Journal of Food Composition and Analysis 31 (2013) 82–86

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Short communication

The influence of phenolic and phytic acid food matrix factors on iron bioavailability potential in 10 commercial lentil genotypes (*Lens culinaris* L.)

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ARTICLE INFO

Article history:
Received 25 December 2012
Received in revised form 20 April 2013
Accepted 30 April 2013

Keywords:
Ascorbic acid
Biofortification
Chlorogenic acid
Fe deficiency
Gallic acid
Lentil
Phytic acid
Food analysis
Food composition

ABSTRACT

This study aimed to identify lentil (*Lens culinaris* L.) genotypes with superior Fe concentrations and phenolic and phytic acid profiles for enhanced Fe bioavailability. Ten commercial lentil cultivars were analyzed for concentrations of Fe, phytic acid (PA; Fe absorption inhibitor), ascorbic acid (AA; Fe absorption promoter), gallic acid (GA; Fe absorption inhibitor), and chlorogenic acid (CLA; Fe absorption inhibitor). Fe concentrations were measured using inductively coupled plasma-emission spectroscopy. Phytic acid and phenolic compounds were determined using high performance liquid chromatography. Fe concentration across genotypes was 56–70 mg kg^{-1}. Phytic acid was observed at concentrations between 6.3 and 8.7 mg g^{-1}. Mean concentrations of other food matrix factors were as follows: AA = 72.4 mg kg^{-1}; GA = 25.0 mg kg^{-1}; and CLA = 17.4 mg kg^{-1}. Phytic acid to Fe molar ratios in lentil ranged from 8.6 to 13.0, indicating moderate Fe bioavailability. Overall, lentil appears to have considerable potential for Fe biofortification with proper genetic and environmental sourcing. In addition, measurements of food matrix factors (promoters and inhibitors) in conjunction with in vitro digestion/Caco-2 cell cultures are suggested to determine lentil genotypes with superior Fe bioavailability.

1. Introduction

Although highly abundant in the Earth's crust, the insoluble nature of iron (Fe) has led to widespread deficiency and anemia, especially in developing countries (Mayer et al., 2008). Fortifying Fe concentrations in staple foods, as well as increasing Fe absorption in the human gut, could be effective strategies to prevent Fe deficiency. Iron fortification of food is a widely used practice; however, addition of Fe to food is impractical because of cost and technological, cultural and socio-economical limitations in many developing regions where Fe deficiency is prevalent. Compounding this problem is that the utility, or "bioavailability", of Fe is not only a question of the Fe concentration in a given food, but also whether the Fe is plant- or animal-derived and whether other biochemical factors are present within the food matrix (Hunt, 2003; Plaami, 1997; Bravo, 1998). For example, an increase of 67% Fe bioavailability in hybrid corn (*Zea mays* L.) over the control (44.2 ± 4.2 μg ferritin mg^{-1} total protein vs. 26.5 ± 1.6 μg ferritin mg^{-1} total protein) was associated with a mere 12% increase in total Fe concentration, thus demonstrating the impact of other food matrix factors (Hoekenga et al., 2011). Promoters and inhibitors of Fe absorption within the food matrix must be considered with respect to the bioavailability of non-heme Fe in a food crop (Cook et al., 1972). Phytic acid (PA), myo-inositol 1,2,3,4,5,6,-hexakisphosphate, nearly ubiquitous in plants and used as the primary phosphorous (P) storage, inhibits absorption of Fe in the gut (Turnbull et al., 1962). Inhibition is achieved by chelation of $Fe^{2+/3+}$ by PA, but this action can be prevented by ascorbic acid (AA), depending on dosage (Siegenberg et al., 1991). Other notable inhibitors include fiber, heavy metals, and certain polyphenols and tannins (Gillooly et al., 1983; Glahn et al., 2002; Welch and Graham, 2004).

Lentil (*Lens culinaris* L.) is a traditional pulse crop mainly grown in low-rainfall, dry-land cropping systems in rotation with cereals such as wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), and it is used as a staple protein food source in many developing countries and vegetarian populations in the developing world. Annual world lentil production is approximately 4 M tons, of which more than 85% is found in India, Nepal, and Bangladesh (32%); western Canada (29%); Turkey and northern Syria (18%); Australia (4%); and the Midwestern region of the USA, including North Dakota, South Dakota, and eastern Montana (3%) (FAOSTAT, 2011). Little is known about the capacity of many lentil polyphenols to influence Fe bioavailability. Several studies have been conducted on the phenolic profiles of common beans

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http://dx.doi.org/10.1016/j.jfca.2013.04.003

(*Phaseolus vulgaris* L.) (Ying et al., 2006; Ariza-Nieto et al., 2007; Laparra et al., 2008); however, relatively little is known about the phenolic profiles of lentil. Both gallic acid (GA) and chlorogenic acid (CLA) have an inhibitory action on Fe bioavailability in humans. Other phenolic Fe promoters and inhibitors in lentils include derivatives of quercetin and kaempferol (Ying et al., 2006; Ariza-Nieto et al., 2007; Laparra et al., 2008).

Taking these factors into account, biofortification is a novel approach by which staple food crops grown in developing regions can be bred through conventional breeding techniques to deliver more bioavailable Fe to the consumer (Welch and Graham, 2004). We characterized Fe concentration and a choice of food matrix factors present in commercial lentil genotypes grown in North Dakota, USA, in order to predict and select those genotypes with potentially higher bioavailable Fe for future genetic and breeding efforts on Fe biofortification. The objectives of this study were to (1) characterize genetic and environmental variation of Fe and Fe-bioavailability promoters and inhibitors in 10 commercial lentil genotypes grown in ND, USA and (2) identify lentil genotypes with food matrix factors that may predict high Fe bioavailability for human health.

2. Materials and methods

2.1. Materials

Chemical standards, reagents and high purity solvents used for Fe, PA, and phenolics extractions were purchased from Alfa Aesar, A Johnson Matthey Company, VWR International and Sigma-Aldrich Co. (St Louis, MO, USA) and used without further purification. Water (distilled and deionized; ddH_2O) was purified by a Milli-Q Water System (Millipore, Milford, MA, USA) to a resistance of 18.2 MΩ cm or greater.

2.2. Samples

Lentil seed samples of 10 commercial genotypes were obtained from regional variety trials conducted by the North Dakota State University Pulse Breeding Program. These lentil genotypes fell into six market classes: small red, extra-small red, French green, small green, medium green and large green. CDC Redberry, CDC Red Rider, and CDC Rouleau were all representatives of small red lentils; CDC Greenland, Pennell, and Riveland were representatives of the large green market class. The remaining market classes each had only one cultivar: extra-small red, CDC Rosetown; French green, CDC Lemay; small green, CDC Viceroy; and medium green, CDC Richlea. Seed subsamples for analysis were randomly selected from entire harvested plots from randomized field studies at two locations (Ward and McLean Counties, ND) for each of 2 years (2010 and 2011) with three replications. Total of 120 whole lentil seed samples (with seed coat) were collected and analyzed for Fe, PA, and phenolic concentrations. Approximately 10–20 g of each sample were cleaned and ground into a fine powder using a top-loading UD grinder (Unholtz Dieckie Corporation, USA) and stored at −40 °C until analysis.

2.3. Fe extraction and quantification

Total Fe concentration in lentil seeds was determined using a previously-described, modified HNO_3-H_2O_2 method (Thavarajah et al., 2009a). Ground seed samples (500 mg) were digested in nitric acid (70% HNO_3) at 90 °C for 1 h. Samples were further digested with hydrogen peroxide (30%) before being diluted to 10 mL with nano-pure water. Fe concentrations were measured using inductively coupled plasma emission spectroscopy (ICP-ES; ICP-6500 Duo, Thermo Fisher Scientific, Pittsburg, PA, USA). Measurements of total Fe using this modified method were validated using National Institute of Standards and Technology (NIST) standard reference material 1576a (wheat flour; [Fe] = 14.1 ± 0.1 mg kg^{-1}). A homogenized laboratory reference material (CDC Redberry: Fe = 64 mg kg^{-1}) was also used periodically for quality control. A calibration curve for Fe concentration was produced using serial dilutions from 0.5 to 5.0 mg L^{-1}. The detection limit was 5 μg L^{-1}.

2.4. Phytic acid (PA) extraction and quantification

Lentil seed samples were prepared using a modified PA extraction method described by Thavarajah et al. (2009b). Ten milliliters of 0.5 M hydrochloric acid (HCl) were added to powdered lentil samples (100 g), then the flasks were placed in a boiling water bath with stirring for 5 min. After centrifugation (4000 × *g*, 3 min), PA-cation complexes were disrupted with the addition of 1.5 mL of 12 M HCl. Analysis of PA was achieved using high performance anion exchange (HPAE) with a conductivity detector (ICS-5000 Dionex, Sunnyvale, CA, USA). Briefly, an Omnipac Pax-100 anion exchange column (250 mm × 4 mm I.D.), in series with an Omnipac Pax-100 (8 μm) guard column, was employed for PA separation (Dionex, Sunnyvale, CA, USA). A gradient mobile phase with a flow rate of 1.0 mL min^{-1} was applied using the following eluents: (A) 130 mM sodium hydroxide; (B) deionized water–isopropanol (50:50, v/v); and (C) water. Prior to PA detection, eluents were suppressed by an anion suppressor (ASRS® 300, 4 mm: Dionex, Sunnyvale, CA, USA). Pure PA standards were used to produce a calibration curve and for peak identification and quantification of PA. The detection limit of PA was 5 mg L^{-1}. Low PA laboratory reference samples were used periodically to ensure measurement consistency (CDC Redberry = 4.5 ± 0.1 mg g^{-1} and CDC Robin = 4.6 ± 0.1 mg g^{-1}). Tolerance of error for all lab references samples was <0.1%.

2.5. Phenolic extraction and quantification

Extraction and quantification of phenolic compounds was carried out using a previously described method (Duenas et al., 2002). Phenolic compounds were extracted from finely ground lentil seed samples (1.0 g) using 10 mL of methanol:water:acetic acid (899:100:1). Individual vials were vortex mixed and shaken in an incubator for 1 h. Samples were centrifuged at 2000 × *g* for 5 min. The supernatant was passed through a filter (0.45 μm) before the extracts were injected into the high performance liquid chromatography (HPLC) system with conductivity detection. Chemicals were separated using a C18 column (250 mm × 4.60 mm) in series with a guard column (30 mm × 4.60 mm). A gradient mobile phase with a flow rate of 1.0 mL min^{-1} was applied using the following eluents: (A) water:acetic acid (98:2) and (B) water:acetonitrile:acetic acid (78:20:2). Pure phenolic standards were used to create calibration curves for each analyte and for peak identification and quantification. The detection limits for AA, GA, and CLA were 0.1, 0.1, and 0.2 mg kg^{-1}, respectively. Measurement of AA was conducted at a wavelength of 254 nm, GA at 271 nm, and CLA at 321 nm. Standards for quercetin dihydrate and kaempferol were also analyzed. A laboratory reference (CDC Redberry) and pure standards were periodically used to ensure consistency and <5% error.

2.6. Statistical analysis

The experimental design was a randomized complete block design with three replicates of 10 commercial lentil genotypes at two locations over 2 years (n = 120). For combined analysis, the General Linear Model procedure (PROC GLM) of SAS version 9.3