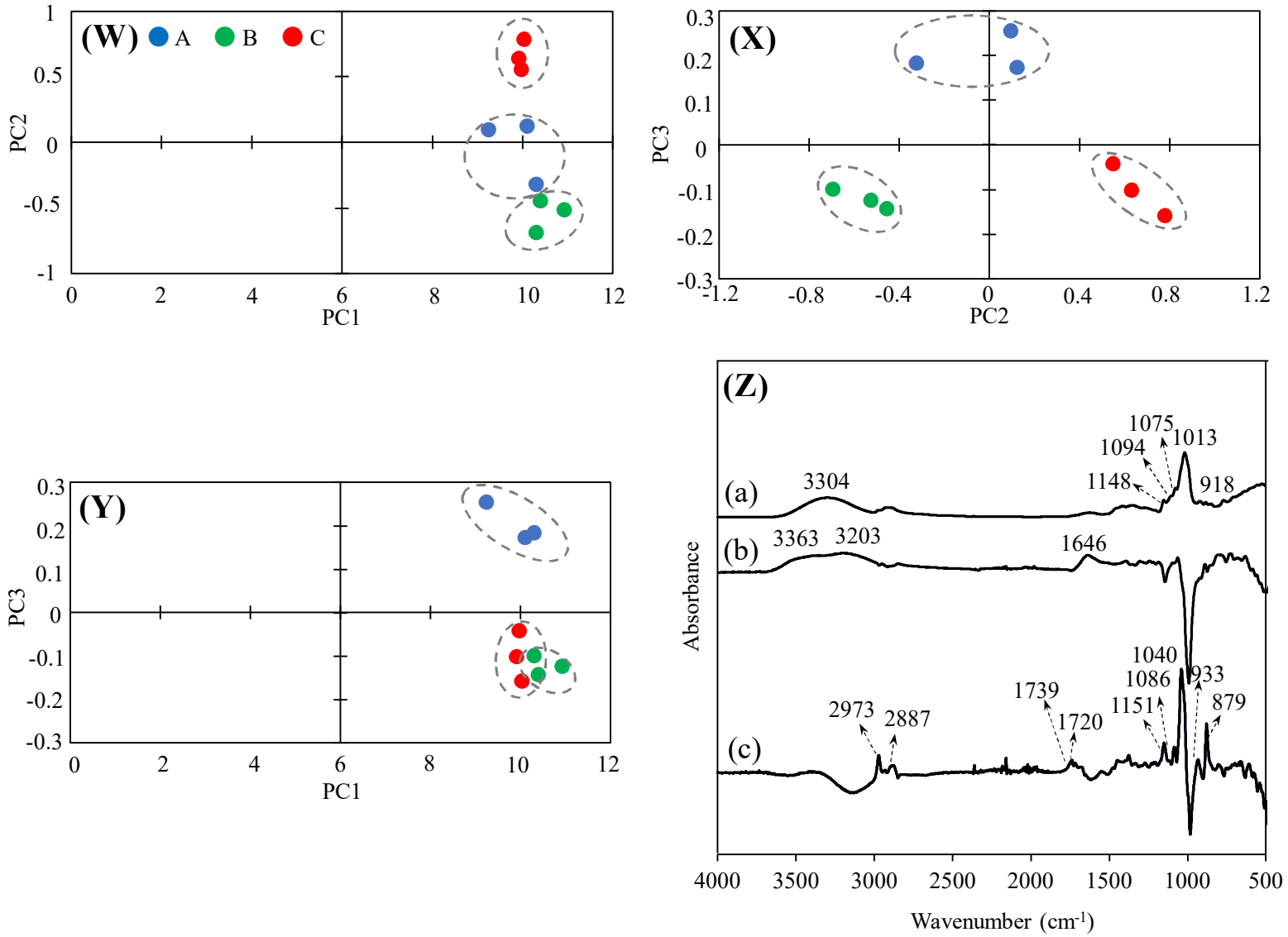

(X)
(a)
(b)
(c)
Absorbance
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)
(Y)
1048
C-O-C
-OH
3375
H2O
1634
(d)
1047
3367
(e)
1626
1046
3376
(f)
1635
Absorbance
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)

Figure. 4


(W)
A
B
C
PC2
PC1
(X)
PC3
PC2
(Y)
PC3
PC1
(Z)
(a)
(b)
(c)
3304
3363
3203
2973
2887
1739
1720
1646
1148
1094
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1086
1040
1013
1151
933
918
879
Absorbance
Wavenumber (cm-1)

Figure. 5

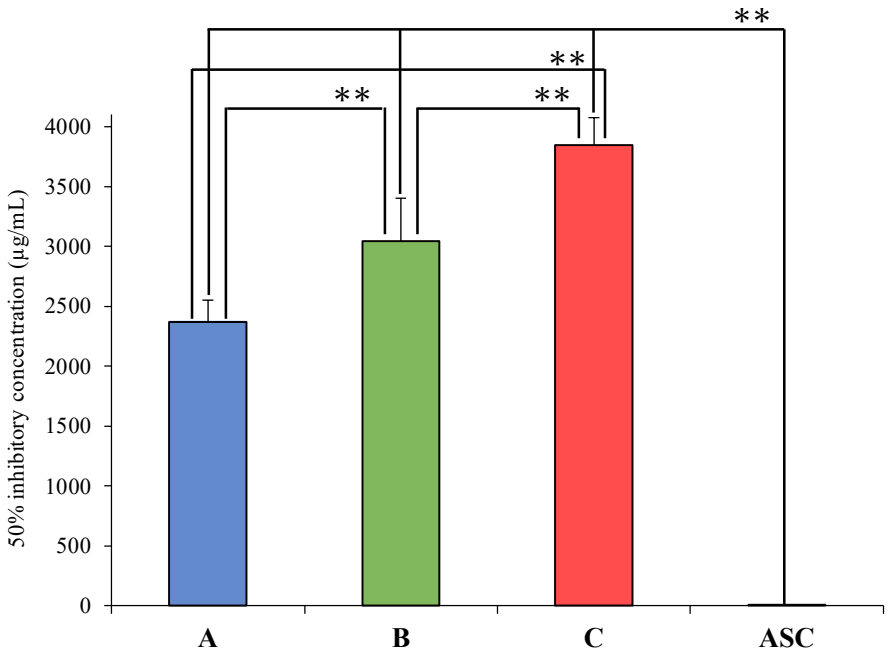
**
**
**
**
4000
3500
3000
2500
2000
1500
1000
500
0
50% inhibitory concentration (µg/mL)
A
B
C
ASC

Figure. 6