

Photo-oxidation using UV radiation as a sample preparation procedure for the determination of copper in fruit juices by flame atomic absorption spectrometry

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Received 22nd July 2011, Accepted 9th December 2011

DOI: 10.1039/c2ay05438h

The present work proposes the photo-oxidation using UV radiation/H₂O₂ as an alternative procedure for pretreatment of industrialized fruit juice samples for determination of copper by flame atomic absorption spectrometry (FAAS). For optimization of the photo-oxidation procedure the following variables were studied: pH and volume of buffer solution, volume of concentrated hydrogen peroxide and irradiation time. The sample degradation was monitored by molecular absorption spectrometry in the spectral range of 200–800 nm. The established conditions were: pH 10, volume of buffer of 1.0 mL, volume of concentrated hydrogen peroxide of 1.5 mL and irradiation time of 90 min, for a 5 mL volume of the photo-digested sample. An external calibration technique with aqueous standard was used for copper quantification. Under these conditions, the method allows the copper determination with limits of detection and quantification of 7 and 22 µg L⁻¹, respectively. The precision, expressed as relative standard deviation (RSD), was 1.43 and 0.27% for copper concentrations of 0.2 and 2.0 µg mL⁻¹, respectively. The accuracy of the method was evaluated for five industrialized fruit juice samples (orange, grape, mango, passion fruit and peach) and the results compared with those obtained after acid digestion of the samples and determination by FAAS. No significant difference was observed between the results obtained by both procedures, applying a paired *t*-test at the 95% confidence level.

Introduction

A knowledge of the mineral content in beverages and foods is important due to their toxicity or essentiality.^{1,2} Copper is known to be an essential nutrient for humans, but can cause serious health problems depending on the concentration. It is an essential element in hemoglobin synthesis, in catalysis of metabolic oxidation and as a constituent of some metalloenzymes.^{1–3} Copper deficiency in humans can cause bone demineralization, depressed growth and gastro-intestinal disturbances. However, an excessive intake has been shown to cause liver cirrhosis, dermatitis and neurological disorders.¹

Fruit juices are widely consumed in all ages due to their flavor and nutritional value, being a good source of vitamins and minerals.^{2,4,5} Although fruit juice is a liquid matrix, the direct determination of metals by spectro-analytical techniques is not a simple task due to the elevated content of organic compounds present in this matrix type, such as sugars, proteins, amylums and others.⁶ The direct analyses of fruit juices by flame atomic

absorption spectrometry (FAAS), for instance, can cause problems related to sample transport, such as aspiration and nebulization, due to their high viscosity, flame fluctuations and accumulation of solid deposits in the burner head, due to the high content of soluble solids.^{6–8} Thus, a sample pretreatment step is often required for the determination of metals in fruit juices when these analytical techniques are employed for detection.^{4,5,9,10}

In this context, UV radiation-assisted mineralization appears as an efficient and simple alternative to conventional methods of sample decomposition. The use of UV radiation as a sample pretreatment step usually offers the following advantages: low consumption of reagents; use of less toxic reagents; possibility of conducting the process at low temperature, avoiding the loss of volatile elements; and in some cases, the possibility of conducting the process at weakly acidic or alkaline pH levels.^{11,12} This technique of sample mineralization has been extensively used in association with electro-analytical techniques for the determination and speciation analysis of metals,^{13–16} being comparatively less applied in association with spectro-analytical techniques for analyses.^{17–19}

For all these reasons, the present paper proposes the photo-oxidation using UV radiation/H₂O₂ as a step of pretreatment of industrialized fruit juice samples for the determination of copper by FAAS.

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Table 1 Operational parameters of the spectrometer

Parameter	Value
Lamp current/mA	4.0
Wavelengths/nm	324.8
Spectral bandwidth/nm	0.5
Acetylene flow rate/L min ⁻¹	2.0
Air flow rate/L min ⁻¹	13.5
Burner height/mm	13.5

Experimental

Instrumentation

A laboratory-made UV-digester equipped with three 20 W low-pressure mercury lamps has been used for the photo-oxidation of the industrialized fruit juice samples.

Measurements of molecular absorption were carried out using a molecular absorption spectrophotometer model Cary 5E UV-Vis-NIR from Varian (Mulgrave, Victoria, Australia).

A flame atomic absorption spectrometer model SpetraAA 220 from Varian equipped with a deuterium background corrector and hollow-cathode lamps was used for the determination of copper. The operating conditions were chosen as recommended by the manufacturer, as shown in Table 1.

A Digimed DM 20 pH meter (Santo Amaro, Brazil) was also used.

Reagents, solutions and samples

All reagents were of analytical grade unless otherwise stated. Ultrapure water was obtained from an Easypure RF purification system (Barnstedt, Dubuque, IA, USA). Nitric acid and hydrogen peroxide were of Suprapur quality and obtained from Merck (Darmstadt, Germany). Laboratory glassware was kept overnight in 10% (v/v) nitric acid solution. Before use, glassware was rinsed with deionized water and dried in a dust-free environment.

A 50 µg mL⁻¹ copper solution was prepared from a 1000 mg L⁻¹ copper stock solution (Merck) by appropriate dilution with 1% (v/v) nitric acid solution.

Calibration solutions in the range of 0.2–2.0 µg mL⁻¹ were prepared from a 50 µg mL⁻¹ solution by appropriate dilution with 1.0 mol L⁻¹ nitric acid solution.

Phosphate buffer solutions of pH 6.0, 7.0 and 8.0 were prepared by dissolution of 24.20, 10.76 and 1.46 g of NaH₂PO₄·H₂O (Merck) with 3.56, 17.67 and 27.44 g of Na₂HPO₄ (Merck) in ultrapure water, respectively.

Ammonium buffer solutions of pH 9.0 and 10.0 were prepared by dissolution of 8.56 and 3.21 g of ammonium chloride (Merck) with 5.5 mL and 19.3 mL of ammonium hydroxide (Merck) in ultrapure water, respectively.

All buffer solutions were prepared with the final concentration of 1.0 mol L⁻¹ for a volume of 200 mL.

Different industrialized fruit juices (orange, grape, mango, passion fruit and peach) were purchased in a local supermarket. Aliquots of 100 mL of each sample were stored in polyethylene bottles decontaminated previously, before analysis.

Procedure for photo-oxidation of fruit juices

A volume of 5.0 mL of fruit juice was introduced into a Petri dish, and 1.5 mL of 30% (v/v) hydrogen peroxide and 1.0 mL of 1.0 mol L⁻¹ ammonium buffer solution (pH 10.0) were added. The samples were subject to UV photo-oxidation for 90 min. Then, the samples were transferred to 10 mL volumetric flasks and diluted to volume with 1.0 mol L⁻¹ nitric acid solution. All samples were analyzed in triplicate and blank solutions were prepared with all the reagents used for preparation of the samples.

Procedure for acid digestion of fruit juices

A volume of 5.0 mL of fruit juice was introduced into a 125 mL Erlenmeyer flask, and 5.0 mL of concentrated nitric acid and

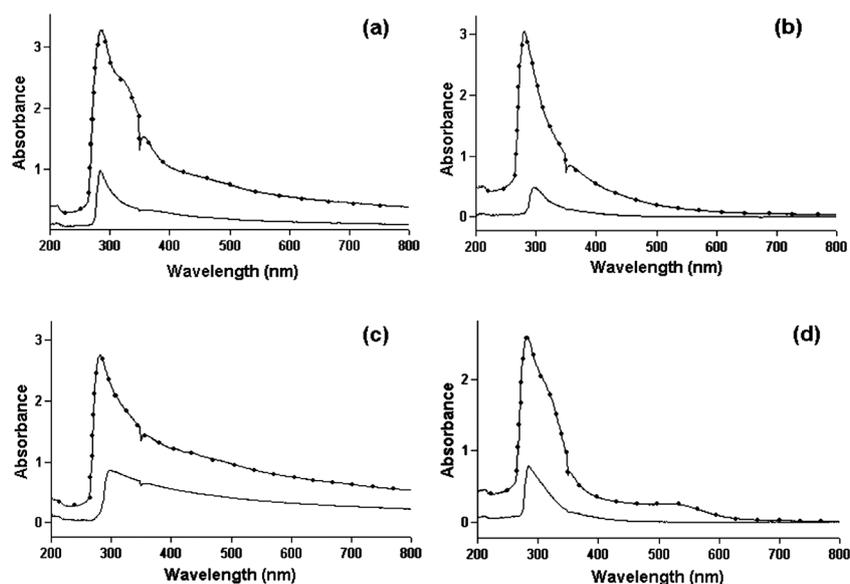


Fig. 1 Spectra of molecular absorption achieved for different fruit juices: (a) orange juice, (b) apple juice, (c) passion fruit juice and (d) grape juice. Line: sample after the procedure of proposed photo-oxidation. Line with points: sample without treatment.

4.0 mL of 30% (v/v) hydrogen peroxide were added. The samples were heated to 120 °C and evaporated to dryness on a hot plate, after which the residue was dissolved in ultrapure water, transferred to 10.0 mL volumetric flasks and diluted to volume with 1.0 mol L⁻¹ nitric acid solution.

Results and discussion

Optimization of the experimental conditions for the photo-oxidation of fruit juices

The univariate methodology was adopted for optimization of the experimental conditions involving the following variables: pH and volume of buffer solution, volume of concentrated hydrogen peroxide and irradiation time. All the experiments were carried out using a 5 mL volume of an industrialized orange juice sample and the process of sample degradation was monitored by molecular absorption spectrometry in the spectral range of 200–800 nm for a final volume of 25 mL.

Initially, the pH effect of the buffer solution was evaluated by the photo-oxidation process in the range of 6–10. This variable was studied using a volume of buffer solution of 1.0 mL, volume of concentrated hydrogen peroxide of 1.0 mL and irradiation time of 60 min. It was observed that the sample degradation was maximum at pH 10. Similar results were obtained by Buldini¹⁵ and dos Santos¹⁷ for wine samples.

The volume of buffer solution used for the fruit juice degradation was also tested. That variable was studied in the interval of 0.25–1.5 mL and a maximum of sample degradation is already obtained for a volume of buffer solution from 1.0 mL. There is no significant increase in the sample degradation to larger volumes of buffer solution.

The influence of the hydrogen peroxide volume used for sample photo-oxidation was evaluated in the range of 0.5–2.5 mL and a maximum of sample degradation is already achieved at a volume of 1.5 mL, above which no significant increase was observed for the degradation process.

Finally, the influence of the UV irradiation time on the fruit juice degradation was studied in the interval of 30–150 min. An irradiation time of 90 min has been found sufficient for the process of fruit juice degradation.

This way, the experimental conditions established during the optimization process for the photo-oxidation procedure were: a volume of 1.0 mL of buffer solution of pH 10.0, a volume of hydrogen peroxide of 1.5 mL and an irradiation time of 90 min.

Evaluation of the photo-oxidation process

In order to evaluate the extension of applicability of the proposed procedure, spectra of molecular absorption were obtained for different fruit juices (orange, passion fruit, apple and grape) under the optimized conditions, as shown in Fig. 1. The spectra were compared with those obtained for samples without pretreatment. In all the cases, a significant reduction of the spectral area was observed. This demonstrates that the procedure of proposed photo-oxidation is efficient for organic compounds degradation present in different fruit juices.

Analytical characteristics of the method

The calibration technique used for copper quantification was evaluated by comparison of slopes obtained using aqueous standard solutions with those obtained by an analyte addition technique in the sample after the acid digestion, in the sample after the photo-oxidation process and in the sample without pretreatment. Table 2 shows the slopes obtained for the different studied systems and the respective correlation coefficients, using the confidence interval. Obviously, a significant difference was observed for the slope obtained in the sample without pretreatment when compared with the aqueous curve slope. However, no significant difference was observed between the slopes of the curves in the sample after acid digestion or photo-oxidation with the slope of the aqueous system. These results demonstrate again that the proposed photo-oxidation procedure using UV radiation and H₂O₂ is efficient for degradation of industrialized fruit juices and also that the determination of copper can be done using an aqueous standard for calibration.

The limits of detection ($3\sigma/S$) and quantification ($10\sigma/S$) of the method were 7 and 22 $\mu\text{g L}^{-1}$, respectively, these being determined according to IUPAC recommendations, where σ is the standard deviation of the blank ($n = 10$) and S is the slope of the analytical curve. The precision of the method, expressed as relative standard deviation (RSD), was 1.43 and 0.27% (both $n = 10$) for copper concentrations of 0.2 and 2.0 $\mu\text{g mL}^{-1}$, respectively. The linearity of the method was in the range of 0.022–4.0 $\mu\text{g mL}^{-1}$ of copper.

Table 2 Equations and correlation coefficients of the analytical curves obtained for different systems

System	Slope/ $\text{mL } \mu\text{g}^{-1}$	Correlation coefficient
Aqueous standards	0.0576 ± 0.0025	0.9994 ± 0.0017
Sample without treatment	0.0471 ± 0.0064	0.9955 ± 0.0043
Photo-digested sample	0.0539 ± 0.0042	0.9984 ± 0.0029
Digested sample	0.0572 ± 0.0053	0.9977 ± 0.0036

Table 3 Determination of copper in industrialized fruit juice samples ($n = 3$, a confidence level of 95%)^a

Fruit juice	Copper concentration/ $\mu\text{g mL}^{-1}$	
	Method proposed	Acid digestion
Grape (A)	0.49 ± 0.01	0.52 ± 0.03
Grape (B)	0.26 ± 0.02	0.30 ± 0.01
Grape (C)	0.23 ± 0.03	0.24 ± 0.02
Orange (A)	0.12 ± 0.01	0.12 ± 0.01
Orange (B)	0.06 ± 0.01	0.06 ± 0.01
Orange (D)	0.08 ± 0.01	0.08 ± 0.01
Mango (C)	0.27 ± 0.05	0.29 ± 0.02
Passion fruit (D)	0.05 ± 0.02	0.04 ± 0.01
Peach (E)	0.09 ± 0.02	0.11 ± 0.01

^a Application of a paired *t*-test: the calculated *t* is 0.17, while that the critical *t* is 2.12.

Application

The proposed method based on photo-oxidation using UV radiation and H₂O₂ was applied for analysis of industrialized fruit juice. The samples also were analyzed after the acid digestion procedure with nitric acid and hydrogen peroxide and copper determined by FAAS. These results are shown in Table 3 using the confidence interval. The application of a paired *t*-test, at a confidence level of 95%, demonstrates that there are no significance differences between the results obtained for both the procedures, considering that the calculated *t* is 0.17, while that the critical *t* is 2.12.

Conclusions

The application of the photo-oxidation using UV radiation and H₂O₂ as a step of sample decomposition is shown to be an efficient alternative for the pretreatment of industrialized fruit juices for the determination of copper by FAAS.

Furthermore, the proposed method presents the following advantages: simplicity, low consumption of reagents and use of reagents less corrosive in comparison to conventional methods of samples decomposition. These characteristics are inherent to methods based on use of the UV photo-oxidation as a step of samples preparation.

Acknowledgements

The authors gratefully acknowledge the Brazilian agencies PRONEX/Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for grants and fellowships.

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