Food Chemistry 178 (2015) 287–291

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Homogeneity study of a corn flour laboratory reference material candidate for inorganic analysis

Ana Maria Pinto dos Santos [a,*], Liz Oliveira dos Santos [a], Geovani Cardoso Brandao [a], Danilo Junqueira Leao [a], Alfredo Victor Bellido Bernedo [b], Ricardo Tadeu Lopes [c], Valfredo Azevedo Lemos [d]

[a] Universidade Federal da Bahia, Instituto de Química, Grupo de Pesquisa em Química e Quimiometria, Campus Ondina, 41170-115 Salvador, Bahia, Brazil
[b] Universidade Federal Fluminense, Centro de Estudos Gerais, Instituto de Química, Niterói, Rio de Janeiro, Brazil
[c] Universidade Federal do Rio de Janeiro, Instituto Alberto Luiz Coimbra de Pós Graduação e Pesquisa de Engenharia, Programa de Engenharia Nuclear, Rio de Janeiro, Brazil
[d] Universidade Estadual do Sudoeste da Bahia, Laboratório de Química Analítica (LQA), Campus de Jequié, 45206-190 Jequié, Bahia, Brazil

ARTICLE INFO

Article history:
Received 14 June 2014
Received in revised form 30 August 2014
Accepted 3 January 2015
Available online 19 January 2015

Keywords:
Corn flour
Reference material candidate
Homogeneity study
Analysis of variance
Principal component analysis

ABSTRACT

In this work, a homogeneity study of a corn flour reference material candidate for inorganic analysis is presented. Seven kilograms of corn flour were used to prepare the material, which was distributed among 100 bottles. The elements Ca, K, Mg, P, Zn, Cu, Fe, Mn and Mo were quantified by inductively coupled plasma optical emission spectrometry (ICP OES) after acid digestion procedure. The method accuracy was confirmed by analyzing the rice flour certified reference material, NIST 1568a. All results were evaluated by analysis of variance (ANOVA) and principal component analysis (PCA). In the study, a sample mass of 400 mg was established as the minimum mass required for analysis, according to the PCA. The between-bottle test was performed by analyzing 9 bottles of the material. Subsamples of a single bottle were analyzed for the within-bottle test. No significant differences were observed for the results obtained through the application of both statistical methods. This fact demonstrates that the material is homogeneous for use as a laboratory reference material.

1. Introduction

Corn is one of the most consumed cereals in the world and is the second most grown cereal in Brazil. It is a very nutritive food and is utilized as a food for both humans and animals. In the form of flour, it is greatly utilized in Brazilian cuisine in several ways, including in the preparation of cakes, breads, cookies, and other baked goods (Schwanz et al., 2012). Therefore, the development of analytical methods for assessing the content of essential and toxic elements in corn flour is relevant.

The usual process for accuracy evaluation of an analytical method is the analysis of a certified reference material (CRM) that is recommended by the International Union of Pure and Applied Chemistry (IUPAC) (dos Santos, Lima, & de Jesus, 2011; IUPAC, 2002; Lima et al., 2010; Valente, Sanches-Silva, Albuquerque, & Costa, 2014). However, CRMs of many types of matrices are not available, and they have high costs. Thus, there has been a growing requirement for the development of new reference materials (Kato et al., 2013; Spisso et al., 2013; Ulrich & Sarkis, 2013; Valente et al., 2014). The homogeneity test is one of the most important steps in the development and production of a CRM (dos Santos et al., 2011; Lima et al., 2010). According to the International Organization for Standardization (ISO) Guide 35 (ISO, 2006), homogeneity is related to various factors, such as the type of material, the size of the sample, and the types of analytes in addition to the accuracy and precision of the determination. Under these guidelines, a material is considered homogeneous with respect to a given property if the trials of subsamples of a material batch are in accordance with the assigned value and the uncertainty (ISO, 2006).

In homogeneity studies, the various units of a material batch are important for establishing the properties of the batch. To evaluate the units, the choice of the unit should be performed randomly for that assessment to be representative of the total quantity. In many studies of homogeneity, this test is performed in 2–5% of the total number of units (bottles) during the storage procedure, in which the variation should be included in the certified reference material (CRM)/reference material (RM) uncertainty. Additionally, the homogeneity of a unit (bottle) is used to determine the recommended minimum amount of representative sample that should be used in an analysis. In addition to these studies, a review of the different quantities of the reference material candidate is recommended prior to conducting laboratory tests (ISO, 2006).

* Corresponding author. Fax: +55 71 32374117.
E-mail address: amps@ufba.br (A.M.P. dos Santos).

http://dx.doi.org/10.1016/j.foodchem.2015.01.024
0308-8146/

According to ISO Guide 35, the certification process for a reference material, similar to a homogeneity study, is performed using univariate statistical method, such as a one-way analysis of variance (ANOVA) (ISO, 2006). In recent years, multivariate data analysis has been applied to chemical data with the goal of evaluating different types of results (Rocha, Nogueira, da Silva, Queiroz, & Sarmanho, 2013). Multivariate statistical methods allow the extraction of information that is not clearly shown by the data set (do Nascimento et al., 2010). Among these methods, principal component analysis (PCA) has been most widely used in analytical chemistry (Anunciação, Leao, de Jesus, & Ferreira, 2011; do Nascimento et al., 2010; dos Santos et al., 2013a,b; Lima et al., 2010; Liu et al., 2014; Oliveira et al., 2014; Rocha et al., 2013; Šelih, Šala, & Drgan, 2014). PCA can be applied in data analysis with the following goals: data reduction, structural simplification, object grouping, modeling, outlier detection, variable selection, and prediction. Furthermore, PCA using a correlation matrix is not influenced by factors with high variance because PCA displays the data in the same measurement scale (Wold, Esbensen, & Geladi, 1987). This feature can be very useful for homogeneity studies of reference material candidates due to the high variability of the data obtained in the determination of macro- and microelements. However, only a small number of works reported in the literature have employed PCA to evaluate the homogeneity of reference material candidates, such as wheat flour (Lima et al., 2010), active pharmaceutical ingredients (Rocha & Nogueira, 2011) and sodium diclofenac (Rocha et al., 2013).

This work aims to present a homogeneity study for a corn flour reference material candidate by considering the quantitative results of nine elements in the samples, whose main function is to ensure the measurement reliability. Both univariate and multivariate statistical methods (ANOVA and PCA) were used to evaluate the homogeneity of the reference material candidate.

2. Experimental

2.1. Instrumentation

The multi-element determination of Ca, K, Mg, P, Zn, Cu, Fe, Mn and Mo was performed using the following equipment: an inductively coupled plasma optical emission spectrometer (ICP OES) model Vista PRO from Varian (Mulgrave, Australia) with axial viewing and a charge-coupled device detector. The instrumental parameters used for the multi-element determination were as follows: RF generator of 40 MHz, power of 1.3 kW, plasma gas flow rate of 15 L min^{-1}, auxiliary gas flow rate of 1.5 L min^{-1} and nebulizer gas flow rate of 0.7 L min^{-1}. The elements and the analytical spectral lines (nm) used were: Ca II (422.673), K I (766.491), Mg II (279.553), P I (213.618), Zn II (202.548), Cu II (327.395), Fe II (238.204), Mn II (257.610), and Mo II (204.598), where "I" is the atomic emission line and "II" is the ionic emission line.

A digester block model TE-040/25 from Tecnal (São Paulo, Brazil) was used for the acid digestion of the samples of corn flour and the rice flour CRM.

2.2. Reagents and solutions

Ultrapure water produced from a Milli-Q purification system from Millipore (Bedford, MA, USA), with resistivity of 18 MΩ cm^{-1}, was used throughout the experiments. The reagents nitric acid and hydrogen peroxide were of analytical grade and obtained from Merck (Darmstadt, Germany). A working standard solution was prepared fresh daily by serial dilution from stock solutions containing 1000 mg L^{-1} (Merck) of the elements Ca, K, Mg, P, Zn, Cu, Fe, Mn and Mo.

2.3. Preparation of the reference material candidate

The samples of corn flour used in this work were obtained from the same manufacturer but from different batches and were acquired in a supermarket in Salvador City, Bahia, Brazil. Seven kilograms of corn flour were used to produce the reference material candidate. After irradiation with 15 kGy gamma radiation to prevent the development of fungi and bacteria, the material was placed in a polyethylene bucket for homogenization. Six subsamples were taken from the bulk material for the preliminary study of homogeneity. After a satisfactory level of homogeneity was achieved, the corn flour was then transferred to 100 polyethylene flasks. Approximately 80 g of the material was transferred to each flask. A total of 100 bottles were distributed into batches containing 10 bottles each, resulting in a total of 10 batches. The bottles used allowed airtight storage, and they were labeled with the name of the material and numbered from 1 to 100.

2.4. Digestion of the samples and determination of the elements

Triplicates of each sample were run for the determination of the total contents of the elements. Approximately 2.0 g of the sample were placed in a digester tube, and 2.0 mL of 65% (w/w) nitric acid and 1.0 mL of 30% (w/w) hydrogen peroxide were added. Cold finger was used as reflux system (Ferreira et al., 2013). Then, the mixture was heated on a digester block until a limpid solution was obtained. The digested solution was then quantitatively transferred to centrifuge tubes and diluted with ultrapure water up to a final volume of 12.0 mL. A blank digest was carried out in the same way as the samples of interest. The multi-element determination of Ca, K, Mg, P, Zn, Cu, Fe, Mn and Mo was performed using the inductively coupled plasma optical emission spectrometry (ICP OES) technique.

2.5. Homogeneity study

The homogeneity study evaluated representative samples of the entire lot. After remixing the sample inside the bottle, three subsamples from each unit were taken for analysis, and twenty bottles of the corn flour were randomly selected for this study. The steps of this test were as follows:

(1) The influence of the sample mass on the homogeneity of the reference material candidate was examined by analyzing mass quantities of 100, 200, 300, 400 and 500 mg.
(2) The between-bottle homogeneity was studied using the measurement results obtained from 9 units of the reference material candidate.
(3) The within-bottle homogeneity was analyzed using subsamples of one selected unit.

All experiments were performed to determine the concentrations of the elements Ca, K, Mg, P, Zn, Cu, Fe, Mn and Mo. These elements were chosen because they are important macro- and micronutrients for the human diet and their contents are often determined in cereals for nutritional and toxicological studies. All homogeneity study was performed during a time interval of two weeks for ensure the stability of the samples.

2.6. Evaluation of the homogeneity

The homogeneity study was performed with 9 flasks of the reference material candidate. The flasks were selected in a random way and analyzed as described in the previous section. Then, the variance analysis (ANOVA) was applied to the measurement results to evaluate the homogeneity. According to the