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Sample Preparation for Trace Analysis of Iron, Copper and Zinc in Thai Fruit Wines by ICP-AES

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ABSTRACT

Trace analysis of iron (Fe), copper (Cu) and zinc (Zn) in local Thai fruit wines was conducted by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The sample preparation of the fruit wines prior to analysis by optimized ICP-AES conditions was studied by comparing the de-alcoholization and acid digestion methods using conc. HNO_3, conc. H_2SO_4 and H_2O_2. It was found that both methods of sample treatment gave slightly different metal contents in the fruit wines, but the ratio of conc. HNO_3/H_2O_2 (10:1, v/v) was preferred, resulting in the contents of Fe, Cu and Zn in the wine samples ranging from 1.3 - 4.8, 0.050 - 1.3 and 0.37 - 3.4 mg/l, respectively. The mean recoveries of these metals were in the range of 97.9 - 101 %. Similar precision with RSDs of < 4.5 % were obtained. The standard curves showed good linearity with a correlation coefficient of > 0.9956. The LOD (3SD) and LOQ (10SD) for the acid digestion method, estimated from the noise on the signal obtained for its blank, were also investigated.

Keywords: Fruit wine, iron, copper, zinc, ICP-AES

INTRODUCTION

Wine is a well-known alcoholic product with worldwide commercial economics. Its composition and properties are related to the wine's origin and age. The constituents of wine are water, ethanol, saccharides, amino acids, phenolic compounds and other pigments, and trace metals [1-6]. Most metals in wine are also present naturally and their concentration pattern has already been used for origin determination. The mineral contents of wine depend on several factors including soil, location, grape varieties, weather or environmental conditions and viticultural practices [7-9].

At present, increasing global pollution of the environment requires a systematic monitoring of all kinds of food including common beverages. Heavy metals in wines are of interest because of their essential or toxic nature. Some metals such as Fe, Zn, Cu, Cr, Co and Mn are essential, while Pb, As, Cd, Ni and Hg are toxic at certain levels. The excessive presence of Al, Fe, Zn and Cu has found to be a negative effect on the organoleptic properties of wine [10-13]. The compositions of various metals in different wines of various countries have been the subject of many studies [14-17]. Such data are also not readily available for most wine, especially the locally produced fruit wines in Thailand. The study is important because a lot of people consumes large amounts of wine, beer and other alcoholic products. Sometimes, this results in increasing the daily intake of heavy metals above maximum permissible levels. When wine or wine products are consumed in large quantities, the toxic effects of their pollutants may have an additive effect inducing alcoholism. Thus, there is interest in trace metal determinations in wines.

Many analytical methods can be used to determine trace metals in wine. In most methods extensive sample preparations are involved. Some are time-consuming and require many chemicals and sophisticated instruments [10,12,14,18-22]. Recently, very sensitive techniques for trace element analysis have been introduced [23]. ICP-AES is a multi-element method with very good detection power and offers the right conditions for reliable and rapid determination [4,14]. Due to the high temperature of the plasma, less matrix interference is observed. Therefore, the relatively complex organic matrix of wine should have no influence on the measurements and the analysis of the sample may be possible without any pretreatment.

The aim of this study was to compare the treatment methods for sample preparation of wine between a simple de-alcoholization method and acid digestion method with oxidizing agents such as concentrated nitric and sulfuric acid, and hydrogen peroxide prior to ICP-AES measurement. The results of the spiked standard solution and measurement of the wine samples were compared. The present study focused on the determination of Fe, Cu and Zn contents in local Thai wines made from various edible fruits available in markets.

MATERIALS AND METHODS

Reagents

All chemicals used (concentrated HNO_3, concentrated H_2SO_4, and 30 % (v/v) H_2O_2) and buffer solutions pH 4.01 and 7.00 were AR grade from Merck (Germany). The stock standard solutions with 100 mg/l of Cu, Fe and Zn, ICP grade, were from Spex Certiprep (USA). Deionized water was prepared by the Nanopure UV, D7332 (Barnstead, USA).

Apparatus

The ICP spectrometer JY 2000 (Jobin Jyon, France) was used. The ICP-AES instrument was equipped with an RF variable power (800 - 1,200 W) including RF frequency (40.68 MHz), 2,400 grooves grating, cyclonic spray chamber, Meinhard nebulizer and PMT spectrophotometer. In addition, an R3000 rotavapor (Buchi, Germany), pH meter, 520A (Orion, USA), hotplate and stirrer, Cimarec (Thermolyne, USA) ribbed watch glass (Pyrex, UK) and filter membrane 0.45 μm (Whatman, UK) were used.

Wine Samples

Eight wine samples produced in the same year were purchased individually from local retail shops in Khon Kaen, Kalasin province and Bangkok, Thailand. They were commonly classified as a local Thai fruit wine named with different kinds of the raw materials used. Four table wines which are commonly made from grape both white and red were purchased from wine shops in Khon Kaen province, used as labeled sources of the samples (**Table 1**).

In this study, there are 2 groups of local Thai fruit wines, white and red, which are from various production areas both in the Northeast and in Bangkok, Thailand. White wines are generally made from mango, pineapple and carambora fruits. The red ones come from native colored fruits such as mulberry, “maow” known as compiled wild fruit and roselle. Grape wines, used as reference here, are also both white and red originally imported from Europe. The organoleptic tests for these wines have not yet been done by a wine expert, but they look naturally different in their physical and sensory properties such as apparent color and pH. Acidity was therefore estimated by pH meter immediately after opening the wine bottle. **Table 1** shows a description of the apparent color and pH of the wine samples. Certainly, differences in the apparent colors of the white and red wines were found but the color among each group was somewhat distinguishable. The pH values of these wines show no trend in difference ranging from 2.6 to 3.1, except the rather low value found in “maow” wine (pH 2.2), indicating an acidic taste for this common fruit wine.

Table 1 Apparent color and pH of some local wine and market-table wine samples.

Sample	Apparent color	pH
White wine		
Mango wine	Golden yellow	3.1
Pineapple wine	Light white	3.9
Carambora wine	Golden yellow	3.0
Grape wine 01	Light golden yellow	3.0
Grape wine 02	Dark golden yellow	2.6
Red wine		
Mulberry wine 01	Brownish orange	2.9
Mulberry wine 02	Brownish orange	2.8
Maow wine	Pink-red	2.2
Roselle wine 01	Reddish brown	3.1
Roselle wine 02	Reddish brown	2.9
Grape wine 03	Reddish brown	3.1
Grape wine 04	Reddish brown	2.7

Sample Preparation

In preparation of aqueous calibration standards, the organic components of the wine (in particular the alcohol contents) must be removed from the samples by 2 selected methods as follows.

De-Alcoholization Method

Wine samples (50 ml) were de-alcoholized at 80 °C using a reduced pressure evaporator until its total volume was approximately a quarter of its initial volume. It was then kept cool, filtered through Whatman No. 42 filter paper into a 50 ml volumetric flask and the final volume was adjusted with deionized water.

Acid Digestion Method

The wine samples were decomposed according to the following procedure: 25 ml sample volume with 10 ml concentrate HNO_3 in a 50 ml beaker containing a boiling bead covered with a ribbed watch glass was gently evaporated on a hot plate at 70 °C until its total volume was approximately a quarter of its initial volume. It was then kept cool and 1 ml of concentrated H_2SO_4 was added. The mixture was again heated for a while, kept cool, filtered through Whatman No. 42 filter paper into a 25 ml volumetric flask and the final volume was adjusted with deionized water. The digesting procedure was done by HNO_3/H_2O_2 (10:1, v/v) and was carried out with the same manner except for the addition of 1 ml of 30 % (v/v) H_2O_2 instead of concentrated H_2SO_4.

Method Validation

The calibration curves for each metal were obtained from each concentration range used: 0.02 - 3 mg/l Fe, 0.02 - 0.4 mg/l Cu and 0.05 - 2 mg/l Zn. Three replicates of 10 % (v/v) ethanol solution were used as a control for wine samples also digested by

both de-alcoholization and acid digestion methods as mentioned above for the limit of detection (LOD) and limit of quantitation (LOQ) estimation [24]. Percentage recoveries for the analysis of wine samples were carried out using the spiked standard solution valued in mg/l of 1.5 Fe, 0.12 Cu and 0.50 Zn by ICP-AES.

Optimization of ICP-AES

In order to obtain the highest sensitivity, the experimental conditions employed with ICP-AES instrument were preliminary optimized with a radio-frequency (RF) power between 800 - 1,200 W including the flow rates of plasma gas (8 - 14 l/min), sheath gas (0.1 - 0.4 l/min), sample flow (0.8 - 1.8 ml/min) and pressure of nebulizer (2.4 - 3.2 bar).

RESULTS AND DISCUSSION

Optimized Conditions of ICP-AES

The optimum conditions obtained for ICP-AES measurement of Fe, Cu and Zn in Thai fruit wines are summarized in **Table 2**.

Table 2 Operating conditions of ICP-AES for Fe, Cu and Zn determination.

Parameter	Plasma condition
RF power	1,000.0 Watt
Pressure of nebulizer	3.0 Bar
Plasma gas flow	12.0 l/min
Sheath gas flow	0.2 l/min
Sample flow	0.8 ml/min
Element	Wavelength (nm)
Fe	259.940
Cu	324.754
Zn	213.856

Analytical Methods

Concerning the sample preparation, the contents of Fe, Cu and Zn obtained from the de-alcoholization method, found in both white and red fruit wines, were comparable to those of grape wines (**Table 3**). This trend was also observed when the wine samples were treated by acid digestion with a ratio of HNO_3/H_2SO_4 (10:1, v/v). The trace amounts of these metals which were digested by the same ratio of HNO_3/H_2O_2, were considerably higher than those of both procedures. These data were statistically compared, resulting in no significant difference at 95 % confidential level. Therefore,

their contents (mg/l) were found in the ranges of 1.3 - 4.8, 0.050 - 1.3 and 0.37 - 3.4 for Fe, Cu and Zn, respectively.

The standard curves showed a good linearity with r^2 as following: Fe, $y = 140.3x + 5.82$ ($r^2 = 0.9994$); Cu, $y = 1868.8x + 20.76$ ($r^2 = 0.9956$) and Zn, $y = 145.9x + 6.60$ ($r^2 = 0.9998$). On this basis the acid digestion method described here was found to be more suitable. However, the de-alcoholization method is preferred because it is environmentally friendly and has reduced analysis time.

The blank solution that was prepared from 10 % (v/v) ethanol solution, used as a control for wine sample, was also investigated by both methods. Both LOD (3SD) and LOQ (10SD) estimated from the noise on the signal were obtained for its blank. It was found that the LODs (LOQs) for Fe, Cu and Zn as prepared by the de-alcoholization method were 173 (392), 21.9 (35.4) and 48.2 (78.1) μg/l, respectively. Digestion by HNO_3/H_2SO_4 (10:1, v/v) gave LODs (LOQs) for Fe, Cu and Zn to be 123 (360), 17.8 (26.7) and 43.7 (88.5) μg/l, respectively. Digestion by HNO_3/H_2O_2 (10:1, v/v) gave LODs (LOQs) for Fe, Cu and Zn to be 20.4 (30.1), 17.6 (36.0) and 20.2 (32.6) μg/l, respectively.

The method recovery was carried out using the spiked wine samples. The average values (%) for the data (**Table 4**) of different wine samples were as follows: Fe 92.7, Cu 94.0, Zn 91.4 (preparation by de-alcoholization); Fe 102, Cu 95.2, Zn 95.6 (digestion with HNO_3/H_2SO_4) and Fe 101, Cu 100, Zn 99.7 (digestion with HNO_3/H_2O_2). Similar precision with RSDs of 0.40 - 4.5 % for these metals were obtained.

Based on the obtained results of method recovery of these heavy metals, the sample treatment by de-alcoholization method gave considerably low values (< 95 %) for all metals studied. This is due to labile species of Fe, Cu and Zn organically bound matrices [22]. Thus the fruit wine samples are still needed to digest by strong oxidizing agents like those mixtures of the acids used in this experiment. However, it is somewhat difficult to get the highest percentage recovery depending on both trace level analysis of the metals and the nature of fruit wines. Analysis of certified materials for these metals is therefore considered to make comparing found in almost recent data [7].

Table 3 Iron, copper and zinc contents found in wine samples as prepared by 3 different preparations and followed by ICP-AES.

Sample/	**Metal concentrations (mg/l)**								
Preparation method/	**De-alcoholization method**			**HNO_3/H_2SO_4 (10:1, v/v) digestion**			**HNO_3/H_2O_2 (10:1, v/v) digestion**		
Metal	**Fe**	**Cu**	**Zn**	**Fe**	**Cu**	**Zn**	**Fe**	**Cu**	**Zn**
White wine									
Mango wine	1.81 ± 0.01	0.09 ± 0.002	0.32 ± 0.01	1.65 ± 0.01	0.08 ± 0.002	0.31 ± 0.01	2.13 ± 0.01	0.10 ± 0.002	0.37 ± 0.01
Pineapple wine	1.77 ± 0.01	0.05 ± 0.002	1.62 ± 0.09	1.75 ± 0.01	0.04 ± 0.002	1.21 ± 0.09	1.79 ± 0.01	0.06 ± 0.002	2.01 ± 0.09
Carambora wine	4.50 ± 0.08	0.11 ± 0.002	1.96 ± 0.05	4.35 ± 0.08	0.08 ± 0.002	1.66 ± 0.05	4.79 ± 0.08	0.15 ± 0.002	2.44 ± 0.05
Grape wine 01	1.93 ± 0.01	0.16 ± 0.001	0.33 ± 0.02	1.89 ± 0.01	0.11 ± 0.001	0.33 ± 0.02	2.02 ± 0.01	0.19 ± 0.001	0.99 ± 0.02
Grape wine 02	3.62 ± 0.03	1.11 ± 0.006	0.57 ± 0.01	3.59 ± 0.03	0.92 ± 0.006	0.55 ± 0.01	3.91 ± 0.03	1.24 ± 0.006	0.60 ± 0.01
Red wine									
Mulberry wine 01	3.79 ± 0.04	0.04 ± 0.002	2.91 ± 0.05	3.76 ± 0.04	0.03 ± 0.001	2.52 ± 0.05	3.92 ± 0.04	0.05 ± 0.002	3.40 ± 0.05
Mulberry wine 02	4.46 ± 0.09	1.10 ± 0.008	1.30 ± 0.06	4.25 ± 0.09	1.10 ± 0.008	1.29 ± 0.06	4.76 ± 0.09	1.30 ± 0.008	1.32 ± 0.06
Maow wine	2.39 ± 0.01	0.05 ± 0.003	0.47 ± 0.01	2.35 ± 0.01	0.05 ± 0.00	0.41 ± 0.01	2.45 ± 0.01	0.07 ± 0.003	0.56 ± 0.01
Roselle wine 01	3.42 ± 0.02	0.05 ± 0.001	2.62 ± 0.13	3.38 ± 0.02	0.04 ± 0.001	2.58 ± 0.13	3.57 ± 0.02	0.07 ± 0.001	3.39 ± 0.13
Roselle wine 02	1.30 ± 0.05	0.07 ± 0.002	1.52 ± 0.06	1.29 ± 0.05	0.07 ± 0.002	1.46 ± 0.06	1.32 ± 0.05	0.09 ± 0.002	1.59 ± 0.06
Grape wine 03	1.88 ± 0.02	0.12 ± 0.005	0.32 ± 0.01	1.82 ± 0.02	0.10 ± 0.005	0.31 ± 0.01	1.97 ± 0.02	0.17 ± 0.005	1.07 ± 0.02
Grape wine 04	1.60 ± 0.01	1.07 ± 0.009	0.65 ± 0.02	1.58 ± 0.01	1.03 ± 0.06	0.43 ± 0.02	1.62 ± 0.01	1.12 ± 0.009	1.07 ± 0.02

Table 4 Recoveries of Fe, Cu and Zn in the spiked wine samples using 3 preparation methods.

Wine sample/	**Recovery (%)**								
Preparation method/	**De-alcoholization method**			**HNO_3/H_2SO_4 (10:1, v/v) digestion**			**HNO_3/H_2O_2 (10:1, v/v) digestion**		
Metal	**Fe**	**Cu**	**Zn**	**Fe**	**Cu**	**Zn**	**Fe**	**Cu**	**Zn**
Mango wine	92.0	100	88.0	96.0	91.7	88.0	97.3	91.7	98.0
Pineapple wine	92.7	100	96.0	103	83.3	96.0	101	108	98.0
Grape wine 01	98.7	91.7	86.0	109	91.7	90.0	96.0	108	86.0
Grape wine 02	89.3	91.7	94.0	101	91.7	96.0	109	108	108
Maow wine	86.7	91.7	84.0	107	108	106	90.7	83.3	110
Roselle wine 01	95.3	91.7	94.0	102	91.7	108	103	91.7	106
Grape wine 04	94.0	91.7	98.0	95.3	108	92.0	107	108	92.0

CONCLUSIONS

It is concluded that the determination of Fe, Cu and Zn in local Thai fruit wines can be conducted with the sample preparation by either a simple de-alcoholization or acid digestion method prior to the ICP-AES measurement. An approach on the sample preparation of wine for trace metal analysis has been so far studied by different analytical techniques. That is indeed needed a procedure with less time-consuming and reduction of chemicals used. Thus, the reliable method with accuracy and precision should be rapid and convenient for almost wine matrices. From this study, it was found that the results obtained from the de-alcoholization method were comparable to those of the acid digestion method. Although both procedures gave slightly different metal contents, digestion with a ratio of HNO_3/H_2O_2 (10:1, v/v) gave the highest values. However, the metal contents in the fruit wine samples varied depending on the fruit species. In both white and red wine samples, the values are somewhat differences among themselves as well. Therefore, the applicability of both methods for the sample treatment becomes a clear matter of choice for trace analysis of these heavy metals in different wine matrices.

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บทคัดย่อ

ริกาญจน์ คุณโน เฉลิม เรืองวิริยะชัย และ ศักดิ์สิทธิ์ จันทร์ไทย

การเตรียมตัวอย่างสำหรับวิเคราะห์เหล็ก ทองแดง และสังกะสีปริมาณน้อย ในไวน์ผลไม้ไทยโดยเทคนิค ICP-AES

ได้ทำการวิเคราะห์โลหะเหล็ก ทองแดง และสังกะสีที่มีปริมาณน้อยๆ ในตัวอย่างไวน์ผลไม้ของไทยโดยใช้เทคนิค ICP-AES โดยศึกษาเปรียบเทียบวิธีเตรียมตัวอย่างไวน์ผลไม้ด้วยวิธีระเหยอัลกอฮอล์ และวิธีย่อยด้วยส่วนผสมของกรดไนตริกเข้มข้น กรดซัลฟูริกเข้มข้น และไฮโดรเจนเปอร์ออกไซด์ ก่อนนำไปตรวจวัดภายใต้สภาวะที่เหมาะสมของ ICP-AES พบว่า การเตรียมตัวอย่างทั้งสองวิธีนี้ให้ผลการวิเคราะห์ปริมาณโลหะในไวน์แตกต่างกันเพียงเล็กน้อย แต่ส่วนผสมของกรด ไนตริกเข้มข้นและไฮโดรเจนเปอร์ออกไซด์ในอัตราส่วน 10 ต่อ 1 โดยปริมาตรนั้นให้ผลการวิเคราะห์สูงกว่า คือหาปริมาณเหล็ก ทองแดง และสังกะสีในตัวอย่างไวน์ผลไม้อยู่ในช่วง 1.3 - 4.8, 0.050 - 1.3 และ 0.37 - 3.4 มิลลิกรัมต่อลิตร ตามลำดับ ให้ค่าปริมาณสารคืนกลับโดยเฉลี่ยของโลหะเหล่านี้อยู่ในช่วง 97.9 - 101 เปอร์เซ็นต์ แสดงความแม่นยำของวิธีในเทอมค่าเบี่ยงเบนมาตรฐานสัมพัทธ์เหมือนกันในช่วงต่ำกว่า 4.5 เปอร์เซ็นต์ ได้กราฟมาตรฐานที่ให้ช่วงเส้นตรงด้วยค่าสัมประสิทธิ์สหสัมพันธ์มากกว่า 0.9956 นอกจากนี้ได้ประเมินค่าขีดจำกัดต่ำสุดของการตรวจวัด (3SD) และขีดจำกัดต่ำสุดของการหาปริมาณ (10SD) ของวิธีย่อยด้วยกรดโดยพิจารณาจากการเทียบสัญญาณที่ได้กับสัญญาณรบกวนของพื้นหลัง

ศูนย์ความเป็นเลิศด้านนวัตกรรมทางเคมี ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002