



Analytical Methods

Development of spectrophotometric method for iron determination in fortified wheat and maize flours



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ABSTRACT

The determination of iron in fortified foods is mandatory by many global regulatory agencies. However, the spectroscopic techniques require elevated investments limiting their applicability especially in developing countries. Therefore, simple, viable and analytical methods with sufficient sensitivity can become an alternative. In this work, a sensitive, simple and viable spectrophotometry method to determine iron in wheat and maize flours was developed following a cloud point extraction (CPE) procedure. The analyte was first complexed with 2-(5-Bromine-2-pyridylazo)-5-diethylaminophenol (Br-PADAP) in the presence of the surfactant octylphenoxypolyethoxyethanol (Triton X-114). For the CPE optimization the variables: pH of the medium, stoichiometry of the complex, surfactant, and salt concentrations were evaluated. Linearity in the analytical blank was obtained by using the square root of absorbance ($\sqrt{\text{Abs}}$) in order to adjust the residues of the curve. The precision was lower than 5% and the accuracy ranged from 97 to 101%. The limits of detection and quantification were $0.004 \mu\text{g mL}^{-1}$ and $0.01 \mu\text{g mL}^{-1}$, respectively. The method was applied to investigate the content of iron in 14 brands of fortified flours. The concentrations of iron varied from 0.435 to 3.62 mg/100 g and 0.570 to 3.15 mg/100 g in wheat and maize flour, respectively. The content of iron in all brands investigated in this study was approximately 10-fold lower than the value required by (ANVISA). The amount of iron in fortified foods was satisfactorily determined by using a simple, sensitive, and low cost spectrophotometric method.

1. Introduction

Undernutrition is highly prevalent in low-income and middle-income countries, resulting in substantial increases in mortality and overall disease burden (Black et al., 2008). In such scenario, a diet poor in minerals is associated with many chronic diseases. The iron deficiency anemia, for example, is one of the most prevalent forms of malnutrition is, which is close related with increased maternal mortality, decrease of work capacity, poor cognitive, motor development, and behavioral problems in children (Grantham-McGregor & Ani, 2001; WHO, 2011). In order to bring this form of micronutrient deficiency under control, many countries have adopted the low cost strategy to fortify the foods with iron (WHO, 2011).

Two types of iron are basically employed for food fortification: heme iron, and non-heme iron (Hallberg, 1981; Hurrell, 1997). The wheat and maize flours, as well as breakfast cereals products, are present in the iron-fortified foods. According to the Brazilian regulations, the wheat and maize flours should be fortified with 4.2 mg iron per 100 g of flour (ANVISA, 2002). Therefore, in order to check if the requirements of the current legislation are being complied it is important

to have analytical methods capable to detect and quantify the iron in such goods.

Among the spectroscopic techniques commonly applied to determine iron and other metal species, the Flame (F AAS), Graphite Furnace Atomic Absorption Spectrometry (GF AAS), Inductively Coupled Plasma Optical (ICP AES) and Inductively Coupled Plasma – Mass Spectrometry (ICP – MS). Despite of being well-established techniques, they require a minimum of infrastructure and are of highly effective cost, which limits their applications by many laboratories (Ojeda & Rojas, 2004). The UV–VIS spectroscopy can be used as an alternative method to determine metal species in distinct matrices, including foods (Peng et al., 2015). This well-established technique has low acquisition and maintenance costs; good analytical frequency, precision and accuracy. However, it lacks a sufficient sensitivity to determine trace of metals, such as iron. Thus, in order to increase the sensitivity, it is necessary to pre-concentrate the species of interest (Madrakian, Afkhami, Siri, & Mohammadnejad, 2011).

The cloud point extraction (CPE) has been widely used as an alternative to liquid-liquid extraction for subsequent determination of metal ions (Madrakian & Ghazizadeh, 2008). This technique is based on the

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properties of surfactants, like the octylphenoxypolyethoxyethanol (Triton X-114), to form micelles once reached the critical micelle concentration (CMC) (Khammas, 2009; Souza, Teixeira, & Bezerra, 2016). The micelles can interact directly with metal ions or metallic complexes obtained from a hydrophobic chelating ligand (Youcef, Benabdallah, & Reffas, 2015). This phenomenon can be observed by the appearance of turbidity on the solution followed by the development of two phases. The aqueous phase, of greater volume, is called “poor phase” and it is discharged. The other one, called “rich phase”, with small volume, contains the analyte of interest (Khammas, 2009).

A CPE method based on Br-PADAP complexation followed by spectrophotometric quantification of iron in beer samples has been already reported (Filik & Giray, 2012). However, hitherto, application CPE approach followed by UV–VIS spectrophotometric analysis to quantify iron in wheat or maize flours has not been reported. Therefore, this study aims to develop and validate an alternative analytical procedure using CPE and spectrophotometry to measure the iron content in fortified flours marketed in Brazil.

2. Materials and methods

2.1. Reagents

All solutions were prepared using deionized water, and all the reagents were analytical grade. The standard solution of Fe^{+3} $1000 \mu\text{g mL}^{-1}$ from Merck (Gernsheim, Darmstadt, Germany) was further diluted to $100 \mu\text{g mL}^{-1}$ and $10 \mu\text{g mL}^{-1}$, respectively. Fresh solutions of reducing agent ascorbic acid ($1\% \text{ w v}^{-1}$) and sodium chloride ($30\% \text{ w v}^{-1}$) were prepared before each experiment. The buffer pH 8 consisted of 0.2 mol L^{-1} of boric acid, 0.2 mol L^{-1} of potassium chloride, and 0.2 mol L^{-1} of sodium hydroxide. The Br-PADAP ($10^{-5} \text{ mol L}^{-1}$) complexing agent solution comprised a mixture containing $0.00036\% \text{ (w v}^{-1}\text{)}$ of Br-PADAP and $5\% \text{ (w v}^{-1}\text{)}$ g of Triton X-114. Finally, the surfactant solution contained $5\% \text{ (w v}^{-1}\text{)}$ of Triton X-114.

2.2. Optimization of experimental conditions

A 2^4 plan with central point was conducted in order to evaluate the following variables: medium pH (3.0–8.0), Triton X-114 surfactant concentration (0.2 – $1.0\% \text{ w v}^{-1}$), Fe^{2+} /Br-PADAP stoichiometry (1:1–3:1) and NaCl concentration (0.0 – $3.0\% \text{ w v}^{-1}$) (Table 1 – supplementary material).

2.3. CPE procedure

The CPE experiments were performed by adding 1.0 mL of digested wheat and maize flours samples, 0.5 mL of ascorbic acid $1\% \text{ w v}^{-1}$, 2.0 mL of buffer pH 8.0, 1.0 mL of NaCl $30\% \text{ w v}^{-1}$, and $223 \mu\text{L}$ of Br-PADAP $10^{-5} \text{ mol L}^{-1}$ solutions into 15.0 mL falcon tubes. The tubes were vortexed during 30 s and let over 20 min at room temperature for complex formation. The volume was adjusted to 10.0 mL with deionized water and $400 \mu\text{L}$ of Triton X-114 ($5\% \text{ w v}^{-1}$) surfactant solution were added. The tubes were kept in water bath at about 60°C until the formation of the cloud point and then cooled in a refrigerator to assure the phase separation. The phases were manually separated and the rich phase was diluted to 2.0 mL with ethanol. The absorbances at 550 nm , corresponding to maximum of absorption, were measured through a UV–VIS spectrophotometer (Thermo Scientific, Genesys 10S).

2.4. Samples decomposition

Seven brands of wheat and maize flours were acquired in distinct markets of Divinópolis city – Minas Gerais, Brazil. The brands entitled 1A, 1B, 1C, 1D, 1E, 1F and 1G correspond to maize flours and the brands entitled 2A, 2B, 2C, 2D, 2E, 2F and 2G correspond to wheat

flours. For moisture removal, the samples were dried at 110°C until constant weight and stored in stoppered polyethylene bottles until analysis. The sample preparation was based on wet decomposition by adding 30.0 mL of concentrated nitric acid ($65\% \text{ w v}^{-1}$) and 5.0 mL of concentrated hydrogen peroxide ($30\% \text{ w v}^{-1}$) in 3.0 g of sample, subsequently. The mixture was heated on a mantle at 120°C during 30 min . After cooling at room temperature, the pH was adjusted to 8.0 with the buffer solution. One milliliter of the resulting solution was used for the CPE procedure.

2.5. Method validation

The evaluation of the merit parameters was performed following the INMETRO's Guide (2011). The parameters of merit were: linearity, matrix effect, limit of detection (LOD) and quantification (LOQ), precision and accuracy.

2.5.1. Linearity and matrix effect

Studies to evaluate the linearity were performed by using analytical blanks and the digested pooled samples of wheat and maize flours. The analytical curves were prepared, independently, with an Fe^{3+} standard solution in concentration levels of 0.05; 0.1; 0.15; 0.2; 0.25 and $0.3 \mu\text{g mL}^{-1}$. Each level was prepared in triplicate and the absorbances were determined randomly. The Ordinary Least Squares Regression Method (OLS) was applied in order to estimate the linear regression equation. The slopes of the curves prepared in the digested pools were compared with those from the analytical blanks in order to evaluate the matrix effect.

2.5.1.1. Residual plots for outlier. The presence of outliers was evaluated by Jackknife standard residual and also by visual analysis of graphs of regression residues versus Fe^{2+} concentration levels (Souza & Junqueira, 2005).

2.5.1.2. Normality, independence and homoscedasticity. The following premises required by OLS were evaluated: (a) normality by Ryan–Joiner test, (b) independence of residues by Durbin–Watson statistic and (c) homoscedasticity of residues by modified Levene test.

2.5.2. Limit of detection and limit of quantification

Analytical blanks were prepared independently as described in Sample decomposition section, excepting the dried samples. The limits of detection (LOD) and quantification (LOQ) were obtained according to INMETRO's Guide 2011.

The limit of detection (LOD) and the limit of quantification (LOQ) were estimated according to the following equations: $\text{LOD} = X + t \times s$ (equation 1) and $\text{LOQ} = X + 10 \times s$ (equation 2), where X = average of 10 analytical blank concentrations, s = averaged standard deviation of the concentrations, and $t = t$ student ($t_{(0.05; n-1)}$) (INMETRO's Guide, 2011). The LOQ was confirmed experimentally. The results were evaluated considering the recovery and coefficient of variation (% CV).

2.5.3. Precision and accuracy

Analytical blank solutions were prepared and fortified with standard solution of Fe^{+3} at the concentration levels of 0.15 and $0.25 \mu\text{g mL}^{-1}$. Each one was prepared in seven replicates. The digestion was conducted in three different days, and the determinations were performed in the same day. The intermediate precision was assessed according to the % CV. Accuracy was evaluated by addition and recovery methods (Magnusson & Örnemark, 2014).

3. Results and discussion

3.1. Optimization of cloud point extraction method

Protocols for iron quantification in fortified wheat flour and other

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