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Analytical Methods

Multi-elemental analysis of ready-to-eat "baby leaf" vegetables using microwave digestion and high-resolution continuum source atomic absorption spectrometry

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ABSTRACT

The mineral content (phosphorous (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu)) of eight ready-to-eat baby leaf vegetables was determined. The samples were subjected to microwave-assisted digestion and the minerals were quantified by High-Resolution Continuum Source Atomic Absorption Spectrometry (HR-CS-AAS) with flame and electrothermal atomisation. The methods were optimised and validated producing low LOQs, good repeatability and linearity, and recoveries, ranging from 91% to 110% for the minerals analysed. Phosphorous was determined by a standard colorimetric method. The accuracy of the method was checked by analysing a certified reference material; results were in agreement with the quantified value. The samples had a high content of potassium and calcium, but the principal mineral was iron. The mineral content was stable during storage and baby leaf vegetables could represent a good source of minerals in a balanced diet. A linear discriminant analysis was performed to compare the mineral profile obtained and showed, as expected, that the mineral content was similar between samples from the same family. The Linear Discriminant Analysis was able to discriminate different samples based on their mineral profile.

1. Introduction

Vegetables have almost all essential nutrients for human metabolism, if consumed as recommended, and are linked with the promotion of good health (Miller-Cebert, Sistani, & Cebert, 2009). Baby leaf salads are a relatively new ready-to-eat product, which is gaining popularity among the consumers (Clarkson, O'Byrne, Rothwell, & Taylor, 2003). It is prepared with young leaves, harvested at a very early stage of maturation while still metabolically active. These leaves offer a softer texture and add a variety of colours and shapes to the meal (Martínez-Sánchez et al., 2012). They have an appealing appearance due to their 3-D structure, and experience lower levels of oxidative damage because of their small stem diameter. These products have greater stability during shelf life, despite being packed without modified atmosphere or any other compounds to delay deterioration. They rely, almost exclusively, on strict control of storage temperature to preserve quality (Martínez-Sánchez et al., 2012; Wagstaff et al., 2007). Lowering the temperature of the product will slow the leaf metabolism, decreasing their respiratory rate (Rico, Martín-Diana, Barat, & Barry-Ryan, 2007). Minimal processing (washing, cutting and packaging) increases vegetables metabolic rate, leading to faster deterioration, which could result in rapid loss of components influencing flavour and nutritional value (Conte et al., 2008; Martínez-Sánchez et al., 2012). Maturity can also affect composition and the stability of fresh-cut vegetables (Martínez-Sánchez et al., 2012).

Accurate nutritional information is needed by public agencies and agricultural/food industries to promote products and healthy eating (Borah, Baruah, Das, & Borah, 2009; Miller-Cebert et al., 2009). Although green leafy vegetables are less rich in protein and carbohydrates, they are a good source of most minerals and vitamins (Altundag & Tuzen, 2011; Grusak & DellaPenna, 1999). Minerals are among the micronutrients that must be obtained through diet (Reddy & Bhatt, 2001). The basic functions of minerals in biological systems include participation in proteins, lipids and carbohydrates metabolism, as well as cellular and skeletal structure and a role in osmotic pressure and acid/base regulation (Chekri et al., 2012). Plants accumulate minerals accordingly to their requirements; however, the mineral content can be affected by genetic factors, soil and weather conditions, the use of fertilisers, and the plant's maturity at harvest (Grusak & DellaPenna, 1999; Sanchez-Castillo et al., 1998). Therefore, mineral quantification of

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vegetables must be done to assure the nutritional values stated on package label.

Mineral analyses in vegetables are common, and there are numerous techniques described in the literature. These include Atomic Absorption Spectrometry (AAS) (Chekri et al., 2012) with flame (FAAS) or electrothermal (EAAS) atomization and the Inductively Coupled Plasma Spectrometry (ICP) (Cooper et al., 2011) with atomic emission spectrometry (ICP-AES) (Lisiewska, Gębczyński, Bernaś, & Kmiecik, 2009), optical emission spectrometry (ICP-OES) (Altundag & Tuzen, 2011; Miller-Cebert et al., 2009), or mass spectrometry (ICP-MS) (Noël, Leblanc, & Guérin, 2003). Although, AAS is not the newest technique, its use has expanded with the development of High-Resolution Continuum Source Atomic Absorption Spectrometry (HR-CS-AAS) (Oliveira, Neto, Nóbrega, & Jones, 2010; Welz, 2005). The equipment has a continuous radiation source between 190 and 800 nm and a double mono-chromator with double-Echelle grating, which allows rapid change of wavelength during a sequential multi-element analysis. The linear CCD array detects not only the analytical line, but also, its spectral environment at high resolution, producing an atomic spectrum with 200 pixels. The central pixels correspond to the analyte line, including the spectral environment of about ±0.2 nm around that chosen analyte line, i.e. the noise. This feature allows monitoring the baseline and avoids signal interferences by correcting the baseline automatically, creating a very stable system with low noise levels and significantly lower detection limits (Oliveira et al., 2010; Welz, 2005). Although there are a few studies using HR-CS-AAS for food analysis (Oliveira et al., 2010, 2012; Welz, 2005), determination of a wide group of macro- and micro-minerals in green baby leaf vegetables has not been done previously. Therefore, the aim of this study was to (i) optimise and validate a HC-CS-AAS method for determining macro- (K, Na, Ca and Mg) and micro- (Fe, Mn, Zn and Cu) mineral composition of ready-to-eat "baby leaf", which included microwave mineralization of the samples; (ii) study the mineral content (phospurous, potassium, sodium, calcium, magnesium, iron, manganese, zinc and copper) of different green "baby leaf" vegetables; and (iii) the stability of the mineral content during a 10 days storage period.

2. Materials and methods

2.1. Reagents and analytical solutions

The ultrapure water (18.2 MΩ cm resistivity) used was from a Simplicity 185 water purification system (Millipore, Molsheim, France). Standard solutions of potassium, calcium, magnesium, iron, manganese, zinc and copper were prepared from 1000 mg/l stock solutions (Panreac Quimica SA, Barcelona, Spain). The phosphorous standard solution was made using potassium dihydrogen phosphate from Riedel-de Haën (Seelze, Germany) and the sodium from the 1000 mg/l stock sodium chloride from Merck (Darmstadt, Germany). Standards and samples solution were acidified with 1% (v/v) of 65% nitric acid (Sigma–Aldrich, Steinheim, Germany), except for phosphorous analysis. To avoid ionisation and chemical interferences in potassium, sodium, calcium, magnesium, iron, manganese and zinc HR-CS-FAAS analysis, caesium chloride (1% w/v; Sigma–Aldrich, Steinheim, Germany) was used as ionisation buffer. To avoid interference from elements that could form stable oxy-salts and enhance sensitivity, 1% (w/v) of $LaNO_3$ (Panreac Quimica SA, Barcelona, Spain) was added to the standards and samples solution for calcium determinations. Copper was analysed by HR-CS-EAAS using a matrix modifier prepared from the relevant salts ($Pd(NO_3)_2$ (0.1%), $Mg(NO_3)_2$ (0.05%) in water (Merck, Darmstadt, Germany and Panreac Quimica SA, Barcelona, Spain, respectively)). Phosphorous was quantified using a spectrometer method according to 4500-P standard method (Greenberg, Clesceri, & Eaton, 1992); the colour development reagent was prepared with ammonium molybdate tetrahydrated (99.0%) and ammonium metavanadate (99.0%) (Merck, Darmstadt, Germany). All glassware and plastic material were soaked in 10% nitric acid for 24 h, rinsed with ultra-pure water and dried before use.

2.2. Samples

Minimally processed baby leaf vegetables (washed and packaged) were supplied by a producer (Odemira, Portugal). The samples were received at the laboratory one day after processing. The samples were baby leaves of green lettuce (*Lactuca sativa* var. crispa), swiss chard (*Beta vulgaris*), watercress (*Nasturtium officinale*), lamb's lettuce (*Valerianella locusta*), wild rocket (*Diplotaxis muralis*) from conventional and organic production, spinach (*Spinacia oleracea*) and parsley (*Petroselinum crispum*). About 1 kg of baby leaf samples were freeze-dried (Telstar Cryodos-80, Terrassa, Barcelona), on arrival and after 10 days of refrigerated storage (3 ± 1 °C, monitored with a EL-USB 2, Lascar Electronics, Salisbury, UK). The freeze-dried leaves were reduced to a fine powder in a knife mill (GM 200, RETSCH, Haan, Germany) and stored protected from light, oxygen and high temperatures. This procedure generated a composite sample to exclude individual differences and ensure that the test sample was representative.

2.3. Microwave digestion

The sample mineralization was carried out by microwave assisted digestion MARS-X (CEM, Mathews, NC, USA). The procedure was adjusted to achieve complete mineralization of the sample within the shortest time possible. The microwave program (Table 1) was adjusted using 0.1 g of the freeze dried wild rocket baby leaf digested with 9 mL of nitric acid. A four stage program with a maximum temperature of 180 °C was chosen. Different portions of the samples (0.1, 0.15 and 0.2 g of freeze-dried baby leaf) and mixtures of nitric acid/ultrapure water (9:0; 6:3; 7:2; 5:4) and nitric acid/hydrochloric acid (7:2) were tested. The best conditions were 0.15 g of freeze dried sample, nitric acid/ultrapure water (6:3) and 15 min prior to microwave digestion. Samples were quantitatively transferred to a graduated plastic tube and diluted to a final volume of 15 ml with ultrapure water. Four replicates of each sample from the two sampling days (day 1 and day 10) were digested. Blank digestion was carried out without addition of any sample. A certified reference material BCR ®-679 (freeze dried white cabbage), obtained from Institute of Reference Materials and Measurements, was digested and analysed with the same protocol.

2.4. Mineral analysis

Potassium, sodium, calcium, magnesium, iron, manganese and zinc were analysed using a HR-CS-FAAS Analytik Jena ContrAA 700, equipped with a xenon short-arc lamp of 300 W (XBO 301, GLE, Berlin, Germany), operating in a hot-spot mode. An air/acetylene oxidising flame (Linde, Portugal) was used and the equipment was coupled to an AS52S autosampler (Analytik Jena, Germany).

Table 1
Microwave digestion program.

Stages	1	2	3	4
Power (W)	600	600	600	600
Time (min.)	3	5	8	2
Temperature (control)	50 °C	90 °C	170 °C	180 °C
Hold (min.)	5	10	20	10