Microchemical Journal 126 (2016) 509–514

Contents lists available at ScienceDirect

Microchemical Journal

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

Synchrotron based high throughput screening method for mineral analysis in cereal and pulse grains meal

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ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form 18 January 2016
Accepted 19 January 2016
Available online 27 January 2016

Keywords:
VESPERS (Very Sensitive Elemental and Structural Probe Employing Radiation from a Synchrotron) beamline
Minerals
Grain crops
Screening method

ABSTRACT

A rapid high throughput non-destructive method, using an X-ray microprobe beamline (Very Sensitive Elemental and Structural Probe Employing Radiation from Synchrotron, VESPERS, CLS Saskatoon), has been developed to screen multiple minerals in cereal and pulse meals. Starch spiked with commercial mineral standards cocktail at different concentrations was optimized to develop standard curve to determine mineral concentrations in grain meal samples. A grid sample holder to hold 84 samples was built for high precision and rapid analysis of multiple samples. The developed analytical method can be used for quantitative determination of eleven minerals including Ca, Cr, Mn, Fe, Ni, Co, Cu, Zn, Se, Rb and Mo at mg Kg^{-1} or ppm concentrations. Method adaptability was assessed using a cereal crop wheat and pulse crop chickpea grain meal. The method was successfully used for Ca, Mn, Fe, Ni, Cu, Zn, Se, Rb and Mo determination in wheat and chickpea and measurements were cross analyzed using inductively coupled plasma mass spectroscopy ICP-MS technique for the same samples. The method is highly sensitive (sensitivity at ppm level) and can be adapted to analyze mineral concentrations in grain meal from crops of interest. The precise and reproducible high throughput screening method can be used in crop improvement to develop bio-fortified grains with increased mineral concentrations.

1. Introduction

Carbohydrates and storage proteins are the major constituents in cereals and pulse grains. Additionally, several minor constituents present in small concentrations play important roles in plant growth and development, grain utilization and human nutrition. The minor constituent minerals form an essential group that is important in plant and human metabolism. Macronutrient minerals (N, P, K, Ca, S and Mg) are present in relatively large (mg/g) quantities and micronutrients (B, Cl, Mn, Fe, Zn, Cu, Mo, Se and Ni) are present in very small (<1 mg/g) quantities. Environmental conditions for growing plants and genotypes within the same species influence the uptake and accumulation of minerals and result in significant variation of the macro- and micronutrients in grains [1,2]. In human diet, grains and vegetables are major sources of minerals required for optimal human metabolism for normal daily functions. At least 22 minerals are needed for human growth and development [3,4]. Grains with increased mineral concentrations will allow humans to meet their daily dietary minerals [5].

Analytical methods used to determine mineral concentration in plant materials were originally developed for soil analysis [6,7] and are not fully optimized for analyzing plant materials. A desired method for the analysis of plant minerals would include a rapid, non-destructive analytical method which requires minimum sample processing. A rapid and accurate analysis of minerals and micronutrients in grains would provide robust data sets required to develop grains with desired mineral concentrations.

Spectroscopic methods used for characterization generally reflect the molecular composition and nature of chemical bonds in a sample. UV-visible, near infrared (NIR) and mid infrared (MIR) parts of electromagnetic spectrum are used in absorption techniques to determine the molecular composition of samples. These techniques have a serious limitation to determine mineral content, since they depend on correlation between mineral nutrient and presence of spectroscopically active compound [8]. Therefore, this indirect relationship requires strict validation and specificity tests before they can be used in plant mineral analysis [9]. Additionally, the calibrations used in these indirect techniques are limited by the variation of the composition and distribution of spectroscopically active compounds within and between plant species [10,11]. Particle size also affects the distribution of minerals and small focused beams would result in non-uniform distribution of minerals. In some cases, because of the low concentrations of minerals, these would not be detected due to low signal to noise ratio, even by techniques that are highly sensitive [12,13].

Laser-induced breakdown spectroscopy uses laser beam to create plasma from sample surfaces. The light emission spectrum generated

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http://dx.doi.org/10.1016/j.microc.2016.01.011
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after relaxation of excited plasma is element-specific [14] and has been used to determine K, P, Mg, Ca, Mn, Fe, Zn and B concentrations in wheat and other crops [15,16]. Although this technique requires limited processing, it is destructive. The major limitation of the technique is that the laser vaporizes very small quantities of sample from the surface, thus the method is prone to non-homogeneous sampling for analysis.

Other analytical techniques such as atomic absorption spectroscopy (AAS) [17], inductively coupled plasma mass spectroscopy (ICP-MS) [18], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [19], and inductively coupled plasma optical emission spectrometry (ICP-OES) [20] need elaborate sample preparation and processing. Extensive sample preparation introduces high degree of errors in the data by sample contamination during processing, digestion and analysis. A non-destructive rapid analysis technique that collects data on several minerals in the same sample will be very useful to develop mineral composition profiles in grain samples. X-ray fluorescence (XRF) analysis is an ideal technology, but laboratory methods using benchtop XRF are not sufficiently rapid to meet the needs of large data collection.

X-ray fluorescence analysis has been successfully used for multi-elemental analysis of plant samples [8]. In XRF spectroscopy, X-rays are used as a source of energy to excite all possible elements in a sample. Relaxing to the ground state, X-rays of lower energy are emitted from the sample. The emitted X-rays are element specific and the sensitivity of XRF depends on the differential cross-section of X-ray absorption and non-elastic scattering of the element. Heavy elements such as Mn, Fe, Cu, Ni, and Zn are easily detected, while light elements such as S, P, K, Mg, Ca, Cl and Na need to be present in higher concentrations. B and N are beyond detection limit in XRF [8] when high energy photons are used for excitation and detection in air.

Synchrotron radiation provides high intensity photon sources, which are qualitatively far superior to conventional X-rays, to detect a wide range of elements including trace elements, heavy metals or metalloids [21,22]. X-ray beams from synchrotron radiation are intense and tunable. Detection limit at mg Kg^{-1} level is of physiological importance and can be achieved for most elements of interest. [23]. Synchrotron based techniques require minimal time for sample preparation and can be applied to hydrated sample or *in-situ* analysis for elemental speciation and mapping. There are two main types of synchrotron based techniques used in plant research: X-ray fluorescence (SXRF, S for synchrotron) and X-ray absorption spectroscopies (XAS). SXRF is used for elemental determination and fast imaging, while XAS is used for chemical speciation analysis of specific elements. XRF data acquisition with synchrotron could be very fast, but there are limitations on the loading of samples in the beamline. Furthermore, the type and quality of sample influence the selection of beam type used for analysis. Generally two types of beams are used to analyze elements in plant samples, millimeter X-ray beam and micro focused X-ray beam. Millimeter X-ray beam analyses average speciation of elements in homogenized samples while micro-focused beam is used to analyze spatial information of intact samples at micron scale [24]. A larger beam gives a better average distribution of minerals while the micro beam is susceptible to the size of the crystallites, inhomogeneity and localization of minerals within crystallites.

A high throughput non-destructive rapid mineral analysis method was optimized using a synchrotron beamline (Very Sensitive Elemental and Structural Probe Employing Radiation from a Synchrotron, *i.e.*, VESPERS) at the Canadian Light source, Saskatoon, Canada [25,26]. Special sample holders (Fig. 1) and stages were configured for rapid analysis in both micro-focused and mm-sized beams. A novel starch based standard material spiked with known concentration of minerals was used to prepare calibration standards. The suitability of the method was assessed using grain meal from two important grain crops, wheat and chickpea.

2. Materials and methods

2.1. Preparation of standards

Mineral element standards (Ca, Mn, Cu, Co, Ni, Fe, Zn, Mo, Se and Rb) dissolved in 5% nitric acid (part number 4400-151119NB01) were purchased from CPI International, Santa Rosa, CA, USA. Except Ca (1000 μg mL^{-1}) all the other minerals were present at 100 μg mL^{-1} in the stock solution. Serial dilutions were prepared from the stock solution. Commercial wheat starch (1 g) (Baker and Adamson, General Chemical Division, NY, USA) was spiked with 1.0 mL of each dilution and dried overnight in the fume hood. Starch mixed with deionized water (conductivity 0.056 μΩ cm^{-1} at 25 °C) and dried was used as control. Dried starch was homogenized with a mortar and pestle used as standard powder. The values of standard weight mixed with starch were converted to mg Kg^{-1} (ppm) to prepare a standard curve. Control starch (spiked with deionized water) spectra was processed to obtain a baseline.

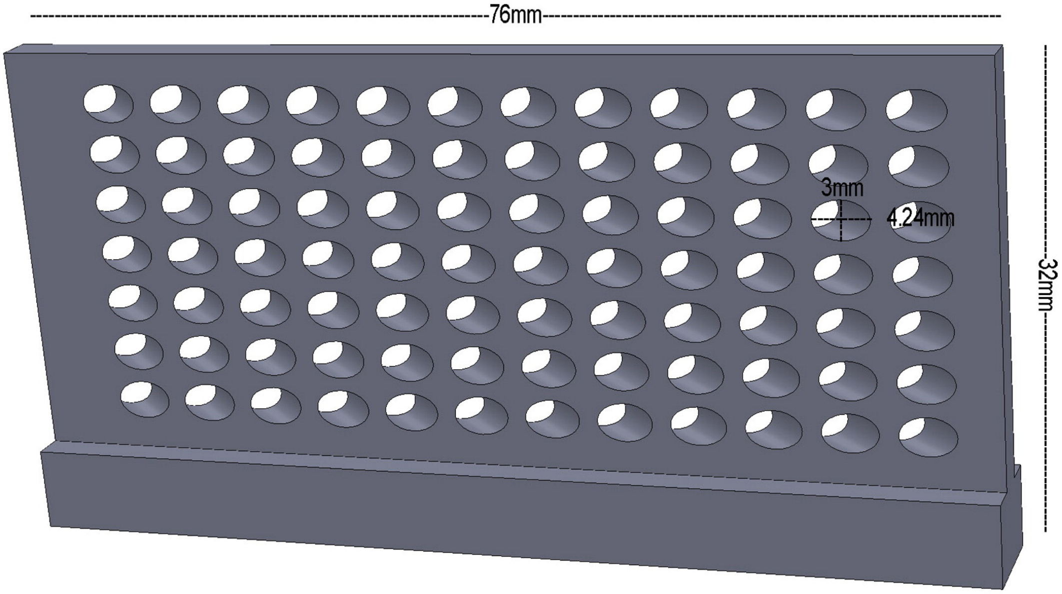

Fig. 1. Aluminum alloy sample holder with 84 holes, which appear elliptical from 3 mm holes drilled at 45° to the surface, for sample loading. The sample holder was specifically designed for mineral screening in grain meals using VESPERS.