

TECHNICAL NOTE

Simple differentiation of sake (Japanese alcoholic beverage) based on trace inorganic components using colorimetric methods

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Several colorimetric methods were combined and used for the discrimination of commercial sake samples, based on their constituent inorganic components. The method was very rapid, simple, and did not require expensive equipment. Further, we showed that this method has potential application in immediate differentiation of sake by the visual inspection.

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In recent years, the exportation of the Japanese traditional alcoholic beverage, sake, has increased remarkably owing to the escalating demand worldwide. Sake is brewed using sake yeast (*Saccharomyces cerevisiae*) with steamed rice, *koji* (*Aspergillus oryzae*, cultured on steamed rice), and a significant amount of water (1). This alcoholic beverage is known to contain various components (e.g., carbohydrates, organic acids, nitrogenous compounds, inorganic species), which contribute to the quality of the product (2).

The inorganic components of various alcoholic beverages are often studied owing to their effects on product quality (3–5). In addition, such analyses have recently been used not only for quality control, but also to confirm the authenticity of the products and detect counterfeit goods (5–7). These discrimination techniques are widely demanded in order to decrease economic loss owing to counterfeit products; however, no studies have been conducted regarding the discrimination of sake based on constituent inorganic components. Moreover, the direct determination of inorganic species in sake is typically carried out using techniques such as inductively coupled plasma spectrometry (8), atomic absorption spectrometry (9,10), and ion chromatography (11); such analyses are very difficult for sake brewers, because they require both expensive equipment and technical skills.

Another analytical method used in the determination of food/beverage components is colorimetric determination, which has been widely utilized for some time (12). Colorimetric assays are excellent, simple, and rapid methods, and the results can be immediately analyzed by the naked eye. Therefore, in this study, we combined several colorimetric methods for the discrimination of commercial sake products, based on the composition of constituent trace elements. The method was very rapid, easy to set up, and did

not require expensive equipment. Further, we showed the possibility that by this method, commercial sake products could be differentiated visually.

Commercial sake samples from different sake breweries in Japan were purchased at retail liquor stores. The colorimetric assays for trace elements such as Ca^{2+} , Mg^{2+} , and Zn^{2+} in sake were carried out using the Metallo Assay LS series (Metallogenics, Chiba, Japan). Specifically, Ca^{2+} was determined using the calcium assay kit by the *o*-cresolphthalein method, Mg^{2+} was determined using the magnesium assay kit by the xylydyl blue-I method, and Zn^{2+} was determined using the zinc assay kit by the 5-Br-PAPS [2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfo-propylamino) phenol] method. Cl^- and PO_4^{3-} were measured using the QuantiChrom Assay kit series (BioAssay Systems, Hayward, CA, USA). Cl^- was determined using the chloride assay based on the Fried method, and PO_4^{3-} was determined using the phosphate assay kit based on the Malachite Green method. For all determinations, sake was used directly in the assays without extraction or purification, and according to the kit instructions.

Instrumental determinations of Ca^{2+} , Mg^{2+} , and Zn^{2+} were carried out using an atomic absorption spectrophotometer AA-7000 (Shimadzu, Kyoto, Japan), according to the diluted injection method described by Namba et al. (9,10). Cl^- and PO_4^{3-} were detected using an ion chromatograph IC-2001 (Tosoh, Tokyo, Japan) according to instructions provided by the manufacturer. The sake sample was diluted 20-fold with deionized water, and then was passed through a Toyopak IC-SP S cartridge (Tosoh). The following conditions were used for the ion chromatographic analysis: column: TSKgel SuperIC-AZ column (4.6 mm I.D. × 150 mm, Tosoh); eluent: 1.9 mM NaHCO_3 and 3.2 mM Na_2CO_3 ; flow rate: 0.8 mL/min; column temperature: 40°C; injection volume: 30 μL ; detection: conductivity detector.

First, to test whether or not the colorimetric assays could be used to detect the amounts of the various trace elements, we compared the results of the colorimetric methods to those of the

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instrumental analyses. Ca^{2+} , Mg^{2+} , Zn^{2+} , Cl^- , and PO_4^{3-} were analyzed in 25 samples of commercial sake produced in Japan. The results are shown in Fig. 1. For all of the target ions in the sake samples, linear correlations between the amount measured by the colorimetric methods and by the instrumental techniques were

observed (atomic absorption spectrometric method vs. colorimetric method: Ca^{2+} , $r = 0.949$; Mg^{2+} , $r = 0.997$; Zn^{2+} , $r = 0.951$; ion chromatographic method vs. colorimetric method: Cl^- , $r = 0.966$; PO_4^{3-} , $r = 0.991$). Table 1 shows the limit of detection, limit of quantification, relative standard deviation, and recovery of all ions

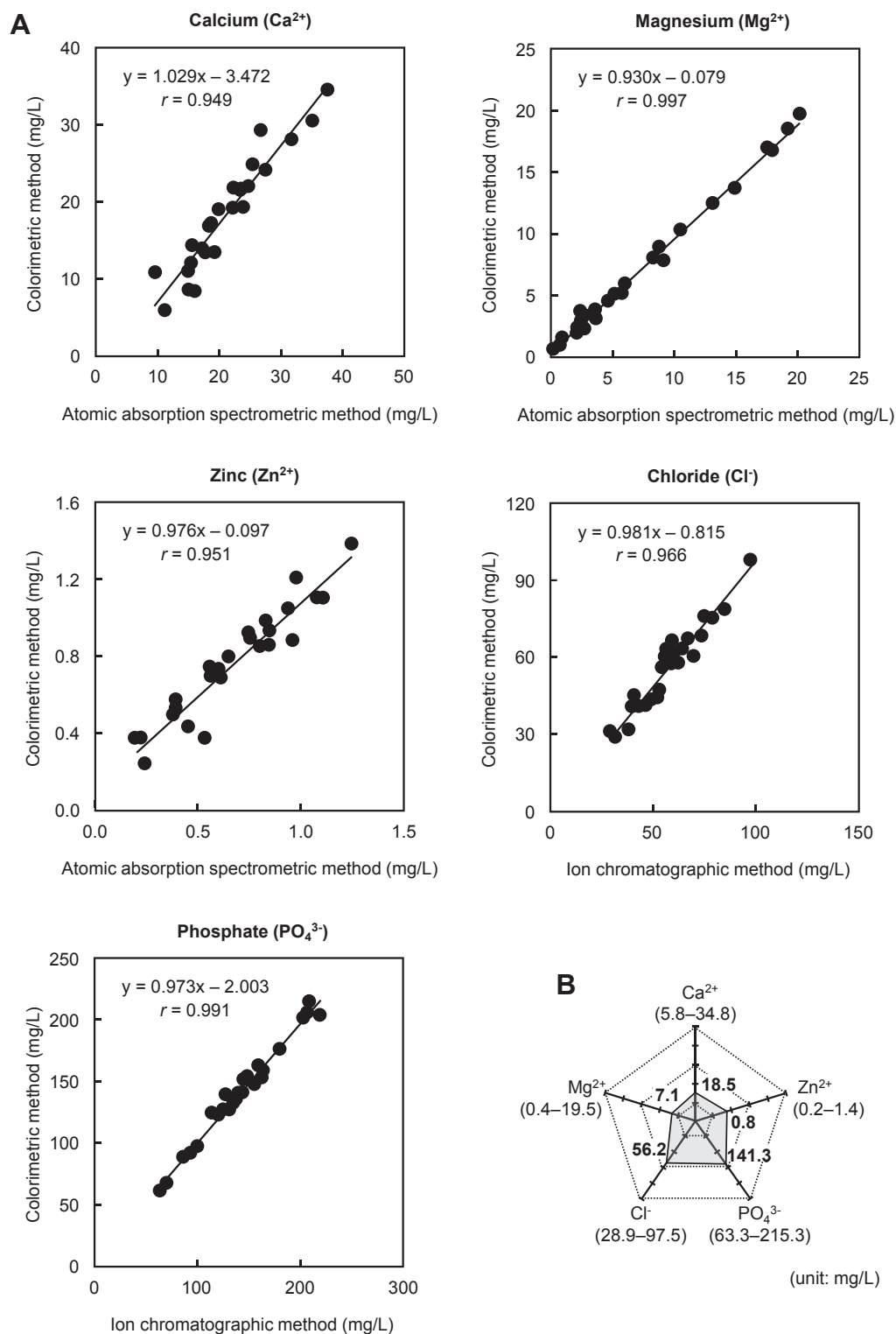


FIG. 1. Determination of five inorganic components in sake using colorimetric methods. Twenty-five samples of commercial sake from Japan were used in this experiment. (A) Correlation between instrumental and colorimetric methods in the measurements of inorganic components of sake. The assays are described in the text. (B) Pentagram diagram of average values of five ions in commercial sake samples determined using colorimetric methods. In the parentheses near each ionic formula, minimum–maximum values of the ions are indicated.

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