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Determination of sugars and amino acids in japanese wine using core-shell liquid chromatography tandem electrochemical detection

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ABSTRACT

In this study, sugars (glucose, fructose, and sucrose) and amino acids in plum and yuzu wines were quantified with a new and simple method involving an electrochemical detector (ECD) and core-shell column [S-30/70=St (styrene)/DVB (divinylbenzene)-5TMDAH (tetramethyldiaminohexane)]. Analysis was conducted under the following conditions: A column temperature (40°C), flow rate (0.3 mL/min), injection volume (20 μL), and mobile phase (0.1 mol/L NaOH). Glucose, fructose, and sucrose had retention times of 14.68 min, 16.22 min, and 19.29 min, respectively, when measured using an ECD. When the sugar content was compared among the plum wines, plum wine A had the lowest sugar content. A comparison of the sugar content of the different types of yuzu showed that yuzu wine C had the lowest sugar content. In addition, plum wine C showed higher values of amino acid components (2.27 μmol/mL of alanine, 0.67 μmol/mL of GABA, and 5.28 μmol/mL of glutamic acid) compared with A and B. This straightforward LC technique is reliable in the determination of carbohydrates and amino acid contents in wine, useful criteria for consumers in selecting wines parallel to their ideal health outcomes.

Keywords: Amino acids, core-shell column, electrochemical detector, ion chromatography, sugars

INTRODUCTION

As one of the culturally important industries in Japan, the production of sake uses fruits that are locally produced in a particular region. In regions where plum and yuzu (a Japanese citrus fruit) are abundant, it is customary to prepare and enjoy these wines at home.[1] At present, fruit wine is prepared industrially by many brewing companies, and the sugar and amino acid content varies. Globally, the market size of sake is expected to reach 10.47 billion (USD) by 2026 (Ref).[2]

One of the most famous Japanese fruit wines is plum wine. Plum wine contains Vitamin B_2 and Vitamin B_6 as well as citric acid, which helps to alleviate fatigue. Citric acid promotes recovery from fatigue by producing energy in the body and breaking down lactic acid, the source of fatigue.[3] It also helps the absorption of minerals and promotes metabolism. The alcohol contained in plum wine promotes blood circulation and warms the body, while the pleasant aroma of plum wine helps to relax the body. In this way, a small number of aperitifs can nourish one, both mentally and physically.

There are different types of plum wine, and sugar is commonly added during its production process. One gram of alcohol (ethanol) is approximately equal to 7 Kcal and the addition of sugar means that plum wines are considered to be high-energy foods.[4] Therefore, there is clinical interest in the amount of sugar and carbohydrates in aperitifs. A carbohydrate-restricted diet is expected to have benefits not only for metabolic syndrome but also for various diseases and conditions such as dementia, locomotive syndrome (sarcopenia and frailty), and aging.[5-9] It can be assumed that carbohydrate restriction reduces the glycation of proteins in organs associated with carbohydrate intake, thereby maintaining and improving organ function.[10] Providing consumers with information on the sugar and carbohydrate contents of the fruit wine they choose as an aperitif allow them to enjoy the unique Japanese alcoholic beverages in the Japanese food culture more in the context of increasing health consciousness.

Ion chromatography (IC) is a separation technique for the analysis of carbohydrates using a column packed with ion exchangers to measure the conductivity of the target analyte with an electrical conductivity detector.[11] The use of a suppressor reduces the background conductivity of the eluent in the measurement and allows for the sensitive detection of ionic substances. Among the advantages of IC as an analytical technique are that dilute concentrations of acid and alkaline solutions can be used as eluent reagents for the measurement, making it relatively safe. Additionally, samples with low contaminants can be analyzed by simple pre-treatments such as dilution or filtration. We have previously reported the determination of cyclodextrins,[12] cyclic polysaccharides, and α-glucosidase inhibitors such as miglitol and acarbose[13] using core-shell columns.[14] The use of core-shell columns in IC with an electrochemical detector (ECD) for the quantification of carbohydrates in fruit wines would be a straightforward and effective method for the evaluation of new fruit wines and provides a positive health impact on the consumers. The application of core-shell IC-ECD in the determination of sugars and amino acids – molecules ubiquitous in the pharmaceutical and food industries – has not been investigated yet. Therefore, the present research aimed to evaluate the sugar, and amino acid contents of locally produced and commercially available fruit wines. Texture (i.e., stickiness) and sourness of fruit wines were also evaluated as these characteristics are thought of as valuable information in the consumption of aperitifs.

MATERIALS AND METHODS

Materials

Plum and yuzu wines were purchased from companies A, B, and C, respectively [Table 1]. All other reagents were purchased from special grade (Fujifilm Wako Pure Chemical Corporation, Tokyo).

LC Measurement Conditions

Sample measurements were performed using an ECD: SU-300, DKK-TOA with a gold electrode and 1V electric potential. The mobile phase was 0.1 mol/L NaOH. The column temperature was maintained at 40, flow rate 0.3 mL/min, using AS8020 autosampler (Tosoh), sample injection volume 20 μL. An ion-exchange column with a core-shell type filler reacted with amine (S-30/70=St (styrene)/DVB (divinylbenzene)-5TMDAH (tetramethyldiaminohexane) (ϕ4.6 mm × 150 mm × 150 mm) was used. The theoretical plate number (N) in the samples was determined using the system integrated processing program.

Preparation of Stock and Standard Solutions Used for LC Measurements

The stock solution was prepared by weighing accurately 20 mg each of glucose, fructose, and sucrose, and added with sufficient amount of distilled water to make 200 μg/mL. It was further diluted with distilled water to obtain different concentrations (6.25 μg/mL, 12.5 μg/mL, 25.0 μg/mL, 50 μg/mL, and 100 μg/mL) of standard solutions. Solutions with known concentrations of glucose (17.3 μg/mL), fructose (18.2 μg/mL), and sucrose (18.2 μg/mL) for each sample were prepared to determine the accuracy (n = 10). The amounts of each compounds recovered were estimated.

Evaluation and Validation of the Proposed Method

Glucose, fructose, and sucrose were quantified using their respective standard solutions respectively and evaluated by calculating the linearity and correlation coefficient (R) from the calibration curve of each sample (n = 10). The reproducibility and precision were evaluated by the relative standard deviation (RSD) of glucose, fructose, and sucrose at repeated concentrations of base (n = 10). The addition recovery test and the degree of separation were evaluated by measuring the known concentrations of glucose, fructose, and sucrose (n = 10). The limit of detection (LOD) and limit of quantification (LOQ) were also calculated from the following equations.

Eq. (1): $LOD=3.3\times(s/a)$
Eq. (2): $LOQ=10\times(s/a)$

Where, s: SD of the intercept for calibration curve; a: slope of the calibration curve.

Sugar Content Measurement

Each fruit wine (plum wine and yuzu wine) was measured using a handheld refractometer (MASTER-M [ATAGO CO., LTD.]) (n = 3).

Surface Tension Measurement

A sample (5 mL) of each fruit wine was placed in a petri dish of 3 cm diameter and set on a measuring table. The measurement

Table 1: Raw materials of fruit wine

Fruit wine	Company	Alcohol content (%)	Additves materials
Plum wine	A	10	Plum, spirits, sugars, acidulant, flavor
	B	10	Plum, sugar, alcohol, brandy
	C	11	Plum, refined sake, sugars
Yuzu wine	A	10	Yuzu, spirits, sugars, acidulant, flavor
	B	7	Fruit juice (yuzu and lime), sugar, alcohol
	C	9	Refined sake, yuzu juice, lemon juice

Company A; Company B; Company C

was performed using a DY500 high-performance surface tensiometer (Dyne Master, Kyowa Interface Science Co., LTD.) (*n* = 3). In addition, 10% ethanol solution and distilled water were measured as reference sample solutions.

Determination of Amino Acids

Each fruit wine was assayed using JLC-500/V (Japan Information Processing Service Co., LTD.). Type B, AN-II, and H amino acid mixture standards were used. As a pre-treatment, the alcohol in each fruit wine was evaporated, and the water content was removed by freeze-drying.

RESULTS AND DISCUSSION

Validity Assessment

Linearity, LOD, and LOQ of LC measurements

The chromatographs of glucose, fructose, and sucrose, representative of a standard mixture of the three sugars, are shown in Figure 1. Specific peaks were identified at retention times of 14.68 min, 16.22 min, and 19.29 min for glucose, fructose, and sucrose, respectively [Figure 1]. To determine the concentration of each sugar, calibration curves were constructed from the standard solutions of glucose, fructose, and sucrose and the correlation coefficients (R) were calculated [Figure 2]. The correlation coefficient of the calibration curves for glucose, fructose, and sucrose was 0.9958, 0.9949, and 0.9958, respectively. Since all the sugars had a correlation coefficient: R > 0.994, it can be inferred that glucose, fructose, and sucrose showed good linearity. The LOD and LOQ of glucose were 1.9 and 5.9 ng/mL; fructose were 3.2 and 9.7 ng/mL; and sucrose were 1.2 and 4.0 ng/mL, respectively [Table 2].

Resolution, trueness, reproducibility, and precision

To investigate the separation of glucose, fructose, and sucrose from the solvent-derived peaks, the degree of separation (Rs) was calculated. To assess trueness, recoveries for known concentrations of glucose, fructose, and sucrose were calculated.[13,15] For the evaluation of reproducibility and precision, the same conditions were used for repeated measurements (*n* = 10) and the RSD was calculated [Table 3]. The resolution of glucose, fructose, and sucrose was Rs = 1.64, 2.21, and 3.62, respectively. The resolution of all sugars was Rs. >1.5, suggesting that sufficient resolution was achieved. The recoveries of glucose, fructose, and sucrose were 104.8 ± 1.10%, 101.2 ± 2.51%, and 103.4 ± 1.73%, respectively. The recoveries of glucose, fructose, and sucrose were 100 ± 5%, and the RSD was within 5%. The RSDs of glucose, fructose, and sucrose were 1.08%, 2.53%, and 1.72%, respectively. Since the RSD of all sugars was <5%, the reproducibility of this method was good and satisfactory precision was obtained. These results indicate that the quantification conditions are suitable and that it is possible to simultaneously quantify glucose, fructose, and sucrose in fruit wine.

Determination of Sugars in Fruit Wine

Determination of sugars in wine using LC-ECD

The chromatographs of plum wine and yuzu wine from Company A, Company B, and the local industry, Company C,

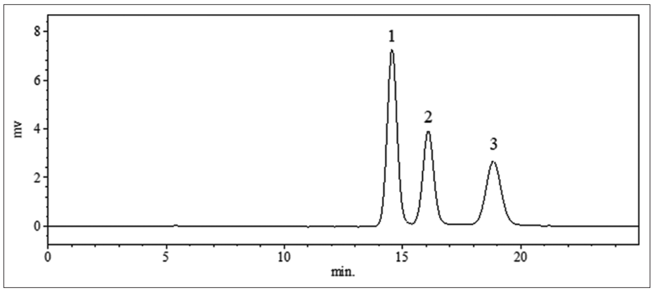

Figure 1: The chromatogram of standard substances peaks: (1) Glucose; (2) fructose; and (3) sucrose

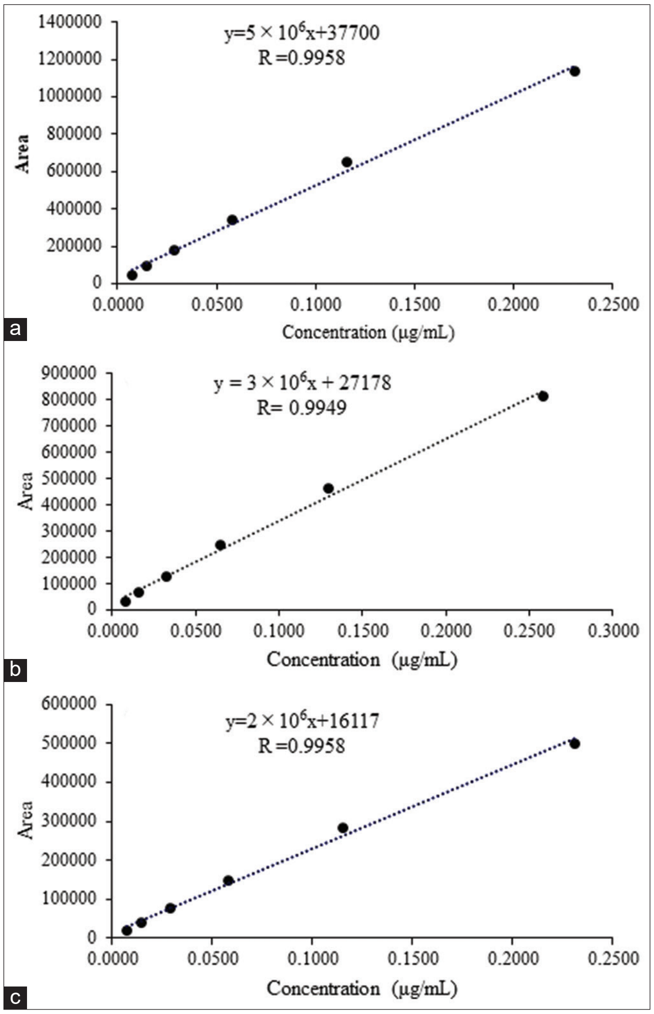

Figure 2: Calibration curves of samples by ECD: (a) Glucose; (b) fructose; and (c) sucrose

Table 2: Limits of determination and quantitation

	LOD (ng/mL)	LOQ (ng/mL)
Standard substance	3.3s/a	10s/a
Glucose	1.9	5.9
Fructose	3.2	9.7
Sucrose	1.2	4

s: SD of absorbance of blank sample, a: The slope of the calibration curve near the detection limit are shown in Figure 3. The composition of the sugars is also summarized in Table 4. Glucose and fructose peaks appeared in all fruit wines, but there were no sucrose peaks in ume wine A and yuzu wine A and C. Glucose and fructose in plum wine A were 2.127 g/100 g and 3.727 g/100 g, respectively. Glucose, fructose, and sucrose in plum wine B were 7.940 g/100 g, 7.801 g/100 g, and 0.47 g/100 g, respectively. Glucose, fructose, and sucrose in plum wine C were 9.353 g/100 g, 8.260 g/100 g, and 0.688 g/100 g, respectively. When the sugar content was compared among the plum wines, plum wine A had the lowest sugar content.

Table 3: Validation of the proposed methods

Standard substance	Rs	Precision	
		Recovery (%)	RSD (%)
Glucose	1.64	104.8±1.10	1.08
Fructose	2.21	101.2±2.51	2.53
Sucrose	3.62	103.4±1.73	1.72

The results are expressed as the mean±SD (*n*=10)

Glucose and fructose of yuzu wine A were 1.881 g/100 g and 3.531 g/100 g, respectively. Glucose, fructose, and sucrose of yuzu wine B were 4.674 g/100 g, 4.707 g/100 g, and 0.452 g/100 g, respectively. Glucose and fructose in yuzu wine C were 1.947 g/100 g and 2.675 g/100 g, respectively. A comparison of the sugar content of the different types of yuzu showed that yuzu wine C had the lowest sugar content. When fruit wines were compared in terms of sugar content, plum wine A and yuzu wine C had the lowest sugar content, suggesting a benefit to the body in terms of sustained drinking. In addition, it was suggested that it is possible to simultaneously quantify the quality of carbohydrates and the carbohydrates contained in fruit wine with a simple method using the separation by core-shell column and the detection method using LC-ECD, and that it is possible to evaluate plum wine from the quantification results.

Determination of sugar (sucrose) using refractometer

In addition to the determination of sugar content as an indicator of sweetness, a comparison was also made using

Table 4: Contents of glucose, fructose, and sucrose in plum wine, yuzu wine, and red perilla wine

Sample	Glucose (g/100g)	Fructose (g/100g)	Sucrose (g/100 g)
Plum wine A	2.127±0.014	3.727±0.037	-
Plum wine B	7.940±0.100	7.801±0.011	0.470±0.019
Plum wine C	9.353±0.095	8.260±0.046	0.688±0.074
Yuzu wine A	1.881±0.039	3.531±0.048	-
Yuzu wine B	4.674±0.028	4.707±0.023	0.452±0.180
Yuzu wine C	1.947±0.010	2.675±0.012	-

The results are expressed as the mean±SD (*n*=10). -: Below limit of detection

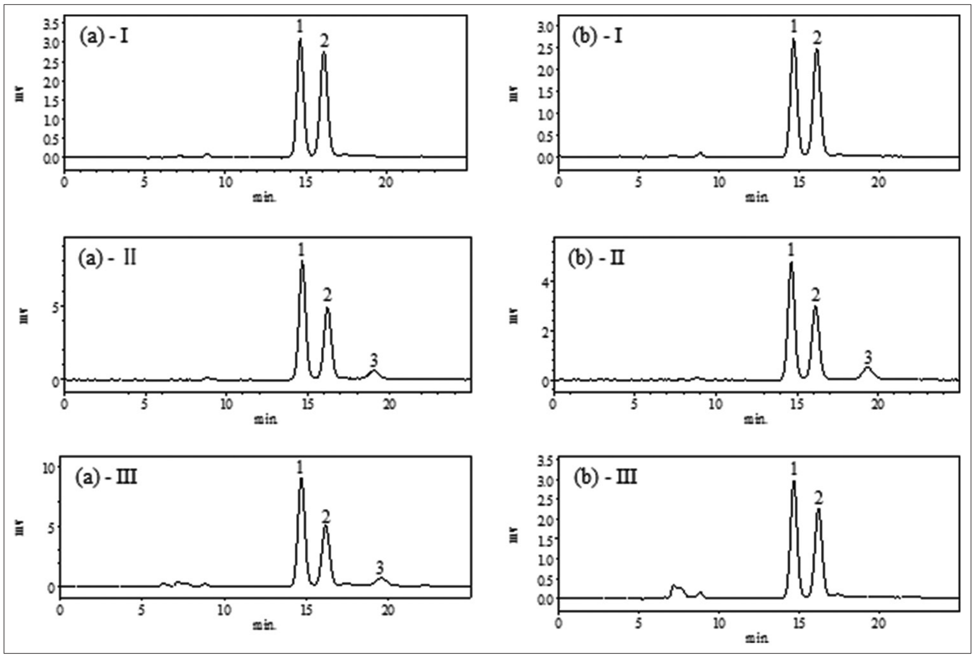

Figure 3: The chromatograms of glucose, fructose, and sucrose in (a) plum wine and (b) yuzu wine. Company: (i) Company A, (II) Company B, and (III) Company C. Peaks: (1) Glucose, (2) fructose, and (3) sucrose

sugar content as a simple method. The sugar content of each fruit wine is shown in Table 5. The sugar content of plum wine was A: 11, B: 20, and C: 25.5. The sugar content of yuzu wine was A: 11, B: 14, and C: 11.5. The sugar content of plum wine C was the highest. The results of the total sugar content determination showed that there is a correlation between the total sugar content of each fruit wine and its sugar content [Table 4], wherein a higher total sugar content [Table 5] reflects high concentrations of glucose, sucrose, and fructose.

Surface tension measurement

The surface tension of each fruit wine was measured to evaluate its relationship with sugar content. The surface tension of each fruit wine is shown in Table 5. No significant relationship was found between sugar content and surface tension, as there was no significant difference in surface tension for plum wine C, which had more sugar content than plum wines A and B. The surface tension of 10% ethanol was 50 mN/m, and the surface tension of yuzu B, which had the lowest alcohol content, showed the highest value, suggesting that the surface tension depends on the alcohol concentration.

pH measurement

The pH of fruit wine samples is shown in Table 5. All the fruit wines had a pH of < 4. Of these, Company A's fruit wine had a particularly low pH, and there was a large difference in the pH of the same fruit wine. The pH of many commercially available beverages is <4, and it is believed that beverages with a pH of <4 could be a cause of dental acid erosion.[16] This is not an exception as the pH of fruit wine is also low. We are keen to ensure that consumers are choosing fruit wines that are beneficial to their health.

Table 5: Brix, pH, surface tension of plum wine, yuzu wine, and red perilla wine.

Sample	Brix	Surface tension (mN/m)	pH
Plum wine A	11	49.36±0.06	2.49±0.02
Plum wine B	20	48.08±0.11	2.76±0.01
Plum wine C	25.5	46.77±0.45	3.12±0.01
Yuzu wine A	11	49.71±0.51	2.43±0.02
Yuzu wine B	14	52.09±0.06	2.68±0.01
Yuzu wine C	11.5	49.33±0.22	3.10±0.01

Amino acid measurement

The amino acid contents of fruit wines are shown in Table 6. In plum wine C, almost all amino acid were found to be of higher values when compared with A and B. Plum wine C contained 2.27 μmol/mL of alanine, 0.67 μmol/mL of GABA, and 5.28 μmol/mL of glutamic acid, which were particularly high compared to other plum wines. It is reported that GABA can relieve tension and stress, calm brain excitement, improve sleep quality, and lower blood pressure in people with high blood pressure.[17] It is also reported that alanine contribute to flavor and sweetness, while glutamic acid contributes to sourness and astringency of wines.[18] Since amino acids give plum wine its flavor, richness, and the range of taste, it can be inferred from the results of amino acid measurements that a fruit wine with a high amino acid content, such as sample C plum wine, is rich, drinkable, and has solid taste. However, if the amount of amino acids is too high, it can be perceived as a miscellaneous taste, and it can also cause premature deterioration of quality such as discoloration. On the other hand, sake with relatively low amino acid content has a light and refreshing taste. In recent years, sports drinks containing amino acids (branched-chain amino acids) have become more popular, and there is a growing interest in understanding the effects of amino acids in the body. It is thought that the amino acids characteristics of plum wine will clarify its relationship with the quality and taste of wine that could aid in the development of new characteristic taste of plum wine.

Different types of plum wines are produced in Japan. In this paper, our laboratory has only analyzed commercially available fruit wines as a case study. In the future, we would like to study fruit wines prepared by local industries (including, for example, *shiso* wine and mandarin wine) using the simple LC-ECD with core-shell column as demonstrated in this study. The choice of beverages (e.g., sugar free and alcohol free) is usually shaped by our health concerns. Hence, the selection of fruit wine in consideration of amino acids as the basis of protein source may also play a role in health and longevity. Finally, fruit wine produced from natural products may vary depending on the raw materials and fermentation process used in production. Each fruit wine in this study was purchased commercially from a lot sold in the market. In the future, we will increase the number of N to plan for the validity of the analytical method and continue to study simpler measurement conditions.

Table 6: Comparison of amino acid composition of plum wine and yuzu wine

Amino acid (μmol/mL)	Plum wine A	Plum wine B	Plum wine C	Yuzu wine A	Yuzu wine B	Yuzu wine C
Ala	N. D	0.238	2.269	N. D	0.232	1.512
Arg	N. D	0.241	0.427	N. D	0.051	0.411
Asp	N. D	0.331	0.776	N. D	0.807	0.381
GABA	N. D	0.169	0.672	N. D	0.141	0.116
Glu	N. D	0.979	5.279	N. D	0.261	0.628
Gly	N. D	0.001	1.095	N. D	0.018	0.845
Leu	N. D	0.069	0.748	N. D	0.009	0.429
Lys	N. D	0.027	0.311	N. D	0.012	0.141
Val	N. D	0.047	0.698	N. D	0.017	0.347

N.D: Not detected

CONCLUSION

The separation method by core-shell column and the detection method using LC-ECD proved that a simultaneous determination of glucose, fructose, and sucrose in fruit wine was possible without the need for derivatization. Differences in the content of glucose, fructose, and sucrose in plum wine samples could be evaluated simultaneously. Core-shell columns in tandem with ECD are a method capable of quantifying amino acids in fruit wines. In addition, the LC-ECD method provides a robust, reliable, and comparable procedure to determine total sugar content in wines with conventional refractometers. This straightforward LC technique is reliable in the determination of carbohydrates and amino acid contents in wine, useful criteria for consumers in selecting wines parallel to their ideal health outcomes.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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