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Minimization of Sample Pretreatment for Al, Cu and Fe Determination in Coconut Water by Electrothermal Atomic Absorption Spectrometry

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Neste trabalho são propostos métodos analíticos rápidos para a determinação direta de Al, Cu e Fe em água de coco não processada por espectrometria de absorção atômica com atomização eletrotérmica. Para a determinação de Fe, 100 µL das amostras foram diluídos com 1400 µL de solução 0,1% v/v HNO_3. Alíquotas de 10 µL desta solução foram injetadas no tubo de grafite sem a adição de modificador químico. Para as determinações de Al e Cu, as amostras foram minimamente diluídas (1250 µL amostra + 250 µL de solução 0,6% v/v HNO_3 + 0,6% m/v Triton X-100) nos frascos do amostrador automático. Nesse caso, alíquotas de 10 µL de uma mistura oxidante (15% v/v H_2O_2 + 1% v/v HNO_3) foram co-injetadas com 15 µL das amostras ou soluções analíticas de referências no tubo de grafite. A mistura oxidante minimizou a formação de resíduos carbonáceos sobre a superfície grafítica e aumentou o tempo de vida do tubo de grafite em 75%, proporcionando mais de 200 ciclos de aquecimento. Os limites de detecção e massas características foram 1,0 µg L^{-1} e 30 pg para Al, 0,7 µg L^{-1} e 20 pg para Cu, e 2,0 µg L^{-1} e 10 pg para Fe, respectivamente. Testes de adição e recuperação foram realizados para estimar a exatidão do método proposto (104 ± 1% para Al, 98 ± 3% para Cu, e 97 ± 1% para Fe).

Fast and reliable methods for the direct determination of Al, Cu and Fe in unprocessed coconut water by electrothermal atomic absorption spectrometry are proposed. For Fe determination, 100 µL of sample were diluted with 1400 µL of 0.1% v/v HNO_3. Aliquots of 10 µL of this solution were injected into the graphite tube without any chemical modifier. For Al and Cu determinations, samples were slightly diluted (1250 µL of sample + 250 µL of 0.6% v/v HNO_3 + 0.6% m/v Triton X-100) directly into the auto sampler cups. In this case, aliquots of 10 µL of an oxidant mixture (15% v/v H_2O_2 + 1.0% v/v HNO_3) were co-injected with 15 µL of samples or analytical solutions into the graphite tube. The oxidant mixture minimized the carbonaceous residues formation and increased graphite tube lifetime in 75%, allowing up to 200 heating cycles. The detection limits and characteristic masses were 1.0 µg L^{-1} and 30 pg for Al, 0.7 µg L^{-1} and 20 pg for Cu, and 2.0 µg L^{-1} and 10 pg for Fe, respectively. The reliability of the proposed methods was evaluated by addition and recovery tests (104 ± 1% for Al, 98 ± 3% for Cu, and 97 ± 1% for Fe).

Keywords: electrothermal atomic absorption spectrometry, coconut water, aluminum, copper, iron

Introduction

Green coconut water is a nutritious, refreshing, isotonic and low caloric drink.[1,2] In addition, it has been advised to gastric disturbs treatment, inhibition of vomit caused by cholera, treatment of dysentery and for infant feeding.[1-6] As a consequence, the green coconut water has been largely consumed all over the world and it has also earned popularity among the soft drink world market. In Brazil, green coconut water consume are increasing, representing 1.4% of total Brazilian market, according to official statistics.[7]

The determination of major and trace elements in coconut water can be a subject of considerable interest due to the relationship between some elements with human nutrition and toxicity. Additionally, some elements and organic constituents, mainly amino acids, can be used to monitor quality, authenticity and origin.[8,9] Trace metal data are potentially more useful than the major elements for identifying the history of food and detecting adulteration.[8] In general, there are a close relationship between trace metals concentration in plants and soil composition. Consequently, some differences in the concentration of samples with different origins could be inferred.[10]

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The complex chemical composition of coconut water includes carbohydrates (fructose and glucose), proteins, lipids, vitamins[3,11] and mineral salts of some cations (Ca^{2+}, Cu^{2+}, Fe^{3+}, K^+, Mg^{2+}, Mn^{2+}, Na^+ and Zn^{2+}) and anions (Cl^-, I^-, SO_4^{2-}, SeO_3^{2-}, PO_4^{3-}).[1,4,12-15]

In general, the determination of elements in coconut water have been performed by using inductively coupled plasma optical emission spectrometry (ICP-OES).[12,13] However, more sensitive techniques are required for the determination of low concentration of elements, such as, electrothermal atomic absorption spectrometry (ETAAS) or inductively coupled plasma mass spectrometry (ICP-MS).

Electrothermal atomic absorption spectrometry is a technique that encompasses several favorable characteristics for the trace elements determination as low detection limits, good selectivity and sensitivity. In addition, the possibility to carry out the *in situ* thermal pretreatment, during pyrolysis step, is one of the most important characteristic of ETAAS that allows the elimination of organic and inorganic concomitants during the heating program.[16] Due to these characteristics, in the majority of cases it is possible to introduce sample without any pretreatment.[17-22]

The determination of Al, Cu and Fe in coconut water is a difficult task because the concentrations of these elements are very low, requiring high sensitivity techniques for detection and, frequently, minimum sample dilution. Acid digestion is time consuming and requires special attention to avoid systematic and random errors that will damage the overall accuracy and precision of the analytical results. Systematic and random errors can be more evident for trace and ultra trace elements determination. Additionally, when chemical digestion are adopted the final acidity of the solution could cause some drawbacks, depending on the analytical technique used for measurements; for example, nebulization interferences caused by elevated solid and acid contents.[14,23] Due to the high sensitivity of ETAAS and the possibility to carry out the matrix elimination during the pyrolysis step, procedures involving acid digestion for Al, Cu and Fe determination in green coconut water are not recommended. Even so, considering these favorable characteristics, strategies can be adopted to eliminate the high organic content, mainly when a minimum dilution is used.

The introduction of air as auxiliary gas[24] or the use of an oxidant mixture (H_2O_2 and HNO_3),[16-21] as matrix modifier, have been successfully used to minimize organic content. These strategies enhance the oxidation of organic compounds preventing the carbonaceous residue build-up on the graphite platform surface. It was observed that oxidant mixture is less aggressive to the graphite tube surface than the introduction of air during the pyrolysis step.[17] This mixture was previously proposed in the literature for the Al, Cr, Mo and Mn determination in milk,[17] Al, Cd, Cr, Mn, Mo, Pb and Se in biological fluids,[18,19] Cd, Pb and Se in baby food,[21] and for the simultaneous determination of Mn and Se in serum.[22]

The aim of this work is to propose fast and reliable methods for the direct determination of trace concentrations of Al, Cu, and Fe in unprocessed green coconut water by ETAAS, using a minimum sample pretreatment. For Al and Cu, it was recommended the use of an oxidant mixture to prevent the carbonaceous residues formation inside the graphite tube.

Experimental

Reagents

All solutions were prepared from analytical reagent grade chemicals and using high purity deionized water obtained by Milli-Q water purification system (Millipore, Bedford, MA, USA). High purity analytical reagent solutions of 1000 mg L^{-1} of Al^{3+} [$Al(NO_3)_3$], Cu^{2+} [$Cu(NO_3)_2$], and Fe^{3+} [$FeCl_3$] from Tritisol (Merck, Darmstadt, Germany) were used to prepare the analytical solutions. Nitric acid (Merck) was purified by distillation in quartz sub-boiling still (Marconi, Piracicaba, SP, Brazil). Nitric acid and 30% m/m H_2O_2 from Titrisol (Merck) were used to prepare the oxidant mixture for the *in situ* thermal sample pretreatment. Triton X-100 Titrisol (Merck) was used to dilute the coconut water samples. A solution of 1% m/v of Triton X-100 was put in the wash bottle of the automatic sampler to clean the sampler probe, avoiding sampling errors.

Samples

Two hundred and thirty two green coconuts samples from different producers were purchased at local supermarkets. A stainless steel punch was used to open the green fruits and the waters were mixed in seven different mixtures (M_1=90, M_2=90, M_3=40, M_4=3, M_5=3, M_6=3, M_7=3 coconuts). These mixtures were prepared according to the conservation processes used after sampling: 90 and 40 coconuts for pasteurization and 3 coconuts for freezing or ultrafiltration. However, in this work it is focused only the development of methods for the determination of Al, Cu, and Fe in unprocessed coconut waters.

Instrumentation

A ZEEnit® 60 model atomic absorption spectrometer (AnalytikjenaAG, Jena, Germany) equipped with hollow cathode lamp, Zeeman-effect background corrector and pyrolytically coated transverse-heated graphite tube with

integrated PIN-platform (Part No. 407-152.013) was used. All measurements were based on integrated absorbance values, controlled by Windows NT® software. Argon 99.998% (v/v) (Air Liquide Brasil, São Paulo, Brazil) was used as purge gas.

The instrumental conditions for the spectrometer and the optimized heating program for the graphite tube atomizer are shown in Table 1.

Procedure

Pyrolysis and atomization temperature curves for Al, Cu and Fe were obtained in absence and presence of coconut water. Pyrolysis temperature curves were obtained fixing the atomization temperatures in 2300 ºC for all elements. Solutions of 50 µg L^{-1} Al^{3+} and 10 µg L^{-1} Cu^{2+} in 0.1% v/v HNO_3, in 0.1% v/v HNO_3 + 0.1% m/v Triton X-100, and diluted coconut water in 0.1% v/v HNO_3 + 0.1% m/v Triton X-100 were used. For Al and Cu determination, 1250 µL of coconut water was mixed with 250 µL of diluent solution (0.6% m/v Triton X-100 + 0.6% v/v HNO_3). In the same way, solution of 10 µg L^{-1} of Fe^{3+} in 0.1% v/v HNO_3 and diluted coconut water in 0.1% v/v HNO_3 were used for heating program optimization. For Fe determination, 100 µL of sample were diluted with 1400 µL of 0.1% v/v HNO_3.

The analytical and sample solutions were directly prepared in the auto sampler cups (volume = 1500 µL).

The analytical solutions were prepared using 1250 µL of deionized water and 250 µL of Al (75 to 300 µg L^{-1}) or Cu (6 to 60 µg L^{-1}) solutions in 0.6% m/v Triton X-100 + 0.6% v/v HNO_3. The matrix-matched analytical solutions were prepared using 1250 µL of coconut waters (n = 3 coconuts) and 250 µL of Al (75 to 300 µg L^{-1}) or Cu (6 to 60 µg L^{-1}) solutions in 0.6% m/v Triton X-100 + 0.6% v/v HNO_3. The solutions homogenization was made using an Eppendorf micropipette (Brinkmann Instruments, Wetsbury, USA).

For Fe determination, the high samples dilution allowed the instrument calibration with aqueous solution (10 to 40 µg L^{-1}) in 0.1% v/v HNO_3.

For Al and Cu determination, aliquots of 15 µL of the analytical or sample solutions were co-injected into the graphite atomizer with 10 µL of oxidant mixture (1% v/v HNO_3 + 15% v/v H_2O_2).

Additions of 15 µg L^{-1} Al^{3+}, 10 µg L^{-1} Cu^{2+}, and 20 µg L^{-1} Fe^{3+} and recovery tests were used to verify the reliability of the entire procedure.

Results and Discussion

Heating program optimization

The thermal behavior of Al, Cu and Fe in presence and absence of coconut water was evaluated from pyrolysis and atomization temperature curves (Figures 1-3). In presence of 0.1% v/v HNO_3, the maximum pyrolysis temperatures obtained for Al, Cu and Fe were 1300 ºC, 1400 ºC and 1200 ºC, respectively.

The addition of 0.1% m/v of Triton X-100 caused absorbance signal reduction of Al (Figure 1). However, this

Table 1. Instrumental conditions and heating program parameters for Al, Cu and Fe determination in unprocessed green coconut water

	Aluminum	Copper	Iron	
Spectrometer setup				
Wavelength (nm)	309.3	224.8	248.3	
Bandpass (nm)	0.5	0.8	0.8	
Lamp current (mA)	4.0	4.0	4.0	
Graphite tube heating program for Al[a] and Cu[b]				
Step	Temperature (ºC)	Ramp (ºC s^{-1})	Hold(s)	Argon flow rate (L min^{-1})
Drying	130	5	20	1.0
Pyrolysis I	400	20	10	1.0
Pyrolysis II	1600[a]/1200[b]	100	10	1.0
AZ*	1600[a]/1200[b]	0	6	0
Atomization	2400[a]/2300[b]	2400[a]/2300[b]	5	0
Cleaning	2600	2500	2	1.0
Graphite tube heating program for Fe				
Step	Temperature (ºC)	Ramp (ºC s^{-1})	Hold(s)	Argon flow rate (L min^{-1})
Drying	110	8	15	1.0
Drying	130	10	10	1.0
Pyrolysis	1200	100	10	1.0
AZ*	1200	0	6	0
Atomization	2300	2300	5	0
Cleaning	2500	200	2	1.0

* AZ = Auto Zero.

surfactant increased the thermal stabilization in absence and in presence of coconut water. The loss of Al in presence of Triton X-100 occurred above 1700 ºC. This thermal stabilization can be related to the formation of various carbides and sub-oxides species prior the atomization temperatures.[25] The high pyrolysis temperature observed for Al dispensed the use of inorganic chemical modifier. The pyrolysis and atomization temperatures adopted for Al determination were 1600 and 2400 ºC, respectively.
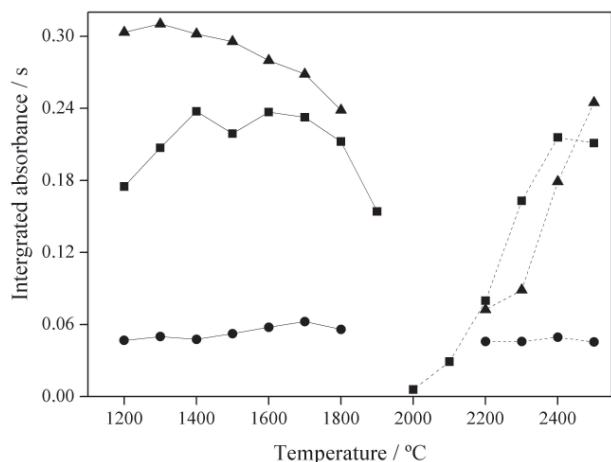

Figure 1. Pyrolysis (—) and atomization (---) temperatures curves for Al: (▲) 50 µg L^{-1} in 0.1% v/v HNO$_3$; (■) 50 µg L^{-1} in 0.1% v/v HNO$_3$ + 0.1% m/v de Triton X-100; and (•) coconut water in 0.1% v/v HNO$_3$ + 0.1% m/v Triton X-100.

The absorbance signals of Cu also decreased in presence of 0.1% m/v Triton X-100 (Figure 2) and a slightly decrease in the pyrolysis temperature was observed. The pyrolysis temperature in presence of Triton X-100 was 1300 ºC. Considering the thermal behavior of Cu in presence of coconut water matrix, the optimized pyrolysis and atomization temperatures were 1200 and 2300 ºC, respectively.

The pyrolysis and atomization curves profiles for Fe are similar in presence and absence of coconut water. In this way, the optimized pyrolysis and atomization temperatures for Fe determination were 1200 and 2300 ºC, respectively. Considering the high dilution and thermal stability of Fe, the use of chemical modifier was not necessary.

In all cases, the atomization temperatures for Al, Cu and Fe were selected based on the integrated absorbance and the repeatability of five consecutive signals.

One of the most important characteristics of the ETAAS is the possibility to perform *in situ* sample decomposition inside the graphite tube. High content of organic matrix can be eliminated by using a suitable diluent and heating program.[17-22]

The high organic content in the minimum diluted coconut water for Al and Cu determination produced an
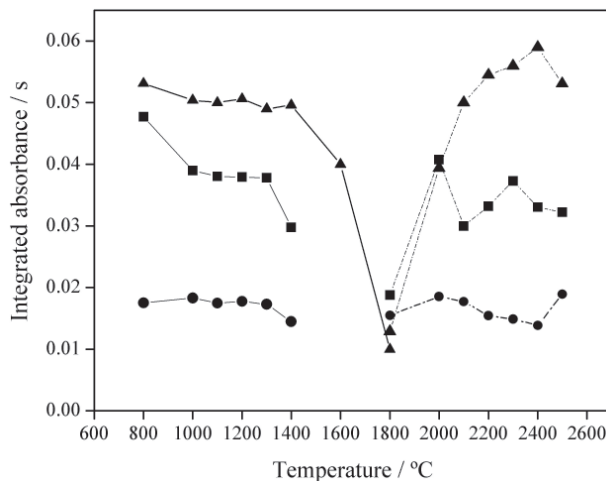

Figure 2. Pyrolysis (—) and atomization (---) temperatures curves for Cu: (▲) 10 µg L^{-1} in 0.1% v/v HNO$_3$; (■) 10 µg L^{-1} in 0.1% v/v HNO$_3$ + 0.1% m/v de Triton X-100; and (•) coconut water in 0.1% v/v HNO$_3$ + 0.1% m/v Triton X-100.

intense background signal during the atomization step and lack of repeatability. Additionally, a carbonaceous residue was accumulated onto the integrated platform surface probably due to the partial organic compound oxidation. The formation of the carbonaceous residues over the pyrolytic coated platform surface was observed after a few heating cycles. As a consequence, the repeatability of the absorbance signals was affected. Under these conditions, the graphite tube lifetime was shortened to less than 25 heating cycles.

The random absorbance signals observed for Al (RSD = 10%) and Cu (RSD = 42%) in the absence of oxidant mixture could be the carbide and oxide species which could be occluded into the carbonaceous residues.[16,25]

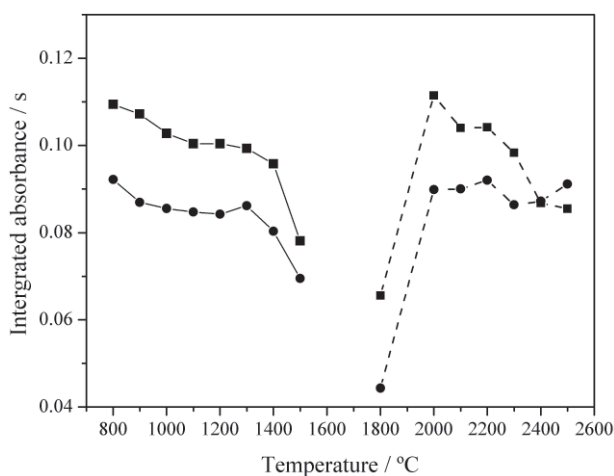Two complementary strategies were implemented to decrease the organic content and minimize the associated

Figure 3. Pyrolysis (—) and atomization (---) temperatures curves for Fe: (■) 10 µg L^{-1} in 0.1% v/v HNO$_3$; and (•) coconut water diluted 15 times in 0.1% v/v HNO$_3$.

complications due to the carbonaceous residues build-up: (*i*) the addition of an oxidant mixture (1% v/v HNO_3 + 15% v/v H_2O_2) as a matrix modifier; and (*ii*) the inclusion of a low temperature pyrolysis step.

This strategy is simple for implementation and less aggressive for the graphite tube surface than the introduction of air during the pyrolysis step as auxiliary gas.[22]

The oxidant mixture action started during the drying step, when the atomizer was warmed up from 20 up to 130 °C and was complemented during the pyrolysis step I (400 °C), Table 1. The background signals for aluminum and copper decreased to acceptable levels after about 10 s of the holding time of the pyrolysis II step.

Unprocessed green coconut water analyses

The influence of concomitants in the Al and Cu determination was investigated by comparison of the analytical curves obtained in presence and absence of the coconut water matrix.

The analytical signals of Al in aqueous solutions were systematically lower than these obtained in presence of coconut water solution. The slope for matrix-matched curve was 0.00252 ± 0.00001 (R^2 = 0.99901) and for aqueous solution curve was 0.00225 ± 0.00003 (R^2 = 0.99408). The slopes ratio was 0.89. Due to this systematic difference between absorbance signals in presence and absence of coconut water, for Al determination the use of matrix-matched calibration is recommended.

The analytical curves of Cu with and without coconut water showed the same slopes (0.00337) and the correlation coefficients were R^2 = 0.9973 and R^2 = 0.9989 for analytical curve in aqueous and matrix-matched solutions, respectively. Therefore, the determination of Cu in coconut water can be performed using aqueous solution for the instrument calibration.

For Fe determination, high dilution of samples (15-fold) enabled the calibration of instrument with aqueous solutions.

Seven different mixtures of coconut water (M_1=90, M_2=90, M_3=40, M_4=3, M_5=3, M_6=3, M_7=3 coconuts) were analyzed. The results obtained are showed in Table 2. The recoveries obtained with the proposed methods are 103 to 105% for Al, 95 to 101% for Cu and 96 to 98% for Fe.

The limits of detection for Al (1.0 μg L^{-1}), Cu (0.7 μg L^{-1}) and Fe (2.0 μg L^{-1}) were calculated based on the standard deviation of 10 measurements of the blank solution, according to 3 S_{blk}/m, where S corresponds to the blank measurement standard deviation and m is the calibration curve slope.

The characteristic masses obtained from the analytical reference curves were 30 pg for Al, 20 pg for Cu and 10 pg for Fe.

Table 2. Mean results and standard deviations (n = 3) for Al, Cu and Fe determinations in unprocessed green coconut water samples

Samples	Concentration (μg L^{-1}) ± SD[a]		
	Al	Cu	Fe
M_1	ND[b]	25 ± 1	323 ± 23
M_2	ND[b]	20 ± 1	250 ± 30
M_3	23 ± 4	5 ± 1	138 ± 11
M_4	25 ± 2	112 ± 13	103 ± 18
M_5	25 ± 4	20 ± 1	296 ± 20
M_6	27 ± 2	3 ± 1	129 ± 5
M_7	26 ± 5	4 ± 1	49 ± 15

[a] SD = standard deviation; [b] ND = Not Determined.

Conclusions

The direct determination of Al and Cu in coconut water by ETAAS can be executed using a minimum dilution in 0.1% v/v HNO_3 + 0.1% m/v Triton X-100 solution. For these elements, the co-injection of an oxidant mixture (15% v/v H_2O_2 + 1% v/v HNO_3) with the sample solution, as matrix modifier was imperative. This oxidant mixture allows the *in situ* thermal sample pretreatment, reducing the background signals and minimizing the formation of carbonaceous residues onto the graphite surface platform. Adopting this strategy, and the matrix-matched calibration for Al, it was possible the determinations of Al and Cu in coconut waters with good precision and accuracy.

Both aspects, the high dilution factor that led to a decrease of matrix effects and the thermal stability, allowed the determination of Fe without adding a chemical modifier and by using aqueous calibration solutions.

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