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Use of fast alkaline solubilisation to determine copper in bovine liver, fish tissues (salmon), and rolled oats by graphite furnace atomic absorption spectrometry using aqueous calibration

Karla Silva Ferreira [a], Weverson Alves Ferreira [b], Juliana M. Mendonça Gomes [c], José Dias Correa-Júnior [c], Cláudio Luis Donnici [b], José Bento Borba da Silva [b,*]

[a] Laboratório de Tecnologia de Alimentos, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes - Rio de Janeiro, Brazil
[b] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte, MG, Brazil
[c] Instituto de Ciências Biológicas, Departamento de Morfologia, Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte, MG, Brazil

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ABSTRACT

In this work, efficient methods to determine Cu in bovine liver, fish tissue, and rolled oats by graphite furnace atomic absorption spectrometry (GF AAS), after fast alkaline solubilisation of the samples with Universol® (a new reagent for sample solubilisation, benzyltrimethylammonium hydroxide in 40 % v/v of water), were presented. The optimum modifiers were permanent Ir (500 μg) plus co-injection of Ru (5 μg) for bovine liver, permanent Nb (500 μg) for salmon, and permanent Ir (500 μg) with co-injection of Pt (5 μg) for rolled oats. The optimum pyrolysis (PT) and atomization temperatures (AT) (obtained by pyrolysis and atomization curves) were of PT of 1300 °C for all matrices, and AT of 1800 °C for bovine liver and 2300 °C for salmon and rolled oats. Aqueous and matrix matching calibration curves (n = 3 curves for each calibration), had average angular coefficients that were not statistically different, i.e. matrix effect was absent for both matrices then the calibration for the three matrices was accomplished with aqueous calibration. The accuracy was checked with six certified materials for bovine liver; four for salmon and two for rolled oats. In all cases, obtained values were in agreement with the certified one. The characteristic masses were of 8.0, 6.4, and 7.8 for bovine liver, salmon and rolled oats, respectively (recommended mass of 10 pg). The LOD and LOQ were of 1.8 and 6.0 μg g^{-1} for bovine liver, 2.0 and 6.6 μg g^{-1} for salmon and 1.4 and 4.6 μg g^{-1} for rolled oats. As the solubilisation is very fast, the methods can be used to determine Cu (and most probably other metals) with simple sample preparation, good accuracy and precision.

1. Introduction

The necessity of copper for human health derives from its involvement in myriad biological processes, including iron metabolism, antioxidant defence and neuropeptide synthesis [1,2]. In adulthood, prolonged marginal copper deficiency has been associated with an increased risk of developing osteoporosis in later life [3,4] and adverse changes in cholesterol metabolism [5,6].

A tight control of Cu homeostasis prevents excess accumulation of Cu in the body; acute and chronic Cu toxicity are relatively rare. However, Cu toxicity may result from exposure to excess Cu caused by accident, occupational hazard, environmental contamination, as well as adrenal gland insufficiency, inborn errors of Cu metabolism, and other factors [7]. Kotulanová and Komárek [8], had compared dry ashing furnace, ashing in low pressure oxygen plasma, acid digestion in pressure vessels, solubilisation with tetramethylammonium hydroxide and slurry sample as sample preparation methods to solubilise animal liver tissue in the determination of Cu by graphite furnace atomic absorption spectrometry (GF AAS). According to the authors, the ashing in the muffle furnace and in the oxygen plasma with sampling in quartz microbowls were recommended for small pieces of liver samples in amounts about 10 mg. In another work, was proposed Cu (among Cd and Zn) in liver (gill and liver) of some fishes using anodic stripping voltammetry. The sample preparation was using a mixture of HNO_3 and $HClO_4$ at 100 °C [9]. According with Jabani et al. [10] these digestion techniques require the use of ultrapure concentrated mineral acids and high temperatures. Flores et al. [11] had developed a new device for direct introduction of solid sample in flame atomizer. This procedure was used to determine Cu in liver tissue by FAAS. The authors conclude that the proposed procedure appears to be attractive for the determination of Cu (and perhaps of other elements) in biological matrices because it combines simple sample treatment, high relative sensitivity and high sampling frequency.

The determination of Cu in anchovies (*Engraules encrasicholus*) from Croatia, were made after digestion with HNO_3 plus H_2O_2 in a microwave

* Corresponding author. Tel.: +55 31 34095708; fax: + 55 31 34095700.
E-mail address: bentojb@yahoo.com.br (J.B.B. da Silva).

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oven system with Cu determination by GF AAS without modifier use [12]. Ahmad et al. [13] had determined Cu (among Cd, Ni, Pb and Zn) in water, sediments and fish tissues (liver, gill, muscle and kidney) in river Panjkora, Pakistan after samples digestion using concentrated HNO_3 and $HClO_4$ and the metals were determined by FAAS. In another work, Cu (Cd and Zn and metallothionein) were determined in water, sediments and fishes (*Aphanius fasciatus*) from unpolluted and polluted areas from Gulf of Gabes in Tunisia. Dried fish tissues (liver, gills, kidney and bone) were digested with HNO_3 at 120 °C and the metals in samples were determined by GF AAS without modifier use [14]. Tuzen [15] developed a method to determine Cu (Cd, Fe, Mn, Pb, and Zn) in five fish species (*Alosa caspia*, *Engraulis encrasicholus*, *Trachurus trachurus*, *Sarda sarda*, and *Clupea sprattus*) from Middle Black Sea coasts in Samsun (Turkey). The author used wet-digestion with concentrated HNO_3 and the copper determination was made by GF AAS using 5 mg Pd + 3 mg $Mg(NO_3)_2$ as modifier. Zn and Cu contents in the edible parts (muscle, fillet) of 19 commercially used fish species from Northeast Atlantic (Tampen, North of Shetland Islands, Faroe Islands and Copinsay) were determined by means of DPSAV (deferential pulse stripping anodic voltammetry) and the samples were lyophilised and milled in a ball-mill made from agate using suprapure sulphuric acid (0.2%, w/w) at pH 2. [16].

Any report was obtained about the determination of Cu (or other metals) in oatmeal (*Avena sativa L*). In a work da Silva et al. [17], had evaluated the bioaccessible fractions of Cu, Fe, Mn and Zn in baby foods and three of the food samples analyzed were based on mixtures of flour. The process of digestion by humans was simulated using *in vitro* digestion as described by Versantvoort et al. [18,19] and the analytes were determined by ICP-MS.

The objective of the present study is the application of a new alkaline agent (Universol®) to the development of a much faster and without heating solubilisation of bovine liver, fish muscle and rolled oats to determine copper by graphite furnace atomic absorption spectrometry (GF AAS).

2. Experimental

2.1. Instrumentation

Integrate absorption (peak area) were measurements in a PerkinElmer Analyst™ 400 atomic absorption spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA), equipped with a graphite furnace (HGA® 800), an autosampler (AS-800), and a deuterium lamp arc continuum background corrector (available in our laboratory). Except for the optimized conditions, the instrument was operated according to the manufacturer's guidelines. GF-AAS was chosen due to its excellent detection limits and low sample demand.

A multi element hollow cathode lamp (Cu, Fe, Mn, and Zn) from Perkin Elmer (Part Number N305-0212) was operated at 25 mA with a slit of 1.8 nm and a wavelength of 324.75 nm for Cu. Argon 99.996% (White Martins, Belo Horizonte, MG, Brazil) was used as the purge gas with a flow rate of 250 mL min^{-1}. Graphite tubes with integrated platforms (Perkin Elmer, Part Number B3001264 and B3001263) were used for all studies. The final volume of samples and calibration solutions placed in the graphite tubes was 20 μL.

2.2. Graphite tube treatment

To study the better permanent modifier for each investigated matrices, each platform inside of the graphite tubes were previously treated with Rh, Ir, Ru, Zr, W, Nb, Ti, and Ta, by applying 50 μL of 1000 μg L^{-1} of each solution and heating the tube with a specific temperature

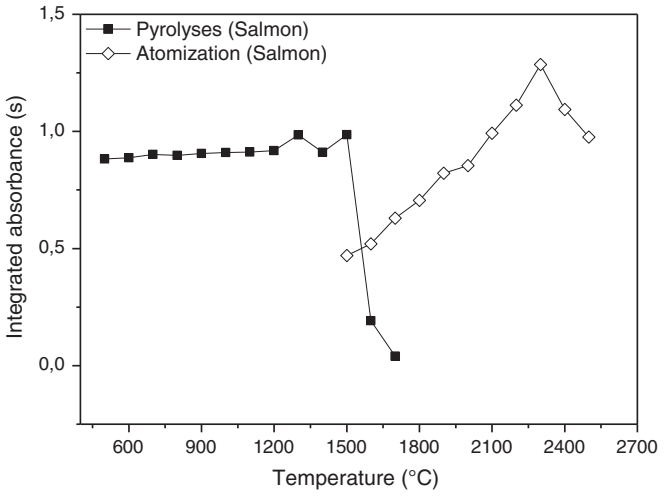

Fig. 2. Pyrolysis and atomization temperature curves for 2 ng of fish tissue (salmon) solubilized with Universol® as described and using permanent niobium (500 μg) as permanent modifier as modifier.

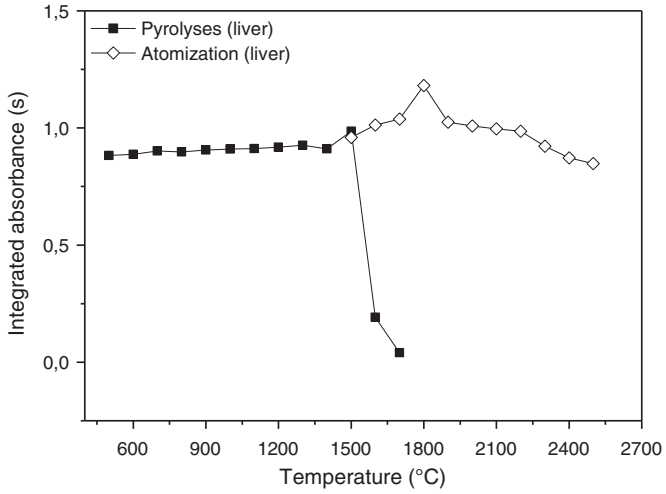

Fig. 1. Pyrolysis and atomization temperature curves for 2 ng of bovine liver solubilized with Universol® as described and using permanent iridium (500 μg) co-injected with 5 μg of ruthenium as modifier.

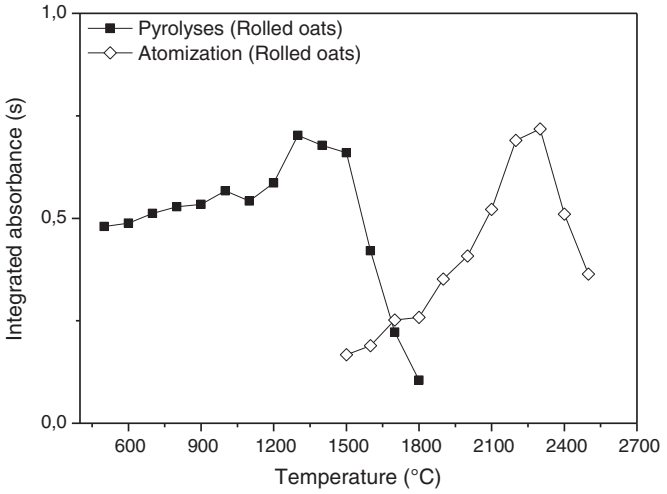

Fig. 3. Pyrolysis and atomization temperature curves for 1.3 ng of oat flake solubilized with Universol® as described and using permanent iridium (500 μg) co-injected with 5 μg of platinum as modifier.