

Determination of Copper in Clarified Apple Juices

MICHAELA ZEINER,^{*,†} IVA JURANOVIĆ CINDRIĆ,[‡] MICHAELA KRÖPPL,[§] AND
 GERHARD STINGEDER[†]

[†]Division of Analytical Chemistry, Department of Chemistry, University of Natural Resources and Applied Life Sciences (BOKU), Muthgasse 18, 1190 Vienna, Austria, [‡]Laboratory of Analytical Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia, and [§]University of Applied Sciences Upper Austria, Campus Wels, Stelzhamerstraße 23, 4600 Wels, Austria

Inorganic copper compounds are not considered as synthetic fertilizers for apple trees as they are traditional fertilizers. Thus, they are used in organic farming for soil or foliar applications. The European Union is for health reasons interested in reducing copper in apple orchards. Because the fertilizer application rate affects the nutrition of apples, the applied copper might also be reflected in the copper concentration of apple juices. Thus, the determination of copper is of concern for investigating the application of copper-containing fertilizers. Samples of clarified apple juice commercially available in the European market were analyzed for their copper content. Prior to quantification by inductively coupled plasma–optical emission spectrometry, the juices were processed by a microwave-assisted digestion system using HNO₃. All samples were also measured directly after dilution with HNO₃. The copper concentrations measured using both methods were all below the limit of detection (17 μg/L).

KEYWORDS: Apple juice; copper; ICP-OES; organic farming; fertilizer

INTRODUCTION

Fruit juices are nutritious and popular drinks. Because of the increased awareness of a healthy lifestyle, the consumption and relevance of these beverages are important parts of human nutrition. Although orange juice is the highest per capita consumption fruit juice in the world, European consumers prefer apple juice to all others (1). The consumption of apple juice rapidly increased in the 1990s and is now constant at a high per capita level of approximately 13 L/year (1).

As an example, apples are the favorite fruit of Austrians. In the seasons 2003/04 in Austria, 422585 tons of apples was produced and 150000 tons was processed to juice and distillate (2). A total of 15000 workplaces in Austria are related to growing apples as well as to downstream work fields such as apple juice production (3). The manufacturing of fruit juice is regulated by EC (European Community) directives and by national ones, such as the “Fruchtsaftverordnung” (fruit juice regulation) in Austria (4). The legal basis of fruit juice manufacturing has fundamentally changed due to Council Directive 2001/112/EC (5). On February 17, 2004, the new “Fruchtsaftverordnung” came into effect. The provisions of article 3 now authorize the addition of vitamins and minerals to the products defined as fruit juices, so that 100 mL of the product contains at least 15% of the recommended daily allowance [RDA; for Cu: United States, 1.5–3 mg; EU, 1.2 mg (6)].

Because of the elevated ecological awareness nowadays, products of organic agriculture are preferably consumed. Organic

farming means the exclusion of using synthetic inputs, such as synthetic fertilizers and pesticides or genetically modified organisms. The inorganic composition of the soils in the orchards, however, is not regulated (7). Furthermore, inorganic copper compounds, such as copper hydroxide, copper oxychloride, (tribasic) copper sulfate, and cuprous oxide, which are traditional fertilizers for apple trees, are not considered to be synthetic fertilizers; thus, they are also used in organic farming for soil or foliar application to prevent malnutrition or as a fungicide. The application of copper in organic farming has often been criticized, since it is more toxic and persistent than some modern synthetic pesticides. However, organic apple growers are highly dependent on the use of sulfur, lime sulfur, and copper fungicides to control apple scab, which causes serious losses in quality and yield of both organically and conventionally grown apples (8). The problem with the accumulation of copper in the soil led to a reduction of copper usage in organic agriculture within the EU after 2002 [Council Regulation (EEC—European Economic Community) No. 2092/91] (9). Alternatives for copper include the application of clay minerals and the use of resistant varieties (10).

Copper is the third most abundant trace element in the human body. Although copper is present in the human body, only in small amounts ranging from 50 to 250 mg, its importance should not be overlooked in various biochemical processes. It forms part of more than 13 enzymes that are involved in energy production, the prevention of anemia and bone disease, and the reduction of cell damage and is necessary in fetal and infant development. Furthermore, copper is necessary for many other functions, for example, maintenance of connective tissue and pigmentation of hair and skin (11). Besides the beneficial effects, copper can cause

*To whom correspondence should be addressed. Tel: +43-1-36006-6547. Fax: +43-1-36006-6059. E-mail: michaela.zeiner@boku.ac.at.

toxic reactions in cases of excessively elevated intake, with the target organs being liver, kidneys, nonstriated and heart muscle tissue, and the brain (12). To avoid an overdose of copper from drinking apple juice, a limit concentration for copper in apple juice is given in the Codex Alimentarius Austriacus (5 mg/L) (13). In the guidelines for mixed fruit juices by the Codex Alimentarius Commission (CAC), the copper limit concentration is stated as 5 mg/kg (14). The Codex Alimentarius (food code) is a joint program of the FAO (the UN's Food and Agriculture Organization) and the WHO (World Health Organization), of which the European Community has been an involved member since the latter half of 2003.

Thus, the determination of copper in commonly consumed food stuffs and beverages is of concern, especially since there is a lack of information on metal contents in apple juices. Only a few recent papers deal with this topic (15). Because of changes in the application rate of copper compounds in apple growing, data from the 1960s cannot be used any more as reference values. At that time, about 0.27 mg/L Cu was found in clarified apple juices from England (16), and a Russian study reported copper contents up to 1.05 mg/kg (17). The Cu concentrations determined in the 1980s were also in that range of magnitude, namely, 0.32 mg/kg (18).

In the present work, commercially available clarified apple juices were analyzed for their copper content after optimization and validation of the appropriate analytical method, inductively coupled plasma–optical emission spectrometry (ICP-OES). The study is focused on copper since this is of concern within the research of the European Community. Other elements that form a significant part of the human diet will be part of further investigations on this topic. Although the maximum allowable copper concentration is 5 mg/kg, much lower concentrations are expected from data given in literature. Furthermore, to leave the door open for determination of a broadened set of elements, ICP-OES was chosen. Other possible methods, but only for single element analysis, are UV–Vis spectrometry with a limit of detection (LOD) of about 5 µg/L (19) or graphite furnace atomic absorption spectrometry [GFAAS; LOD = 0.5 mg/L (20)]. The disadvantage of GFAAS is that it is time-consuming, about 20 min for measurements in triplicate with modifier applications. Regarding UV–Vis spectrometry, the sample preparation (generating a stable-colored complex) is time- and labor-intensive.

Juices were measured on the one hand directly, only diluted with 2% HNO₃, and on the other hand after microwave-assisted digestion using HNO₃. Apple juices contain about 120 g/L sugars, and organic matter is supposed to cause interferences in ICP-OES measurements. By the microwave-assisted digestion procedure, the organic matter is destroyed, and its influence on the results obtained is minimized. Thus, a comparison of these two sample preparation methods seemed useful.

MATERIALS AND METHODS

Chemicals and Glassware. For the experimental work, HNO₃ (65 w% p.a. Merck, Darmstadt, Germany) and a multielement standard (ICP Multielement Standard IV, Merck, Darmstadt, FRG) were applied. All glassware was cleaned by 7 mol/L HNO₃ prior to use.

Samples and Sample Preparation. Thirty-eight clarified apple juices were purchased. Juices commercially available on the European market [Austria, 16; Bosnia-Herzegovina, 2; Croatia, 10; Germany, 6; Slovenia, 2; and Switzerland, 2] were tested and packaged in glass bottles, PET bottles, or tetrapaks.

For the direct determinations of copper, each sample was diluted 1:20 with 2% HNO₃ in triplicate. Furthermore, dilutions of 1:5 and 1:10 were tested for their applicability.

For the microwave-assisted digestion, approximately 6 g of each sample (in duplicate) and 3 mL of HNO_{3,conc} underwent the following procedure

Table 1. ICP-OES Operating Conditions

ICP-OES operating conditions	
instrument spectrometer	Prodigy high dispersive ICP high-resolution echelle polychromator large format programmable array detector (L-PAD)
RF-generator	40 MHz "free-running"
output power	1.1 kW
argon flow	coolant, 18 L min ⁻¹ auxiliary, 0.8 L min ⁻¹ nebulizer, 36 psi
peristaltic pump	1.0 mL min ⁻¹
nebulizer	pneumatic (glass concentric)
spray chamber	glass cyclonic
plasma viewing	axial
replicates for each analysis run	3
sample uptake delay	30 s
hydride generator	Leeman Laboratories, Inc. part #130-1070 three channel peristaltic pump, 0.9 mL min ⁻¹ T-connector reaction coil output power, 1.3 kW plasma viewing, radial

[time (min)/power (W)/T (°C): 3/700/85, 5/500/125, 4:30/1000/160, and 17:30/1000/160; ventilation, 18 min]. The resulting clear solutions were then brought to 20 mL with double-distilled water.

Apparatus. ICP-OES measurements were performed using a Prodigy high dispersive ICP spectrometer working in a simultaneous mode. The instrumental conditions are listed in **Table 1**.

For quality assurance, the same samples were also measured by a second ICP-OES, namely, Ultima 2 (Horiba Jobin Yvon). For microwave digestion of the samples, a high-performance microwave digestion unit (MLS GmbH Mikrowellenlaborsysteme) was applied.

Measurements. All samples, digest solutions, diluted juices, and blank solutions were measured in triplicate at the two different emission lines, namely, 224.700 and 324.754 nm, since these two lines gave the best results in a pilot test.

Quality Assurance. For quality assurance, the same samples were also measured in a second lab using another ICP-OES spectrometer. Furthermore, certified standard reference materials were used for assuring the accuracy of the measurements performed, namely, NIST 1643e (trace elements in natural water) and NIST 1640 (trace elements in water). Because of the dilution of the samples with 2% HNO₃, the amount of organic matter, especially sugars, was reduced, so that the matrix differences between the dilutions and the water CRM were not supposed to influence the results significantly.

Spiking Procedures. Spiking of apple juice samples with copper was carried by addition of aqueous standard solutions to a set of samples prepared as described above. The experiments were performed at four different concentrations, namely, 0.2, 0.5, 1.0, and 2.0 mg/L. All spiked samples were prepared in triplicate and measured by ICP-OES.

Calibration. A multielement standard as well as a single element standard stock solution (1000 mg/L) were used for the preparation of standard solutions in 2% HNO₃ as well as in matrix-matched solutions for the digest solutions. The stock solution was diluted to obtain the following concentrations (mg/L): 0.05, 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10, 15, and 20. Blank solutions were prepared in the same media. The matrix-matched solutions contained 2% (w/w) glucose, 2% (w/w) sucrose, and 7% (w/w) fructose as well as 100 mg/L Ca, 500 mg/L K, 40 mg/L Mg, and 20 mg/L Na.

Validation. A validation including several parameters was performed for both sample preparation methods: limit of determination (3σ), limit of decision (6σ), limit of quantification (9σ), range of determination, linearity, repeatability, intermediate precision (within day), reproducibility (day-to-day), recovery by spiking experiments, and uncertainty of measurement. The last parameter includes sampling, sample storage, sample preparation, and analysis and was determined by the comparison of the

Table 2. Validation Data for Cu by ICP-OES (Prodigy High Dispersive ICP)—Direct Determination

		Cu 224.700	Cu 324.754
limit of detection	$\mu\text{g/L}$	4.12 [20.6] ^a	3.29 [15.9]
limit of decision	$\mu\text{g/L}$	8.24 [41.2]	6.58 [31.8]
limit of quantification	$\mu\text{g/L}$	12.36 [61.8]	9.87 [47.7]
range of determination	mg/L	0.012–1.0	0.010–1.0
linearity ^b	mg/L	$y = 96314x + 1666$	$y = 2097462x + 25460$
r^2		0.99994	0.99994
repeatability	%	<0.5	<0.5
intermediate precision (within day)	%	1.6	1.3
reproducibility (day-to-day)	%	2.6	2.2
recovery (mean of four concentration levels)	%	104.3	105.7
uncertainty of measurement ($n = 11$)	%	5.1	4.3

^a Values in square brackets refer to the juices when diluted 1:5. ^b Note: $y =$ absorbance, and $x =$ concentration.

registered concentration results for 11 (direct measurement) or 35 (microwave assisted digestion) samples, drawn, prepared, and measured in the same way but on different days.

RESULTS AND DISCUSSION

Method Optimization and Validation. No significant interferences by matrix components were observed for both emission lines used (224.700 and 324.754 nm), so that measurements could be performed either at one or both emission lines. No significant influence by the multielement standard in comparison with a single element standard was registered. This offers the possibility for broadening the set of elements determined simultaneously by this method. All figures of merit for the validation of the determination of copper in clarified apple juices directly after dilution with 2% HNO₃ as well as after microwave-assisted digestion are listed in **Tables 2** and **3**, respectively.

Clarified apple juices could be measured directly at all three dilution levels tested, namely, 1:5, 1:10, and 1:20. The organic components, mainly the sugar content of about 120 g/L, did not exhibit any negative effect on the plasma. Thus, the dilution can be adjusted to the concentration ranges expected. Therefore, the lowest dilution level (1:5) is the most appropriate for the quantification of copper. The limits of detection (LOD) determined for Cu in the diluted juices did not vary significantly, approximately 4 $\mu\text{g/L}$ for all three dilution levels. The LODs in the digest solutions were slightly higher, but because of the lower dilution during the sample preparation procedure, the resulting LODs in the juices are in the same range (15–20 $\mu\text{g/L}$).

The pH values of the apple juices were in the range from 3.3 to 3.8. The pH values of all dilutions in 2% HNO₃ (1:5, 1:10, and 1:20) were about 0.7. Thus, even the 1:5 dilution ensured a sufficient low pH for the given analytical task. Using deionized water instead of diluted HNO₃, the resulting pH values were about 3.4 for the 1:5 and the 1:10 dilution and 3.0 for the dilution 1:20.

The calibration curves were linear over the entire range up to 20 mg/L, but the expected concentrations in the juices were much lower. Thus, the calibration curves were calculated only up to 1.0 mg/L to ensure sufficient accuracy in the low concentration range. The correlation coefficients for all curves (digest solutions and diluted juices) were more than 0.9999.

The results obtained for the accuracy determined on the one hand by the analysis of the certified reference materials and on the other hand by the recovery experiments using spiked sample

Table 3. Validation Data for Cu by ICP-OES (Prodigy High Dispersive ICP)—After Microwave-Assisted Digestion

		Cu 224.700	Cu 324.754
limit of detection	$\mu\text{g/L}$	7.03 [23.4] ^a	5.02 [16.7]
limit of decision	$\mu\text{g/L}$	14.1 [46.8]	10.1 [33.4]
limit of quantification	$\mu\text{g/L}$	21.1 [70.2]	15.1 [50.1]
range of determination	mg/L	0.021–20.0	0.015–20.0
linearity ^b	mg/L	$y = 87890x + 4870$	$y = 1938508x + 84400$
r^2		0.99999	0.99997
repeatability	%	<0.5	<0.5
intermediate precision (within day)	%	1.6	1.7
reproducibility (day-to-day)	%	3.5	2.9
recovery (mean of four concentration levels)	%	100.3	102.9
uncertainty of measurement ($n = 35$)	%	4.2	3.5

^a Values in square brackets refer to the juices. ^b Note: $y =$ absorbance, and $x =$ concentration.

solutions were satisfactory. No statistically significant differences between the measured and the certified values were found. The certified and determined concentrations were 85 and 84.6 $\mu\text{g/L}$ (−0.5%) for NIST 1640 and 22 and 21.0 $\mu\text{g/L}$ (−4.5%) for NIST 1643e, respectively. The recoveries (means of the four spiked concentration levels) range from 100 to 106% and are slightly lower for the digested samples than for the diluted ones. The results obtained for the repeatability (<0.5%), the intermediate precision (<1.7%), the day-to-day reproducibility (<3.5%), and the overall uncertainty of measurement (approximately 5%) demonstrated the good applicability of the chosen optimized method for the given analytical task.

Copper Data. The optimized method was then applied for the determination of the copper content of 38 clarified apple juices from six different European countries. Each sample was analyzed after dilution and after a microwave-assisted acidic digestion procedure. Neither in the diluted nor in the digested samples could any copper be found. Thus, the copper concentrations were below the LODs, 15.9 and 16.7 $\mu\text{g/L}$, respectively, at 324.754 nm. Comparing these results with the concentrations found in the 1960s [0.27 (16) and 1.05 mg/kg (17)] as well as with those from the 1980s [0.32 mg/kg (18)], it can be seen that the reduced application of copper compounds in apple orchards nowadays is reflected in lower copper levels in the resulting juices. Even taking into account that the metal content of biological and food sample depends on many environmental factors, such as air, soil, and water, there is at least a factor of 20 between the determined LOD and the literature data, which can be considered as sufficient for the drawn conclusion.

This work evaluated the use of ICP-OES for the determination of copper in clarified apple juices. The method was optimized and validated for the direct measurement of apple juices after dilution with 2% HNO₃ as well as for samples after a microwave-assisted digestion. The LODs, the recoveries, and the precision data obtained are in the same range for both sample preparation methods. Skipping the digestion step allows time and chemicals to be saved and facilitates the analytical procedure. Especially for monitoring purposes, a fast and reliable method is needed. Because ICP-OES is a multielement method when used in simultaneous mode, the proposed method can also be adopted for the determination of other essential and/or toxic major, minor, and trace elements after a specific optimization. Because there were no significant influences observed by the multielement standard and the artificial matrix-matched solutions, problems

arising during broadening the set of elements to be determined are considered to be more or less negligible.

Within in the European Union, the usage of copper compounds in organic farming is regulated and the applicability of substitutes is investigated. However, already, now it can be seen that the copper content in apple juices has decreased significantly in comparison with concentrations measured in the previous decades. Thus, there is currently no negative impact to be expected to human health by copper present in apple juices.

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