Food Chemistry 199 (2016) 782–790

Contents lists available at ScienceDirect

Food Chemistry

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

The effects of hydrothermal processing and germination on Fe speciation and Fe bioaccessibility to human intestinal Caco-2 cells in Tartary buckwheat

Paula Pongrac [a,*], Nathalie Scheers [b], Ann-Sofie Sandberg [b], Mateja Potisek [a], Iztok Arčon [c,d], Ivan Kreft [e], Peter Kump [c], Katarina Vogel-Mikuš [a,c]

[a] Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
[b] Department of Biology and Biological Engineering, Food and Nutrition Science, Chalmers University of Technology, SE-412 96 Göteborg, Sweden
[c] Jožef Stefan Institute, Jamova 37, SI-1000 Ljubljana, Slovenia
[d] University of Nova Gorica, Vipavska 13, POB 301, SI-5001 Nova Gorica, Slovenia
[e] Nutrition Institute, Tržaška 40, SI-1000 Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form 13 November 2015
Accepted 16 December 2015
Available online 18 December 2015

Keywords:
Tartary buckwheat
Iron
Hydrothermal processing
Germination
Bioavailability
X-ray absorption near edge structure

ABSTRACT

Tartary buckwheat is a gluten-free crop with great potential as a wheat substitute. Iron (Fe) is an important mineral element in staple foods which is required in sufficient bioaccessible quantities. The aim of the study was to investigate how processing of grains into groats (hydrothermal processing to remove the husk) and sprouts (7-day-old seedlings) affected Fe speciation (Fe^{2+} or Fe^{3+}), Fe ligand composition and Fe bioaccessibility to human Caco-2 cells. Groats contained the least Fe (23.8 ± 1.65 mg kg^{-1}) and the lowest amounts of Fe^{2+} (8%). Grains and sprouts had comparable Fe concentrations (78.2 ± 2.65 and 68.9 ± 2.73 mg kg^{-1}) and similar proportions of Fe^{2+} (15% and 18%). The main ligands for Fe in Tartary buckwheat material were phytate and citrate. Phytate was less abundant in sprouts, which did not correlate with greater Fe bioaccessibility. Iron bioaccessibility was 4.5-fold greater for grains than groats, suggesting that Fe is more bioaccessible in the husk than in the rest of the grain.

1. Introduction

Grains are a main component in the human diet and a major source of essential mineral elements. The majority of mineral elements (Mg, Mn, Fe, Cu and Zn) in grain are tightly bound in phytate (*myo*-inositol hexakisphosphate) salts and are not readily bioavailable. During the processing of grains, phytates can be broken down, which releases mineral elements and increases their bioavailability. During germination, mineral elements are remobilised in order to be available for active processes of the growing embryo until the first photosynthetically active leaves develop. Soaked and/or germinated grains, sprouts or microgreens presumably have higher total concentrations and enhanced bioavailability of mineral elements, though this is greatly dependent on the particular mineral element and plant species studied (Nelson, Stojanovska, Vasiljevic, & Mathai, 2013).

In humans, Fe is essential for the synthesis of haemoglobin and is involved in oxygen transport through the body. Among mineral elements, Fe is particularly poorly bioavailable. This is especially problematic in cereal-based and vegetarian diets (Collings et al., 2013). The term bioavailability describes the proportion of a nutrient in food that is exploited for normal body functions and can be considered as a measure of nutritional (mineral) quality of the foods. Bioavailability may be estimated by means of *in vitro* or *in vivo* methods. Using *in vivo* studies in humans, the absorption of a mineral element in relation to dietary and physiological factors affecting the bioavailability can be assessed, while *in vitro* intestinal cell studies may predict the trend of absorption and also determine the mechanisms of action (Fairweather-Tait et al., 2005). Human trials are expensive and time-consuming, therefore, *in vitro* cell studies are often the pragmatic choice for estimating bioavailability. Thus, combined *in vitro* digestion/human intestinal (Caco-2) cell systems have been developed to determine Fe bioaccessibility or bioavailability from foods, determined as the amount of the Fe transported into the intestinal cell (bioaccessibility) or

* Corresponding author at: The James Hutton Institute, Errol Road, Invergowrie, Dundee DD2 5DA, UK.
E-mail addresses: paula.pongrac@gmail.com (P. Pongrac), nathalie.scheers@chalmers.se (N. Scheers), ann-sofie.sandberg@chalmers.se (A.-S. Sandberg), mateja.potisek@bf.uni-lj.si (M. Potisek), iztok.arcon@ung.si (I. Arčon), ivan.kreft@guest.arnes.si (I. Kreft), peter.kump@ijs.si (P. Kump), katarina.vogelmikus@bf.uni-lj.si (K. Vogel-Mikuš).

http://dx.doi.org/10.1016/j.foodchem.2015.12.071
0308-8146/

transported across the basolateral membrane (bioavailability) (Glahn, Lee, Yeung, Goldman, & Miller, 1998). This system has been successfully used to predict Fe bioavailability to humans (Au & Reddy, 2000; Yun, Habicht, Miller, & Glahn, 2004) and has recently been improved by including human liver cells (HepG2) to human intestinal Caco-2 cells (Scheers, Almgren, & Sandberg, 2014).

Among the dietary factors that influence the bioavailability of Fe, its chemical form (oxidation state; ferrous, Fe^{2+} or ferric, Fe^{3+} species) and the type of complexing agents (local chemical environment and ligand association) are most important (Theil & Briat, 2004). Both of these characteristics can be studied simultaneously using X-ray absorption spectroscopy (XAS) by which the local structures around the atoms of a selected type in the sample are identified through detection of the photoelectron excited from an inner shell of the absorbing atom when an X-ray photon is absorbed in the process of photoeffect (Vogel-Mikuš et al., 2012). In X-ray absorption near edge structure (XANES) the oxidation state of the selected type of atom in the sample and the local symmetry of its unoccupied orbitals can be deduced from the shape and energy shift of the X-ray absorption edge. XANES analyses of inherent plant material have been particularly valued for their information on the valence state of a mineral element as well as for assessing possible binding scenarios (Zhao, Moore, Lombi, & Zhu, 2014). Such studies, especially on Fe in edible plant parts, are still scarce due to low Fe concentrations in these tissues, which makes analysis of XANES spectra difficult. One of the first evaluations of Fe species using Fe K-edge XANES was performed in wheat (*Triticum aestivum* L.) grain and it demonstrated that the majority (>70%) of Fe is Fe^{3+}, while the rest is Fe^{2+}. The best linear combination fit of the XANES spectra of the samples was obtained with the following XANES spectra of reference Fe compounds: Fe^{2+} phytate, Fe^{3+} phytate and Fe^{3+} sulphate (Singh et al., 2013). However, due to relatively small differences between Fe^{3+} phytate and Fe^{2+} sulphate XANES spectra and the relatively noisy spectra from the grain, estimates of Fe complexes could only be drawn with rather low precision ±10% (Singh et al., 2013). It remains to be evaluated if the relative amounts of Fe species in the grains of the four wheat genotypes studied influence the bioaccessibility and/or bioavailability of grain Fe.

Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) grains are gluten-free and have well-balanced amino-acid and lipid compositions with high dietary fibre contents (Bonafaccia, Marocchini, & Kreft, 2003). As such, Tartary buckwheat has great potential as a wheat substitute, especially in individuals with coeliac disease. In Tartary buckwheat grains, the majority of Fe is located in cotyledons and the correlation with P in cotyledons (Pearson correlation coefficient of 0.736) indicates that not all of the Fe is bound to –O–P ligands, such as phytate (Pongrac et al., 2013). Groats are hydrothermally processed grains with the outer husk removed and thus convenient for cooking. Their consumption is very common in eastern European countries and in Russia. In the last decade, Tartary buckwheat grains have also been used for the production of sprouts (Kim, Kim, & Park, 2004), which have higher concentrations of Na, Mg, P, S, K, Ca, Fe, Cu, Zn and Mo than grains (Hsu, Chiang, Chen, Yang, & Liu, 2008; P. Pongrac et al., unpublished observation). To our knowledge neither the localisation nor speciation of Fe, nor the Fe bioaccessibility in relation to hydrothermal processing or in response to germination and sprouting have yet been studied in Tartary buckwheat. Therefore, the aims of the study were to determine Fe speciation, ligand environment of Fe and Fe bioaccessibility in Tartary buckwheat grains, and hydrothermally processed and germinated grains; groats and sprouts, respectively. In addition, Fe speciation and Fe ligand environment were studied in cotyledons of developing Tartary buckwheat sprouts. For this purpose the following characteristics were determined: (i) Fe speciation and ligand environment of Fe using synchrotron radiation Fe K-edge XANES analysis at whole and at cotyledon level in Tartary buckwheat grains, groats and developing sprouts, (ii) concentrations of phytate in Tartary buckwheat grains, groats and sprouts, and (iii) ferritin formation in Caco-2 cells as an estimate of Fe bioaccessibility.

2. Material and methods

2.1. Plant material

Grains and groats of Tartary buckwheat (*F. tataricum* Gaertn.) were provided by a local grower (Mlin Rangus, Dolenje Vrhpolje at Šentjernej, Slovenia) in 2012. For groat production grains were hydrothermally processed (soaked in water at 95 °C for 20 min, and dried to 20% moisture content using forced ventilation of air at 40 °C by electric fan through a 20 cm thick layer of buckwheat grain for one hour, by grain dryer, A. Rangus, Šentjernej, Slovenia) which enables easier removal of firm husks. Mature, air-dried grain and groats were kept in paper bags, at room temperature and protected from light. Sprouts were grown in an automatic sprouter (EasyGreen® MicroFarm System, EasyGreen Factory Inc., Nevada, USA). The growth containers were kept on a laboratory bench under shaded windows at room temperature (20 °C and watered by misting every 3 h during the day (five times) and twice during the night (with 4–5 h gaps) with tap water. Seven days after germination, sprouts were removed from the sprouter, rinsed with distilled water and blotted. Roots were removed and, for bulk Fe speciation and the local chemical environment of Fe (Fe K-edge XANES) analyses, only cotyledons (without hypocotyls) were frozen in liquid nitrogen and analysed in frozen-hydrated state. For bioaccessibility experiments, only roots were removed since larger amounts of sprout dry weight were needed for analysis. For this purpose, sprouts were frozen in liquid nitrogen, freeze-dried (Alpha Christ 2–4) for 5 days at 0.240 mbar and −25 °C, and homogenised with liquid nitrogen using a pestle and a mortar.

For cotyledon-specific Fe speciation and the local chemical environment of Fe (Fe K-edge μ-XANES) analyses, additional developmental stages were included, namely 24 h soaked grains (imbibition stage), 48 h soaked grain (germination stage, radicle protruded the husk in these samples), 5-day-old sprouts (shedding stage) and fully developed sprouts (7-day-old sprouts; sprouting stage). At the shedding stage cotyledons are not yet fully developed and are still infolded in the husk, but before analyses the husk was manually removed.

2.2. Analysis of Fe bioaccessibility using human intestinal Caco-2 cells

Prior to analyses, initial Fe concentrations and initial phytate concentrations were measured in homogenised grain, groats and sprouts (cotyledons and hypocotyls) of Tartary buckwheat by high-performance ion chromatography. Initial Fe concentrations were measured according to Fredriksson, Carlsson, Almgren, and Sandberg (2002) and initial phytate concentrations according to Carlsson, Bergman, Skoglund, Hasselblad, and Sandberg (2001).

2.2.1. In vitro digestion of grains, groats and sprouts

Homogenised samples of grains, groats and sprouts of Tartary buckwheat (1 g each) were digested *in vitro* by suspending them in double distilled water (10 ml) and digested with pepsin solution (0.3 ml $sample^{-1}$) containing 0.16 mg pepsin L^{-1} of HCl (0.1 M) at pH 2 for 1 h at 37 °C. The pH was gradually adjusted to 7 by the addition of $NaHCO_3$ (1 M). The digested samples were centrifuged at 5000*g* for 30 min. The supernatants were analysed for the soluble Fe Fredriksson et al. (2002) and phytate Carlsson et al. (2001) contents by high-performance ion chromatography.