Microchemical Journal 95 (2010) 222–226

Contents lists available at ScienceDirect

Microchemical Journal

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

Principal component analysis and hierarchical cluster analysis for homogeneity evaluation during the preparation of a wheat flour laboratory reference material for inorganic analysis

Daniel C. Lima [a], Ana M.P. dos Santos [a], Rennan G.O. Araujo [b], Ieda S. Scarminio [c], Roy E. Bruns [d], Sergio L.C. Ferreira [a,*]

[a] Universidade Federal da Bahia, Instituto de Química, Grupo de Pesquisa em Química e Quimiometria 40170-290, Salvador, Bahia, Brazil
[b] Universidade Federal de Sergipe, Departamento de Química, 49.100-000, São Cristóvão, Sergipe, Brazil
[c] Universidade Estadual de Londrina, Departamento de Química, 86055-900 Londrina, PR, Brazil
[d] Universidade Estadual de Campinas, Instituto de Química, 13083-970 Campinas-SP, Brazil

ARTICLE INFO

Article history:
Received 18 November 2009
Received in revised form 4 December 2009
Accepted 4 December 2009
Available online 16 December 2009

Keywords:
Wheat flour
Laboratory reference material
Homogeneity evaluation
Univariate and multivariate analysis techniques
Principal component analysis
Hierarchical cluster analysis

ABSTRACT

The development of a homogeneity study during the preparation of a wheat flour laboratory reference material (LRM) for use in the quantification of metals and metalloids is reported. Inductively coupled plasma optical emission spectrometry (ICP OES) was used with validation performed using a certified reference material of wheat flour furnished by the National Institute of Standards and Technology (NIST). Copper, iron, manganese, phosphor, strontium and zinc were studied in a within-bottle homogeneity test whereas barium, copper, iron, zinc, manganese, strontium, phosphor and calcium were included in a between batch homogeneity study. Standard univariate analysis of variance (ANOVA) was performed for all analytes. Furthermore an alternative multivariate analysis for homogeneity is proposed by performing ANOVA of principal component scores and by inspection of principal component score graphs and hierarchical cluster analysis dendrograms. The ANOVA *F*-tests performed on both, the univariate and multivariate parameters, were not significant at the 95% confidence level and indicated homogeneous wheat flour samples. A 10 kg amount of material was processed, which was distributed in 100 bottles, each containing 100 g. For the between-bottle homogeneity test, three replicates were taken from each of 10 bottles selected of the 100 bottles obtained. The results were evaluated using an *F-test*, which demonstrated no significant difference for the between-bottle results. It is indicative that this material is homogeneous. Afterwards, the influence of the sample mass on the homogeneity of the material was also evaluated by quantification of the elements for 100, 300, 500, 700 and 1000 mg sample masses with all the experiments being performed in triplicate. The *F*-test was also used for evaluation of these results and demonstrated that the material is homogeneous for masses taken in the 100 to 1000 mg range. All these results were further evaluated employing the principal component analysis (PCA) and hierarchical cluster analysis (HCA) multivariate techniques. Both techniques also demonstrated that the material is perfectly homogeneous for use as laboratory reference material.

1. Introduction

The use of certified reference materials (CRM) in analytical chemistry is undoubtedly necessary and actually several CRM have been performed in all world [1,2]. Actually, owing to the International Union of Pure and Applied Chemistry (IUPAC) recommendations [1], it is practically impossible to perform the validation process of an analytical method without the use of these materials. Exceptions are still accepted when there are no specific materials of a certain sample necessary for validation. More recently, CRM's have been used for the establishment of calibration curves during the development of direct analytical methods using solid sampling [3,4] and slurry sampling by electrothermal atomization atomic absorption spectrometry (ET AAS) [5–7], inductively coupled plasma optical emission spectrometry (ICP OES) [8–11] inductively coupled plasma mass spectrometry (ICP-MS) [12] and flame atomic absorption spectrometry (FAAS) [13]. In these cases, CRM's are obligatorily necessary for the investigation of the matrix effect for the proposed method [14]. In this way, CRM's of rice flour have been used in slurry sampling procedures proposed for the determination of copper in powdered chocolate [15] and manganese in wheat flour [16] by FAAS. CRM's of sediments were used for the establishment of calibration curves for the determination of arsenic, mercury and selenium employing the slurry sampling method and ETV-ICP-MS [17]. CRM's of coals were used for the determination of

* Corresponding author. Fax: +55 71 3283 6831.
E-mail address: slcf@ufba.br (S.L.C. Ferreira).

0026-265X/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.microc.2009.12.003

cadmium, copper and lead in mineral coal using solid sampling and ET AAS [18].

Principal component (PC) and hierarchical cluster analysis (HCA) are among the most popular multivariate exploratory methods used in analytical chemistry [19–22]. PCA consists of diagonalization of the covariance or correlation matrix transforming the original chemical measurements into linear combinations of these measurements, the principal components [23–25]. This corresponds to rotating the coordinate space axes so that the explained variance of each PC is maximized. This technique allows data reduction from higher to lower dimensional spaces that can simplify their graphical representation. HCA characterizes similarities among samples by examining interpoint distances representing all possible sample pairs in high dimensional space. The sample similarities are represented on two dimensional diagrams call dendrograms. These techniques complement one another and have been widely used in solving classification problems [26–28].

In the development of candidate to reference material, the homogeneity test is necessary for all of the projects of certification of reference matters, due this to supply information about the possible variability of the non homogeneity of the wrapped material, to detect some impurity, interferences or irregularities that it can be due to problems noticed during the preparation, mainly in matters granulated [29]. The International Organization for Standardization (ISO) Guide 35 supplies information for the certification of candidate material for reference use [30]. This information is restricted to univariate statistical methods. However many modern analytical instruments are capable of making simultaneous measurements for several analytes. In these cases one would like to have both univariate and multivariate statistical procedures to validate laboratory reference material. In this research univariate and multivariate procedures are applied to six analytes in a homogeneity study of the effect of sample mass on material preparation and to eight analytes in a between-bottle homogeneity study for a potential laboratory reference material for wheat flour.

2. Experimental

2.1. Instrumentation

For determination of metals and metalloids an inductively coupled plasma optical emission spectrometer with an axially-viewed configuration (ICP OES, VISTA PRO, Varian, Mulgrave, Australia), equipped with a simultaneous charge-coulped device (CCD) solid-state detector was employed. All measurements were performed using two pixels for each wavelength. The sample introduction system employed using a concentric nebulizer and a cyclonic spray chamber. The calcium, copper, iron, manganese, phosphorous, strontium, barium and zinc elements were determined. All analyses were performed in triplicate and checked by calibration curves. The operational parameters are listed in Table 1.

Table 1
Instrumental parameters used in the axially-viewed ICP OES.

Parameters	Conditions	
RF generator (MHz)	40	
RF power (kW)	1.2	
Plasma gas rate (L min^{-1})	15.0	
Auxilary gas rate (L min^{-1})	1.5	
Nebulizer gas rate (L min^{-1})	0.7	
Injector tube diameter (mm)	2.4	
Spray chamber	Ciclonyc	
Nebulizer	Concentric, type K	
Lines (nm)	Fe (II) 238.264	P (I) 213.618
	Cu (I) 324.754	Zn (II) 202.548
	Ba (II) 493.408	Ca (II) 317.933
	Mn (II) 257.610	Sr (II) 407.771

(I) line of atomic emission.
(II) line of ionic emission.

2.2. Reagents

All reagents were of analytical grade. Ultrapure water was obtained from an EASYpure RF purification system (Barnstedt, Dubuque, IA, USA). Nitric and hydrochloric acid were of Suprapur® quality (Merck, Darmstadt, Germany). Nitric and hydrochloric acid solutions were prepared by direct dilution with water from the concentrated Suprapur® solutions. Laboratory glassware was kept overnight in 10% (v/v) nitric acid solution. Before use, the glassware was rinsed with demineralized water and dried in a dust-free environment. The commercially available metal standard solutions (1.000 ± 0.002 mg L^{-1}) (Titrisol Merck, Damstadt, Germany) were prepared for the standard working range. Calibration curves were obtained within 6.0 to 100.0 mg L^{-1} for calcium and phosphorus, and within 0.02 to 10.0 mg L^{-1} for manganese, zinc, copper, iron, barium and strontium.

2.3. Procedure for decomposition of the wheat flour samples

For acid digestion, about 0.5 g of the wheat flour samples, 3.0 mL of concentrated nitric acid and 3.0 mL of hydrogen peroxide were placed in a glass vessel and heated on a hot plate (at these conditions the samples are decomposed easily). Finally the content was quantitatively transferred to 10 mL volumetric flasks and diluted with 1.0 mol L^{-1} nitric acid solution [15].

2.4. Preparation of the material

Ten kg of wheat flour was made available by a mill, located in Salvador, Bahia. With the intention of inhibiting the precocious loss of material due to infestation, contamination, and development of bacteria and mushrooms, the material was irradiated with Co^{60} at the Institute of Research of Nuclear Energy (IPEN) in São Paulo. Then the 10 kg of wheat flour were transferred to 100 g polyethylene flasks with hermetic covers. Approximately 100 g of wheat flour was transferred to each flask. A total of 100 bottles were used with 10 bottles being distributed to each batch, resulting in 10 batches.

2.5. Validation of the analytical method used for quantification of elements

The accuracy of the ICP OES method employed Cu, Fe, Mn, P, Sr and Zn determination in wheat flour samples was confirmed by analysis of certified reference material furnished by National Institute of Standards and Technology (Gaithersburg, MD, USA), wheat flour NIST 1567a. The results shown in Table 2 demonstrate that this method is statistically satisfactory for the determination of all elements involved in the certification.

3. Results and discussion

3.1. Evaluation of the sample mass on the homogeneity of the material

In this study the influence of the sample mass on the homogeneity of the material was evaluated by determination of the copper, iron,

Table 2
Results obtained for the certified reference materials of wheat flour NIST 1567a analyzed ($n = 3$).

CRM Wheat flour NIST 1567a		
Element	Certified value	Found value
Cu (μg g^{-1})	2.1 ± 0.2	1.9 ± 0.1
Mn (μg g^{-1})	9.4 ± 0.9	8.3 ± 0.3
Fe (μg g^{-1})	14.1 ± 0.5	13.7 ± 0.3
Zn (μg g^{-1})	11.6 ± 0.4	10.9 ± 0.5
P (%)	0.134 ± 0.006	0.122 ± 0.005
Ca (%)	0.0191 ± 0.0004	0.0186 ± 0.0006