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Date:

2018-11-01

Citation:

Zhang, Y. Y., Panozzo, J., Hall, M. S. & Ajlouni, S. (2018). Bioaccessibility of Some Essential Minerals in Three Selected Australian Pulse Varieties Using an In Vitro Gastrointestinal Digestion Model. *Journal of Food Science*, 83 (11), pp.2873-2881. <https://doi.org/10.1111/1750-3841.14377>.

Persistent Link:

<https://hdl.handle.net/11343/284682>

Bioaccessibility of Some Essential Minerals in Three Selected Australian Pulse Varieties Using an *in vitro* Gastrointestinal Digestion Model

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Word count of text 5104 words

Short version of title Bioavailability of minerals in pulses

Choice of journal/section

Journal of Food Science sections:

- Health, Nutrition, and Food

Previous address(es)

N/A

Author disclosures

The authors declare no conflict of interest.

[END PAGE 1]

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/1750-3841.14377](https://doi.org/10.1111/1750-3841.14377).

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ABSTRACT:

Australian produced pulse grains are exported worldwide, predominantly to developing countries where severe essential mineral deficiencies putatively subsist. An *in vitro* digestion model that simulates human gastric, intestinal and colonic digestion and fermentation, was used to examine the bioaccessibility of Fe, Mg, K, Ca, P, Zn, Mn and Cu in commercially available cultivars of Australian field pea, lentil and sweet lupin. The hull and dehulled seeds were prepared following a traditional cooking method, and quantities of bioaccessible minerals were assessed at each stage of *in vitro* digestion using ICP-OES elemental analyses. Results revealed that dehulled field pea (100 g dry weight) had the highest bioaccessible quantity of Fe (2.44 ± 0.73 mg), K (717.10 ± 56.66 mg), P (272.88 ± 9.30 mg), Zn ($1.72.028 \pm 0.28$ mg) and Cu (0.41 ± 0.02 mg). Dehulled lupin was the best source of Mg (138.62 ± 1.53 mg) and Mn (1.28 ± 0.06 mg), and lentil hull showed the greatest Ca bioaccessible quantity (116.33 ± 16.73 mg/100 g dry weight). Additionally, the fed state digestion (11.7 mg bile /ml sample) increased the bioaccessibility of all elements significantly ($P < 0.05$) compared to fasted (1.95 mg bile / ml sample), except for Zn and Mn in lupin and lentils. These results demonstrated that dehulled seeds possess higher mineral bioaccessibility on a percentage basis compared with hulls, and that the fed state of *in vitro* digestion generally improved the mineral solubility significantly ($p < 0.05$).

Keywords: Bioaccessibility, Essential minerals, Legumes, Pulses, *in Vitro* Gastrointestinal Digestion

Practical Application:

This research aimed to assess the prospective biological accessibility of various essential elements in three commercially available Australian pulses. Results of the study provided an insight into the contents of essential minerals in Australian pulses and illustrated the

impact of traditional cooking of dehulled pulses on these minerals bioaccessibility. These findings will provide the consumers with information about some nutritional aspects of major Australian pulses.

Introduction

Dietary essential minerals such as Fe, Mg, K, Ca, P, Zn, Mn and Cu are crucial components for various physiological processes that serve to uphold bodily functions (Gupta & Gupta, 2014). Inadequate intake of these elements can contribute to the development of mineral-dependent diseases associated with egregious monetary and intangible losses (WHO, 2010). However, mineral deficiency remains a prevalent dietary issue hindering the wellbeing of human populations worldwide, particularly in developing countries where dietary diversity is limited (Bailey, West, & Black, 2015; Beal, Massiot, Arsenault, Smith, & Hijmans, 2017). It has been widely documented that only a proportion of total minerals contents are released from the food matrix following digestion, and small amounts become physiologically accessible for absorption within the gastrointestinal tract (Etcheverry, Grusak, & Fleige, 2012). Compared with animal products, minerals from plant foods are known to be less bioavailable due to the presence of anti-nutritional compounds; engendering the widespread consumption of plant-based diets as a major prevailing factor impeding global mineral sufficiency (Platel & Srinivasan, 2016).

The seeds of pulses such as the field pea (*Pisum sativum*), sweet lupin (*Lupinus angustifolius*) and lentil (*Lens culinaris*) are deemed to be good sources of dietary essential

minerals (Curran, 2012; Vaz Patto et al., 2015), as summarised in Table 1. Their routine consumption in diverse cultures, amalgamated with an affordable and nontoxic nature constitutes a sustainable approach to alleviate mineral deficiencies through household consumption (Fiorella, Chen, Milner, & Fernald, 2016). Although mineral bioavailability in pulses are generally considered to be low due to the presence of anti-nutritional compounds (namely phytic acid and polyphenols) that form insoluble complexes during digestion (Alegría - Torán, Barberá - Sáez, & Cilla - Tatay, 2015), the chemical composition of pulses can differ greatly with genetic disposition (species and cultivars), growth environment, and soil conditions (Wang & Daun, 2006). Screening for pulse genotypes with superior mineral bioavailability is thus a promising strategy to exploit natural variation. Such approach has been successfully accomplished for Zn in wheat (*Triticum* spp.) (Welch, House, Ortiz-Monasterio, & Cheng, 2005) and the common bean (*Phaseolus vulgaris*) (Ramírez-Cárdenas, Leonel, Costa, & Reis, 2010).

Existing studies on pulse mineral bioavailability are challenging to compare due to variation in the methods used (Sahuquillo, Barberá, & Farré, 2003), and those focused particularly on Australian pulses are scarce (Karnpanit, Coorey, Clements, Benjapong, & Jayasena, 2017). As a major exporter to developing regions including the Indian sub-continent and Middle East (Pulse Australia, 2015), there is a potential in identifying pulse genotypes appropriate for these markets. This study aimed to compare the mineral bioaccessibility of three high-protein pulses (field pea, lentil and sweet lupin) using an *in vitro* gastrointestinal digestion model. These three pulse cultivars Lupin (cv. Mandelup), lentil (cv. PBA Jumbo) and field pea (cv. PBA Oura) are of high yield and are available through the Australian trade market.

To simulate the conditions in which the pulses are consumed, grains were milled and cooked using a traditional preparation method. Since the discharge of bile as a lipid-digestive agent is affected by the presence of food in the small intestine, this study also examines the effect of fasted (0 - 2.5 mg bile extract/ml digested sample) and fed states (10 and 40 mg bile extract/ml digested sample) on mineral bioaccessibility in individual pulse components (hulls and dehulled grain) (Fu et al., 2015). Minerals were extracted at each stage of gastrointestinal digestion and quantitated using ICP-OES.

Materials and Methods

3.1. Plant samples

Field pea (*Pisum sativum* L.) cv. Oura was sown in Rokewood, Victoria (37°55'0"S 144°08'0"E), on the 26th of April, while sweet lupin (*Lupinus angustifolius* L.) cv. Mandelup and lentil (*Lens culinaris* L.) cv. Jumbo were sown in Ouyen, Victoria (35°06'51"S 142°03'33"E) on the 5th and 6th of May, 2016 respectively. The growing site characteristics are shown in Table 2 (Bureau of Meteorology, 2016). Lentil and lupin were harvested during the first week of December, while field pea was harvested during mid-December. A subsample of each of the three field replicates within a pulse variety was collected immediately following harvest and stored in the dark at room temperature.

3.1.2. Post-harvest treatment

Each sample was dehulled and the cotyledon was split using a ICARDA splitter (International Centre for Agricultural Research in Dry Areas, Beirut, Lebanon). The dehuller/splitting equipment consists of two carborundum disks, with the bottom disk set at

500 rpm and the top stationary. The gap between the two rotating discs were adjusted based on seed size. Hulls and split cotyledons were separated using an aspirator (Kimseed, Wangara, Western Australia). Samples were then ground to a fine powder using the Perten 3100 Hammer Mill fitted with a 0.8 mm screen (Perten Instruments, Hägersten, Sweden), and stored in polyethylene bags until use.

3.1.3. Reagents

Bile extract (bovine), bile salts (for microbiology), guar gum, pectin, CaCl_2 , mucin (porcine stomach), casein (bovine), NaHCO_3 , L-Cysteine HCl, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, were purchased from Sigma-Aldrich (Castle Hill, Australia). Pepsin was acquired from Acros Organics (Morris Plains, United States). Pancreatin (porcine pancreas), nutrient broth (Oxoid®), phosphate buffered saline (Oxoid®), plate count agar (BD™ Difco™), bacteriological peptone (L34), peptone, tryptone and yeast extract were obtained from ThermoFisher Scientific (Scoresby, Australia). Tween 80, NaOH, NaCl, KCl, HNO_3 (70%) and H_2O_2 (30%) were purchased from Chem-supply (Gillman, Australia). KH_2PO_4 and KOH were obtained from Merck (Frenches Forest, Australia).

3.2. Pulse slurries preparation and *in vitro* gastrointestinal digestion

Ground cotyledon and hull ($2 \text{ g} \pm 0.001 \text{ g}$) from lentil, lupin, and field pea samples were weighed into 100 mL glass beakers. Each sample was mixed with tap water at ratios varying from 1:7 for field pea (Eyaru, Shrestha, & Arcot, 2009), to 1:10 for lupin and 1:3 for lentils (Embaby, 2010; Quinteros, Farré, & Lagarda, 2001). However, the final ratio used for

lentils was modified to 1:5.5 ml, as preliminary testing of the reported ratio (1:3) was insufficient to form a wet slurry. Samples were cooked on a hot plate (Haines Educational, Knoxfield, Australia) at the highest setting until the slurries thickened, yet without eye-visible colour change due to Maillard reaction products. From preliminary testing, the cooking times under these conditions were 7, 12 and 15 min for lentil, pea and lupin, respectively. Each pulse sample was cooked three times on the same hot plate, and slurries were then cooled to room temperature. Cooked pulse samples were digested following the *in vitro* gastrointestinal model developed by Fu et al. (2015), with some modification to account for differences in sample volume and scope of this study. The original model comprises of a two-step procedure, simulating digestion environments found in the stomach and small intestine, respectively. In our study, an additional *in vitro* colonic fermentation step was added to the end products from intestinal digestion. All experiments were performed in triplicate, with duplicate blank samples containing 20 mL tap water also digested *in vitro* as negative controls.

3.2.1. Preparation of simulated gastric and intestinal fluids

Simulated gastric and intestinal fluids were prepared according to the original model (United States Pharmacopeial Convention, 2009). For the gastric fluid, 2 g (± 0.01 g) of NaCl and 7 mL of HCl (37%, w/v) were dissolved into 800 mL of MilliQ water, with the pH adjusted to 1.2 using 1 M NaOH solution. Pepsin (6.4 mg/mL) was stirred into the solution for 15 minutes before use.

For the intestinal fluid, a solution containing 5 mM Phosphate Buffered Saline, 0.4 M NaCl and 15 mM CaCl₂ was prepared in Milli-Q water and adjusted to pH 6.8 (± 0.05) with 1 M

NaOH and 1 M HCl (Fu et al., 2015). Prior to use, pancreatin (10 mg/mL) and bile (0.5 or 30 mg/mL) were gently stirred into the solution. The two respective bile quantities are a proxy of the concentrations secreted by the gall bladder during fasting (absence of food) and fed conditions, where the range lies between 2 - 6.4 mM for fasted and 0.5 - 37 mM for fed (Fu et al., 2015; Holm, Müllertz, & Mu, 2013). The selected bile concentrations used in this study were thus equivalent to final volumes of 1.95 mg/mL and 11.7 mg/mL for the fasting and fed states, respectively.

3.2.2. *In vitro* gastric and intestinal digestion

The cooked pulse slurries were transferred into 50 mL polypropylene tubes containing 5 mL of simulated gastric fluid with pepsin (6.4 mg/mL). Samples were incubated for 2 h at 37 °C in a shaking incubator (ZWYR-240, Labwit Scientific, Shanghai, China) at 50 rpm.

Digestion was ceased by adjusting sample pH to 6.8 with 1 M NaOH, where pepsin was denatured (Fu et al., 2015). The digests were centrifuged at 4500 rpm (2490 g) for 30 minutes (Jouan C3i Q5, Waltham, United States) to separate the soluble and insoluble fractions. The pulse hulls, which contained low molecular weight particles that could not be separated at such speed, were filtered using 70 mm ashless quantitative filter paper (No. 40, Whatman, Sigma-Aldrich, Castle Hill, Australia) to obtain the two fractions. A higher speed was not used as centrifugal force above 3000 g may impose unwarranted damage to plant cell walls (Terry & Bonner, 1980), which can in turn influence mineral bioavailability (Glahn, Tako, Cichy, & Wiesinger, 2016). The isolated soluble gastric fraction was frozen at -20 °C for further ICP analysis.

To perform the intestinal digestion at fasted and fed states, the insoluble fraction from gastric digestion was quantitatively transferred and mixed with 6.4 mL of simulated intestinal fluid (pH 6.8) containing pancreatin (10 mg/mL) and bile (1.95 mg/mL and 11.7 mg/mL for the fasting and fed states, respectively) as described before. Samples were incubated for 3 h at 37 °C in a shaking incubator (ZWYR-240, Labwit Scientific, Shanghai, China) at 50 rpm, and digestion terminated by placing samples in ice prior to centrifugation or filtration under the same conditions as gastric digestion. The isolated soluble intestinal fraction was also frozen at -20 °C for further ICP analysis. To ensure consistency through the colonic fermentation process, all insoluble fractions obtained from this step were immediately frozen at -20 °C, until defrosted and pre-warmed to 37 °C for colonic fermentation as a single batch.

3.3. In vitro Colonic fermentation

3.3.1. Faecal culture preparation

Human microflora that has been extracted in the same laboratory in a previous investigation (Sirisena, Ajlouni, & Ng, 2017) and maintained at -80 °C was used in this study. The frozen human microflora was thawed aseptically in a bacteriological cabinet, and activated using nutrient broth to prepare the stock cultures.

3.3.2. Bacterial enumeration

Bacterial enumeration took place prior to, and after 24 h of simulated colonic fermentation. For the initial enumeration, previously prepared faecal stock culture (1 mL) was aseptically introduced into 9 mL of sterile bacto-peptone (0.1%) as the first tube of the dilution series,

thoroughly vortexed and serially diluted again up to 10^{-8} in the same diluent. The same procedures were followed for samples after 24 h of fermentation.

A spread plate technique was used in the bacterial enumeration, where 0.1 mL of selected serial dilutions was spread onto plate count agar in quadruplicate (Sanders, 2012). Half of these inoculated plates (two) were incubated aerobically, and the other half anaerobically at 37 °C for 48 h. The final colony counts were reported as log colony forming units/mL (Log CFU/mL).

3.3.3. Basal medium preparation

The basal medium used for fermentation was based on the method of Dall'Erta et al. (2013) with minor adjustments. Briefly, 2.5 g peptone, 2.5 g tryptone, 2.25 g yeast extract, 2.25 g KCl, 4.5 g NaCl, 2 g mucin, 1 g pectin, 1.5 g casein, 0.75 g NaHCO₃, 0.4 g L-Cysteine HCl, 0.62 g MgSO₄·7H₂O, 0.5 g guar gum, 0.25 g KH₂PO₄, 0.2 g bile salts, 0.55 g CaCl₂ and 0.5 mL Tween 80 were suspended in 500 mL of Milli-Q water and autoclaved at 121 °C for 20 min (3041 VD, Thermoline Scientific, Wetherill Park, Australia). Polysaccharides (soluble starch and arabinogalactan) present in the authors' original medium were eliminated as similar carbohydrates are deemed present from the pulse intestinal digesta.

3.3.4. Batch colonic fermentation

The insoluble fraction remaining from each intestinal digestion replicate was weighed (5 g) into a 50 ml polypropylene tube (Corning CentriStar™, ThermoFisher Scientific, Scoresby, Australia). This was inoculated with 2 mL of previously prepared faecal stock culture and 3

mL of basal medium, both were pre-warmed to 37 °C. Tubes were flushed with N₂ gas (purity 4.0, Coregas, Thomastown, Australia) before the lids were tightened and placed in anaerobic chambers with a gas-pak (AnaeroGen™, ThermoFisher Scientific, Scoresby, Australia) and an anaerobic indicator (BR0055B, ThermoFisher Scientific, Scoresby, Australia). Chambers were placed into a shaking incubator (ZWYR-240, Labwit Scientific, Shanghai, China) at 37 °C and 50 rpm for 24 h.

The pH of samples was measured (HI9124, Hanna Instruments, Keysborough, Australia) before and immediately following fermentation. Samples were then centrifuged at 8000 rpm for 20 min (Jouan C3i Q5, Waltham, United States), with the soluble fraction separated and stored at -20 °C until processed for acid digestion and ICP analysis.

3.4. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Elemental Analysis

3.4.1. Acid digestion

All soluble (bioaccessible) fractions from *in vitro* digested/fermented pulse samples (gastric, intestinal and colonic) were subjected to acid digestion accordance with the closed polypropylene tube method reported by Wheal, Fowles, and Palmer (2011) with some modifications. Aliquots (5 mL) from each soluble gastric, intestinal and colonic digest were transferred into a 50 mL polypropylene tubes (227261, Greiner Bio-One GmbH, Frickenhausen, Germany) containing 2 mL of 70% HNO₃ and 0.5 mL of 30% H₂O₂, thoroughly vortexed, and pre-digested overnight at room temperature. Samples were again

vortexed prior to being placed into a modified aluminium digestion block with proportional–integral–derivative (PID) control (The University of Melbourne, Australia) for 30 min at 80 °C (warming period), then gradually increased to 125 °C for 120 min at 5 °C intervals. Tube caps were loosened to equalise pressure during the initial 30 min at 80 °C, and tightened firmly by the end of the warming period before continuing digestion, and cooling to room temperature when completed.

Reference pulse samples (undigested) were prepared (acid digested) to measure the total minerals present in different pulses and their hulls. This was prepared following the same procedures, except for the first step where 0.3 g (\pm 0.001) of the original dry ground pulse flour was used.

To match the 5% HNO₃ sample matrix of the calibration standards used, all samples were diluted with Milli-Q water to an acid content of \leq 5%. The volumes needed were 20.5 mL for samples involved in the bioaccessibility studies and 22 mL for reference samples (not subjected to gastrointestinal digestion). An accurate volume (9 mL) of each acid digested sample extract was filtered using 0.45 μ m syringe filters (Target2™ Nylon, ThermoFisher Scientific, Scoresby, Australia) and transferred into 10 mL screw-cap polypropylene tubes (Sarstedt, Mawson Lakes, South Australia) for ICP analysis. Nitrile gloves worn during digestion were rinsed prior to use to reduce potential contamination.

3.4.2. ICP-OES specifications

The elemental analyses were performed using an Optima 8300 DV ICP-OES (Perkin Elmer, Glen Waverley, Australia) equipped with the syngistix for ICP 1.0 software, meinhard type K concentric glass nebulizer, glass cyclonic spray chamber, and a radial view optical emission. The operation conditions involved power (1450 W), Ar. flow rate (15 L / min), Ar Auxiliary flow (0.2 L / min) and Ar Nebulizer flow (0.6 L /min). Samples were injected using an automated sampler (S10, Perkin Elmer, Glen Waverley, Australia). The measured elements were observed at different wavelength (Iron, 259.94; Magnesium, 285.21; Potassium, 766.49; Calcium, 317.93; Phosphorous, 213.62; Zinc, 206.2; Manganese, 257.61 and Copper 324.75 nm). Calibration curves for elements were generated using multi-element standards (ICP-AM-17 and ICP-AM-12 Solution A, High-purity standards, Charleston, United States). Background correction was applied to all wavelengths (Table 4), and multiple emission lines were used to check for spectral interference. Random and targeted repeat analysis was performed to improve confidence.

3.5. Bioaccessibility calculation

This current study used the term bioaccessibility to refers to the fraction of mineral that was released from the examined pulses during *in vitro* digestion and becomes accessible (available) for absorption. Bioaccessibility should be distinguished from the term bioavailability, which is defined as the fraction of nutrients or food components that have been efficiently *in vivo* digested, assimilated and then absorbed in the body (Fernandez-Garcia et al., 2009). Consequently, it could be concluded that bioaccessibility of the studied minerals is a prerequisite for their bioavailability.

Total concentrations of Fe, Ca, Mg, Zn, Se, K, P, Cu and Mn were quantified in the soluble (bioavailable) fraction obtained from *in vitro* digestion and fermentation. Bioaccessibility

was determined using the equation proposed by Khouzam, Pohl, and Lobinski (2011) and Sahuquillo et al. (2003);

$$\text{Bioaccessibility} = \left(\frac{A}{B}\right) \times 100$$

where A is the concentration of the element in the bioaccessible fraction following the three-step simulated digestion, and B is the concentration of the element in the raw, uncooked sample of equivalent weight. The minerals present in tap water and reagents were also analysed and corrected for in the final bioaccessible fraction.

3.6. Statistical analysis

Total percentage bioaccessibility of the three pulses were performed using balanced (three-way) ANOVA at 95% confidence. The three factors included pulse type, component (hull or dehulled seed) and digestion state (fasted or fed). Comparisons between the means within each pulse group were assessed using Fisher's least significant difference (LSD) test. All statistical analyses were carried out using Minitab 18 (Minitab Inc., Sydney, Australia).

Results and Discussion

It is well known that only a small fraction of minerals in a food, particularly those plant-based are readily available for absorption by the human body (Platel & Srinivasan, 2016). To our knowledge, this is the first systematic study that compares the bioaccessibility of multiple essential minerals in the major components of different Australian-grown pulses, using *in*

vitro gastrointestinal digestion. In addition, the study quantified and compared essential minerals in two primary components of the seed: the dehulled cotyledon/embryo and seed coat. It should be noted also that tap water was used during cooking of these pulses in order to mimic home practices. However, mineral contents in tap water should not affect the results, or the aims of this investigation, since all experiments included duplicate blank samples containing 20 mL tap water as negative controls (slurries preparation, section 3.2).

4.1. Mineral contents in raw pulses

The absolute contents (mg/100 g dry weight = dwt.) of eight essential minerals (Fe, Mg, K, Ca, P, Zn, Mn, and Cu) in both pulse cotyledon and hulls are shown in Table 3. These data revealed that pulse hulls generally contained greater amounts of Ca relative to its dehulled counterparts, with 5.74-fold more in lupin, 12.93 in lentil and 13.18 in field pea. On the contrary, hulls contained less amounts of P, Mn and Zn in comparison with the cotyledon (dehulled pulses). For example, the average K contents in lupin cotyledon and hull were 1007.75 ± 46.61 and 393.48 ± 31.79 mg / 100 g dwt., respectively. All other tested minerals (Fe, Zn, Mn and Cu) were present in very small quantities in both hull and cotyledon of all pulses. Cu contents in all tested pulses were the smallest in comparison with all other minerals and was below the detection limit (< 0.003 mg / 100 g dwt.) in lupin hull.

Hulls are considered mostly as a fibrous fraction of the seeds (Tosh & Yada, 2010). Data in the literature regarding the mineral content in hulls are very limited as pulses are typically analysed as the whole or dehulled seed (Sparvoli, Bollini, & Cominelli, 2015; Wang, Hatcher, Tyler, Toews, & Gawalko, 2010). Among the studies of hulls and de-hulled seeds was the investigation by Hove (1974) in New Zealand. The author reported that the amounts of Mn,

Zn, Cu and Fe in hulls of a sweet lupin cultivar were 1.7, 3.7, 0.89 and 6.5 mg/100 g dwt, respectively. However, results from this current investigation revealed that sweet lupin hull contained significantly greater amount of Mn (4.49 ± 0.01 mg/100 g dwt), similar amount of Zn (3.05 ± 0.80 mg/100 g dwt), and significantly smaller quantities of Cu (below detection limit) and Fe (3.28 ± 0.82 mg/100 g dwt) (Table 3). These variations in the detected amounts of minerals may be attributed to the usage of different instruments in the analysis (atomic absorption spectroscopy versus ICP-OES) along with other variables including lupin cultivar and growth conditions.

Another study in the United States by Ariza-Nieto, Blair, Welch, and Glahn (2007), on mineral content in the hull of common bean (*Phaseolus vulgaris*) showed a wide range of Fe content (3 – 15.6 mg per 100 g dwt), which is close to the range obtained in this current investigation of the hull from three different pulses [(3.28 (lupin) – 8.99 mg/100g dwt field pea)].

The concentrations of tested minerals (Table 3) in the dehulled seeds (cotyledon) were found to be within the range of those reported in the literature for field pea, sweet lupin and green lentil. The minerals content in dehulled lupin, lentils and field pea were 4.38 ± 0.55 , 5.50 ± 0.52 and 5.65 ± 0.99 mg/100 g dwt for Fe; 1007.75 ± 46.61 , 969.65 ± 45.34 and 1111.18 ± 28.57 mg/100 g dwt for K; and 0.55 ± 0.01 , 0.4 ± 0.1 and 0.55 ± 0.04 mg/100 g dwt, for Cu, respectively. Compared with dehulled lentil and field pea, dehulled lupin was found to be a particularly good source of Mg (217.46 ± 13.78), Ca (113.56 ± 20.5) and Mn (5.03 ± 0.43 mg/100 g dwt). The K content of dehulled field pea (1111.18 ± 28.57 mg/100 g dwt) (Table 3) was greater than the range reported in the literature (920.5 – 966.5 mg/100 g dwt) for Canadian grown dehulled pea (Wang, Hatcher, & Gawalko, 2008). Similarly, minerals contents in dehulled lupin, Ca (113.56 ± 20.5), Zn (4.86 ± 0.95) and Fe ($4.38 \pm$

0.55) were greater than those reported by Karnpanit et al. (2017) (90.66, 2.05 and 3.50 mg/100g dwt, respectively).

4.2. Mineral bioaccessibility

Since mineral solubility is a prerequisite for absorption (Etcheverry et al., 2012), results from this study present the maximum percentage of each element that is released from the food matrix following cooking and *in vitro* gastrointestinal digestion. The reported literature on the minerals bioavailability from pulses, have focused mainly on Fe and Zn and their deficiencies (Platel & Srinivasan, 2016). Investigations of other essential elements often overlooked (Khouzam et al., 2011; Lagarda, Cilla, & Barbera, 2016). Additionally, discrepancies in the bioavailability assessment methodologies used can generate varying results due to systematic differences (Drago, 2017; Sahuquillo et al., 2003). Unsimilar to the *in vitro* reported studies which involved mainly gastric and intestinal digestion (Etcheverry et al., 2012), this current investigation examined all three steps of gastrointestinal digestion including the step of colonic fermentation.

The total percentage bioaccessibility of Fe, Mg, K, Ca, P, Zn, Mn and Cu, as well as the amount released during each of the three digestion steps are shown in Tables 4, 5, 6, 7, 8, 9, 10 and 11, respectively. The highest overall total percentage bioaccessibility was found in Cu (up to 80%, field pea) (table 11), and the lowest in Fe (0.1%, lentil hull) (Table 4). The individual bioaccessibility values for various tested minerals ranged from 0.1 to 45.13% (Fe), 31.21 to 74.19% (Mg), 13 to 61.87% (K), 9.98 to 52.68% (Ca), 12.67 to 68.32% (P), 0.35 to 39.38% (Zn), 0.35 to 69.21% (Mn), and 49.91 to 80.08 (Cu). The data revealed that mineral bioaccessibility varied among the tested pulses. For examples, field pea released the

highest bioaccessible Fe (45.13%, Table 4), Mg (74.19%, Table 5), P (68.32%, Table 8), Zn (37.55%, Table 9) and Cu (80.08%, Table 11), Lupin was the best source of bioaccessible K (61.87%, Table 6) and lentils had the highest % bioaccessible Mn (69.21% Table 10).

Data revealed significant ($P < 0.05$) three-way interactions between the pulse type, component (Hull and Cotyledon), and digestion state for all elements except Mg and Ca, indicating that the three factors are multiplicative in determining the total bioaccessibility. With all tested elements, the pulse type alone displayed a significant ($P < 0.05$) effect on the bioaccessibility of each element with the hulls exhibiting considerably lower bioaccessibility than dehulled pulses (Tables 4 to 11). Additionally, the fed state of digestion (11.7 mg bile /ml sample) generally increased the bioaccessibility of elements significantly ($P < 0.05$) compared to fasted (1.95 mg bile / ml sample) except for Zn in lupin and lentils (Table 9) and Mn in lupin and lentils (Table 10) where the fasted state showed significantly ($P > 0.05$) greater bioaccessibility. This increase in mineral bioavailability with higher bile concentrations may be due to the formation of bile-mineral soluble complexes, which become more available (Sitrin, 2014).

Most of the previously reported studies on minerals bioavailability focused on 2-3 elements and investigated the effect of gastric and intestinal digestion only. For example, Singh, Prasad, and Aalbersberg (2016) applied the *in vitro* gastrointestinal digestion to examine the bioavailability of Fe and Zn only. This current investigation examined the bioaccessibility of eight elements (Fe, Mg, K, Ca, P, Zn, Mn and Cu) during the gastrointestinal digestion and colonic fermentation. As anticipated, including the colonic fermentation improved mineral bioaccessibility *via* the release of more minerals during

fermentation. For example, the bioaccessibility of Fe from *in vitro* gastrointestinal fed state digestion of dehulled and hull lupin reached 10.82 ± 1.2 and $3.27 \pm 0.5\%$, respectively. Adding these two values revealed a total Fe bioaccessibility from both dehulled and hull lupin during fed gastrointestinal digestion of 14.09% (Table 4), which is similar to the % bioavailability of Fe from pulses as reported by Singh et al. (2016). However, adding the amount of bioaccessible Fe from the colonic fermentation of dehulled (13.2 ± 0.95) and hull lupin ($4.71 \pm 1.4\%$) will bring the total bioaccessible Fe to 32.00% , which is much larger than the gastric bioavailable value (14.4%) reported by Singh et al. (2016). Similarly, the total bioaccessibility of Zn from both hull and dehulled lupin (fed state) detected in this current study reached (25.50%) (Table 9), which is much greater than the value (4.02%) reported by Singh et al. (2016).

Another investigation by Das, Raghuramulu, and Rao (2005) revealed that the % ionisable iron (bioavailable) in whole pea was $35.66 \pm 4.44\%$, which was similar to the average value of 37.31% for pea cotyledons detected in this study. It is interesting to observe this resemblance in bioaccessibility, despite differences among the tested cultivars, the methods of sample preparation and extraction, the presence of hulls, and the machine used in mineral quantitation.

Results from this current study revealed also that bioaccessibility of several elements (namely Fe, K, Ca, P and Zn), was very small to no discernible quantities ($<0.1\%$) during intestinal digestion, while larger quantities of these mineral were absorbed in the colon. These findings were in agreement with (Blaine, Chonchol, & Levi, 2014; Gropper, 2008; Khanal, 2008), who reported main absorption of Ca, Mg and K in the human colon. The

small bioavailable fractions of minerals during intestinal digestion may partially explain why Fe from pulses continue to show poor bioavailability in human studies, even in anaemic individuals where absorptive mechanisms are upregulated (DellaValle, Glahn, Shaff, & O'Brien, 2015; Petry et al., 2012). As pulse hulls are known to contain up to 90% of the phenolic compounds (Dueñas, Hernández, & Estrella, 2002; Ramdath & Tsao, 2012), the poor bioavailability of Fe and other cations may be attributed to the formation of insoluble complexes by polyphenols during intestinal digestion, as polyphenols are good chelators of both Fe²⁺ and Fe³⁺ (Bouglé, 2013). However, further studies are required to confirm whether the colon can facilitate the absorption of minerals other than Ca, Mg and K, where some elements (e.g. Zn) have been shown promise in animal studies to be absorbed through the colon (Gopalsamy et al., 2015).

The study's outcome supports the recurring notion that due to quantity and distribution differences in the pro- and anti-nutritional factors in pulses, higher mineral concentrations do not always correlate with higher bioavailability (Karnpanit et al., 2017; Sahuquillo et al., 2003). This pattern of poor correlation was particularly evident for Fe in the hulls, where percentage bioaccessibility was consistently lower than their dehulled counterparts, despite possessing higher concentrations. This observation was not an issue for Mg, since nutritionally significant amounts of Mg (42.3%, 49.66% and 36.44%) were still bioaccessible in the hulls of lupin, lentils and field pea pulses (fed state), respectively (Table 5). In comparison, low levels of total Fe bioaccessibility (< 0.1%) was found in the lentil hull compared with 42.33 ± 2.3% in dehulled lentil (Table 4), regardless of encompassing more Fe in lentil hull (6.99 ± 0.79 mg dwt) compared with its dehulled counterpart (5.50 ± 0.52 mg/100g dwt) (Table 3). Consequently, it should be recommended that dehulled seeds should be consumed where maximal Fe and Mg bioaccessibility is desired. Similarly, the

lentil hull contains significantly ($P < 0.05$) larger quantity of Ca (427.46 ± 40.52 mg/100g dwt) than dehulled lentils (33.07 ± 5.15 mg/100g dwt) (Table 3), however, only small percentage ($27.23 \pm 0.8\%$) of lentil hull Ca was bioaccessible in comparison with $52.68 \pm 0.9\%$ from dehulled lentil (Table 7). Considering the fact that lentils hull contains a huge amount of Ca (427.46 ± 40.52 mg/100g dwt), even the relatively small percentage bioaccessibility ($27.23 \pm 0.8\%$) will endow a decent amount of dietary Ca, despite having low Fe and moderately low Mg bioaccessibility.

Conclusion

The present study confirmed previous suggestions that pulses with high essential mineral content do not always possess greater minerals bioaccessibility. Comparing the three examined pulses, it can be concluded that field pea cultivar contained the highest bioaccessible quantity of Fe, Mg, P, Zn and Cu. Lupin was the best source of K and Ca, and lentil hull contained the most Ca. While dehulling the seed appears to improve the *in vitro* bioaccessibility of most elements, it must be noted that some loss of minerals is expected, particularly for Ca. Results of digested fractions suggested a prevailing factor of low mineral bioaccessibility in pulses. The severe and common known deficiency of some minerals, such as Fe and Zn can be attributed to the low bioavailable fraction found at the site of absorption. Though this is likely to be a result of anti-nutritional compounds that form insoluble complexes, further studies are required to confirm this. *In vivo* studies for elements found in the soluble fractions would also improve the current understanding of essential mineral bioavailability.

Acknowledgments

The authors would like to thank Dr. Jason Brand for providing the pulse samples. We also appreciate Michelle Rhee, Sameera Sirisena, Pamu Kularathna, Martin Ji, Veradina Dharjono and Johnathan Vu for their technical support within the laboratory.

Author Contributions (required for *JFS* original research manuscripts)

All authors have contributed to the planning and coordination of this study. Y.Y. Zhang was responsible for the laboratory work and drafting the manuscript under the supervision of S. Ajlouni and J. Panozzo. M.S. Hall performed the ICP-OES analyses, and assisted in the interpretation of the final ICP results.

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Tables

- a) **Table 1.** Adult nutrient reference values for essential elements in Australia on a daily basis, relative to the amount found in 100g pulses

| | Estimated average requirement (EAR) | Recommended daily intake (RDI) | Amount found in 100g dry pulses |
|-----------------|--|---------------------------------------|--|
| Iron (Fe) | 6 – 8 mg | 8 – 18 mg | 4.82 – 8.80 mg |
| Zinc (Zn) | 6.5 – 12 mg | 8 – 14 mg | 2.62 – 3.96 mg |
| Calcium (Ca) | 840 – 1100 mg | 1100 – 1300 mg | 59.3 – 165 mg |
| Magnesium (Mg) | 255 – 350 mg | 310 – 420 mg | 171 – 250.2 mg |
| Selenium (Se) | 50 – 60 µg | 60 – 70 µg | 171.6 – 223.2 ng |
| Copper (Cu)* | N/A (AI: 1.2 – 1.7 mg) | N/A | 0.77 – 1.43 mg |
| Phosphorous (P) | 580 mg | 1100 mg | 317.4 – 426.5 mg |
| Potassium (K)* | N/A (AI: 2800 – 3800 mg) | N/A | 970 – 2017.3 mg |
| Manganese (Mn)* | N/A (AI: 5 – 5.5 mg) | N/A | 1.13 – 5.43 mg |

* Denotes insufficient current evidence to set an EAR or RDI. an AI (adequate intake) has thus been proposed based on median intakes from national dietary surveys.

Source: Chakravaty, 2003; Khanam & Platel, 2016; National Health and Medical Research Council, 2017.

Table 2. Site characteristics (Bureau of Meteorology, 2016; Victorian Government Data Directory, 2017)

| | Rokewood | Ouyen |
|--|-----------------|--------------|
| Soil type | Heavy red clay | Loamy sand |
| Minimum temperature | 5.7 °C | 5.2 °C |
| Maximum temperature | 25.6 °C | 32.3 °C |
| Approx. average rainfall (1994-2016) | 525 mm | 331 mm |
| Rainfall during the growing season (April – December) | 412 mm | 213 mm |

Table 3. Total mineral concentrations (mg/100 g dry weight) in the pulses studied. Data represent the mean of six measurements \pm SD

| Mineral | Dehulled Pulse | | | Hull | | |
|----------------|-----------------------|--------------------|---------------------|--------------------|--------------------|---------------------|
| | Lupin | Lentil | Field pea | Lupin | Lentil | Field pea |
| Fe | 4.88 \pm 0.55 | 5.50 \pm 0.52 | 5.65 \pm 0.99 | 3.28 \pm 0.82 | 6.99 \pm 0.79 | 8.99 \pm 0.09 |
| Mg | 217.46 \pm 13.78 | 71.36 \pm 4.27 | 81.65 \pm 9.84 | 169.66 \pm 18.76 | 269.41 \pm 23.11 | 285.95 \pm 25.96 |
| K | 1007.75 \pm 46.61 | 969.65 \pm 45.35 | 1111.18 \pm 28.57 | 393.48 \pm 31.79 | 381.02 \pm 27.38 | 1264.83 \pm 98.57 |
| Ca | 113.56 \pm 20.5 | 33.07 \pm 5.15 | 30.47 \pm 4.31 | 652.65 \pm 33.55 | 427.46 \pm 40.52 | 401.72 \pm 50.08 |
| P | 496.98 \pm 28.43 | 259.52 \pm 21.36 | 386.99 \pm 28.85 | 119.76 \pm 16.95 | 195.04 \pm 26.56 | 96.49 \pm 11.74 |
| Zn | 4.86 \pm 0.95 | 1.99 \pm 0.36 | 4.38 \pm 0.72 | 3.05 \pm 0.80 | 1.45 \pm 0.35 | 3.04 \pm 0.60 |
| Mn | 5.03 \pm 0.43 | 1.58 \pm 0.36 | 1.21 \pm 0.20 | 4.49 \pm 0.01 | 0.82 \pm 0.12 | 0.90 \pm 0.27 |
| Cu | 0.55 \pm 0.01 | 0.4 \pm 0.1 | 0.55 \pm 0.04 | * | 0.56 \pm 0.06 | 0.3 \pm 0.56 |

*denotes below detection limits of the ICP-OES at the amount quantified (0.3 \pm 0.001 mg)

Table 4. Percentage iron (Fe) bioaccessibility relative to total quantity present in sample, values reported as means of six measurements \pm standard deviation

| Pulse | Digestion state | % Fe Bioaccessibility | | | | |
|-----------|-----------------|-----------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|
| | | Gastric digestion | Intestinal digestion | Colonic fermentation | Total % Bioavailability | |
| Lupin | Hull | Fasted | 4.23 \pm 0.2 ^b | < 0.1 ^a | 0.76 \pm 0.6 ^a | 4.99 \pm 0.8 ^a |
| | | Fed | 3.27 \pm 0.5 ^b | < 0.1 ^a | 4.71 \pm 1.4 ^b | 7.22 \pm 0.9 ^b |
| | Cotyledon | Fasted | 11.17 \pm 0.4 ^a | < 0.1 ^a | 14.51 \pm 0.47 ^c | 25.68 \pm 0.7 ^c |
| | | Fed | 10.82 \pm 1.2 ^a | < 0.1 ^a | 13.2 \pm 0.95 ^c | 24.02 \pm 0.8 ^c |
| Lentils | Hull | Fasted | < 0.1 ^g | < 0.1 ^a | < 0.1 ^d | 0.10 ^d |
| | | Fed | < 0.1 ^g | < 0.1 ^a | < 0.1 ^d | 0.10 ^d |
| | Cotyledon | Fasted | 5.2 \pm 0.3 ^c | 2.9 \pm 0.1 ^b | 17.47 \pm 1.1 ^e | 25.57 \pm 1.4 ^c |
| | | Fed | 7.4 \pm 1.1 ^d | 13.4 \pm 0.7 ^c | 21.53 \pm 1.38 ^f | 42.33 \pm 2.3 ^e |
| Field pea | Hull | Fasted | 0.9 ^e | < 0.1 ^a | < 0.1 ^d | 0.99 \pm 0.57 ^f |
| | | Fed | 1.2 \pm 0.1 ^f | < 0.1 ^a | < 0.1 ^d | 1.23 ^f |
| | Cotyledon | Fasted | 6.4 \pm 0.1 ^d | 1.66 \pm 0.1 ^d | 21.36 \pm 0.43 ^f | 29.42 \pm 0.66 ^g |
| | | Fed | 9.5 \pm 2.1 ^d | 11.55 \pm 0.6 ^e | 24.08 \pm 2.27 ^f | 45.13 \pm 0.23 ^h |

Means within each column followed by different superscript letters (^{a, b, c, d}) are significantly different (P < 0.05).

Table 5. Percentage magnesium (Mg) bioaccessibility relative to total quantity present in sample, values reported as means of six measurements \pm standard deviation

| Pulse | Digestion state | % Mg Bioaccessibility | | | | |
|-----------|-----------------|-----------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|
| | | Gastric digestion | Intestinal digestion | Colonic fermentation | Total % Bioavailability | |
| Lupin | Hull | Fasted | 17.75 \pm 0.2 ^b | 12.64 \pm 0.1 ^a | 8.92 \pm 0.2 ^a | 39.31 \pm 0.2 ^a |
| | | Fed | 16.98 \pm 2.1 ^b | 14.87 \pm 1.1 ^b | 10.45 \pm 0.2 ^b | 42.3 \pm 1.4 ^b |
| | Cotyledon | Fasted | 29.19 \pm 4.4 ^a | 14.36 \pm 1.8 ^b | 15.09 \pm 0.08 ^c | 58.64 \pm 2.7 ^c |
| | | Fed | 30.22 \pm 3.7 ^a | 14.33 \pm 0.8 ^b | 13.18 \pm 0.99 ^d | 57.73 \pm 0.2 ^c |
| Lentils | Hull | Fasted | 18.73 \pm 0.6 ^d | 19.72 \pm 1 ^d | 4.46 \pm 0.53 ^e | 42.91 \pm 0.8 ^b |
| | | Fed | 17.62 \pm 0.5 ^d | 23.83 \pm 0.9 ^e | 8.21 \pm 0.34 ^a | 49.66 \pm 0.8 ^d |
| | Cotyledon | Fasted | 23.11 \pm 1.8 ^c | 11.6 \pm 3.1 ^b | 20.4 \pm 0.33 ^f | 55.11 \pm 1.3 ^e |
| | | Fed | 24.21 \pm 1.7 ^c | 13.26 \pm 1.5 ^b | 22.58 \pm 1.92 ^f | 60.05 \pm 1.96 ^c |
| Field pea | Hull | Fasted | 19.9 \pm 1.1 ^f | 5.11 \pm 0.3 ^c | 6.2 \pm 0.65 ^g | 31.21 \pm 1.5 ^f |
| | | Fed | 16.66 \pm 0.4 ^g | 13.91 \pm 0.7 ^b | 5.87 \pm 0.39 ^h | 36.44 \pm 0.07 ^g |
| | Cotyledon | Fasted | 28.67 \pm 1.3 ^e | 14.33 \pm 0.8 ^b | 27.46 \pm 2.38 ⁱ | 70.46 \pm 0.87 ^h |
| | | Fed | 29.78 \pm 0.1 ^e | 19.91 \pm 0.2 ^d | 24.5 \pm 1.53 ⁱ | 74.19 \pm 0.14 ⁱ |

Means within each column followed by different superscript letters (^{a, b, c, d}) are significantly different ($P < 0.05$).

Table 6. Percentage potassium (K) bioaccessibility relative to total quantity present in sample, values reported as means of six measurements \pm standard deviation

| Pulse | Digestion state | % K Bioaccessibility | | | | |
|-----------|-----------------|----------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | | Gastric digestion | Intestinal digestion | Colonic fermentation | Total % Bioavailability | |
| Lupin | Hull | Fasted | 40.95 \pm 1.1 ^a | < 0.1 ^a | 15.35 \pm 2.85 ^a | 56.3 \pm 3.8 ^a |
| | | Fed | 38.69 \pm 0.1 ^a | < 0.1 ^a | 14.57 \pm 1.96 ^a | 53.26 \pm 1.7 ^b |
| | Cotyledon | Fasted | 38.61 \pm 2.8 ^a | < 0.1 ^a | 22.66 \pm 0.03 ^b | 61.27 \pm 2.9 ^b |
| | | Fed | 40.66 \pm 3.2 ^a | < 0.1 ^a | 21.21 \pm 1.42 ^b | 61.87 \pm 3.7 ^b |
| Lentils | Hull | Fasted | 10.4 \pm 0.3 ^d | < 0.1 ^a | 2.6 \pm 0.37 ^c | 13 \pm 0.5 ^c |
| | | Fed | 9.6 \pm 0.4 ^d | < 0.1 ^a | 7.14 \pm 0.86 ^d | 16.74 \pm 0.6 ^d |
| | Cotyledon | Fasted | 24.82 \pm 2.4 ^c | 7.6 \pm 0.2 ^b | 20.83 \pm 0.4 ^b | 53.25 \pm 0.4 ^g |
| | | Fed | 25.61 \pm 0.8 ^c | 9.23 \pm 1.3 ^c | 23.45 \pm 1.7 ^b | 58.29 \pm 1.9 ^a |
| Field pea | Hull | Fasted | 17.7 \pm 0.6 ^g | 2.7 \pm 0.39 ^d | 8.76 \pm 0.38 ^e | 29.16 \pm 0.35 ^e |
| | | Fed | 19.4 \pm 0.5 ^d | 5.5 \pm 0.2 ^e | 6.82 \pm 0.7 ^d | 31.72 \pm 0.37 ^f |
| | Cotyledon | Fasted | 15.23 \pm 2.1 ^e | 9.86 \pm 0.65 ^c | 28.7 \pm 1.17 ^f | 53.79 \pm 0.5 ^g |
| | | Fed | 20.9 \pm 0.28 ^f | 14.15 \pm 0.16 ^f | 25.1 \pm 2.11 ^f | 60.15 \pm 0.7 ^a |

Means within each column followed by different superscript letters (^{a, b, c, d}) are significantly different (P < 0.05).

Table 7. Percentage Calcium (Ca) bioaccessibility relative to total quantity present in sample, values reported as means of six measurements \pm standard deviation

| Pulse | Digestion state | % Ca Bioaccessibility | | | | |
|-----------|-----------------|-----------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|
| | | Gastric digestion | Intestinal digestion | Colonic fermentation | Total % Bioavailability | |
| Lupin | Hull | Fasted | 5.2 \pm 0.1 ^b | < 0.1 ^a | 4.78 \pm 0.77 ^a | 9.98 \pm 0.5 ^a |
| | | Fed | 6.79 \pm 0.7 ^b | < 0.1 ^a | 6.04 \pm 0.33 ^b | 12.83 \pm 1.2 ^b |
| | Cotyledon | Fasted | 10.68 \pm 0.4 ^a | < 0.1 ^a | 39.87 \pm 0.04 ^c | 50.55 \pm 3.9 ^c |
| | | Fed | 12.57 \pm 3.1 ^a | < 0.1 ^a | 39.83 \pm 3.8 ^c | 52.4 \pm 6.8 ^c |
| Lentils | Hull | Fasted | 8.48 \pm 0.15 ^g | 10 \pm 0.2 ^b | 3.63 \pm 0.55 ^a | 22.11 \pm 0.2 ^d |
| | | Fed | 8.15 \pm 0.5 ^g | 13.58 \pm 0.4 ^c | 5.5 \pm 1.3 ^a | 27.23 \pm 0.8 ^e |
| | Cotyledon | Fasted | 18.31 \pm 0.7 ^c | 20.9 \pm 0.5 ^d | 11.7 \pm 1.4 ^e | 50.91 \pm 0.5 ^c |
| | | Fed | 20.35 \pm 1.6 ^c | 19.55 \pm 1.2 ^d | 12.78 \pm 0.9 ^e | 52.68 \pm 0.9 ^c |
| Field pea | Hull | Fasted | 11.11 \pm 0.2 ^a | < 0.1 ^a | 4.08 \pm 1.1 ^a | 15.19 \pm 1.1 ^f |
| | | Fed | 11.55 \pm 0.09 ^b | < 0.1 ^a | 3.81 \pm 0.33 ^a | 15.36 \pm 0.55 ^f |
| | Cotyledon | Fasted | 11.11 \pm 0.2 ^a | < 0.1 ^a | 24.92 \pm 0.3 ^f | 36.03 \pm 1.2 ^g |
| | | Fed | 21.9 \pm 3.12 ^f | < 0.1 ^a | 30.6 \pm 2.21 ^g | 52.5 \pm 3.08 ^c |

Means within each column followed by different superscript letters (^{a, b, c, d}) are significantly different (P < 0.05).

Table 8. Percentage Phosphorous (P) bioaccessibility relative to total quantity present in sample, values reported as means of six measurements \pm standard deviation

| Pulse | Digestion state | % P Bioaccessibility | | | | |
|-----------|-----------------|----------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | | Gastric digestion | Intestinal digestion | Colonic fermentation | Total % Bioavailability | |
| Lupin | Hull | Fasted | 24.75 \pm 1.47 ^b | < 0.1 ^a | 10.07 \pm 3.23 ^a | 34.82 \pm 0.4 ^a |
| | | Fed | 20.94 \pm 1.9 ^a | < 0.1 ^a | 22.78 \pm 0.29 ^b | 43.72 \pm 1.0 ^b |
| | Cotyledon | Fasted | 20.59 \pm 2.7 ^a | < 0.1 ^a | 15.6 \pm 1.53 ^c | 36.19 \pm 2.4 ^a |
| | | Fed | 21.24 \pm 1.1 ^a | 4.81 \pm 2.07 ^b | 15.6 \pm 1.53 ^c | 41.65 \pm 0.3 ^c |
| Lentils | Hull | Fasted | 10.96 \pm 0.77 ^c | < 0.1 ^a | 1.71 \pm 0.47 ^d | 12.67 \pm 1.03 ^d |
| | | Fed | 11.26 \pm 0.5 ^c | 23.98 \pm 1.6 ^c | 11.07 \pm 3.01 ^a | 46.31 \pm 1.03 ^e |
| | Cotyledon | Fasted | 11.05 \pm 0.8 ^c | 6.97 \pm 0.4 ^d | 13.48 \pm 0.1 ^a | 31.5 \pm 0.3 ^f |
| | | Fed | 12.85 \pm 0.9 ^c | 21.74 \pm 2.15 ^c | 17.24 \pm 2.77 ^f | 51.83 \pm 0.6 ^g |
| Field pea | Hull | Fasted | 12.98 \pm 0.77 ^c | < 0.1 ^a | 8.07 \pm 0.96 ^e | 21.05 \pm 1.1 ^h |
| | | Fed | 13.21 \pm 0.06 ^c | < 0.1 ^a | 12.34 \pm 2.58 ^a | 25.55 \pm 3.2 ⁱ |
| | Cotyledon | Fasted | 27.85 \pm 2.23 ^d | 17.3 \pm 0.39 ^e | 20.54 \pm 1.8 ^f | 65.69 \pm 0.6 ^j |
| | | Fed | 29.74 \pm 1.1 ^d | 20.02 \pm 1.32 ^c | 18.56 \pm 0.27 ^f | 68.32 \pm 0.28 ^k |

Means within each column followed by different superscript letters (^{a, b, c, d}) are significantly different (P < 0.05).

Table 9. Percentage Zinc (Zn) bioaccessibility relative to total quantity present in sample, values reported as means of six measurements \pm standard deviation

| Pulse | Digestion state | % Zn Bioaccessibility | | | | |
|-----------|-----------------|-----------------------|-------------------------------|------------------------------|------------------------------|-------------------------------|
| | | Gastric digestion | Intestinal digestion | Colonic fermentation | Total % Bioavailability | |
| Lupin | Hull | Fasted | 0.31 \pm 0.04 ^b | < 0.1 ^a | 0.04 \pm 0.03 ^a | 0.35 \pm 0.03 ^a |
| | | Fed | 0.32 \pm 0.04 ^b | < 0.1 ^a | 0.09 \pm 0.01 ^b | 0.41 \pm 0.05 ^a |
| | Cotyledon | Fasted | 24.23 \pm 2.53 ^a | 0.55 \pm 0.57 ^b | 14.6 \pm 2.7 ^c | 39.38 \pm 0.2 ^b |
| | | Fed | 21.83 \pm 0.8 ^a | < 0.1 ^a | 3.26 \pm 0.9 ^d | 25.09 \pm 0.3 ^c |
| Lentils | Hull | Fasted | 10.1 \pm 0.56 ^c | 2.04 \pm 0.09 ^c | < 0.1 ^e | 12.14 \pm 0.4 ^d |
| | | Fed | 10.33 \pm 0.56 ^c | 2.79 \pm 0.24 ^c | < 0.1 ^e | 13.12 \pm 0.4 ^e |
| | Cotyledon | Fasted | 10.27 \pm 0.3 ^c | 9.42 \pm 0.14 ^d | 7.36 \pm 0.4 ^f | 27.05 \pm 0.98 ^f |
| | | Fed | 10.52 \pm 0.6 ^c | 6.03 \pm 0.38 ^e | 7.75 \pm 1.06 ^f | 24.3 \pm 1.0 ^c |
| Field pea | Hull | Fasted | 21.62 \pm 1.8 ^e | 1.64 \pm 0.9 ^f | < 0.1 ^e | 23.26 \pm 0.9 ^c |
| | | Fed | 21.78 \pm 1.17 ^e | 1.73 \pm 1.07 ^f | < 0.1 ^e | 23.51 \pm 0.44 ^c |
| | Cotyledon | Fasted | 18.88 \pm 0.91 ^d | 7.26 \pm 1.26 ^g | 6.18 \pm 0.84 ^f | 32.32 \pm 0.2 ^g |
| | | Fed | 19.66 \pm 1.17 ^d | 10.81 \pm 0.2 ^h | 7.08 \pm 0.99 ^f | 37.55 \pm 0.35 ^h |

Means within each column followed by different superscript letters (^{a, b, c, d}) are significantly different (P < 0.05).

Table 10. Percentage Manganese (Mn) bioaccessibility relative to total quantity present in sample, values reported as means of six measurements \pm standard deviation

| Pulse | Digestion state | % Mn Bioaccessibility | | | | |
|-----------|-----------------|-----------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | | Gastric digestion | Intestinal digestion | Colonic fermentation | Total % Bioavailability | |
| Lupin | Hull | Fasted | 0.19 \pm 0.00 ^b | 0.072 \pm 0.00 ^a | 0.086 \pm 0.02 ^a | 0.348 \pm 0.02 ^a |
| | | Fed | 0.18 \pm 0.10 ^b | 0.073 \pm 0.01 ^a | 0.11 \pm 0.03 ^a | 0.364 \pm 0.07 ^a |
| | Cotyledon | Fasted | 7.09 \pm 3.23 ^a | 2.73 \pm 1.74 ^b | 24.37 \pm 9.3 ^b | 34.19 \pm 1.2 ^b |
| | | Fed | 8.86 \pm 1.03 ^a | 3.6 \pm 1.92 ^b | 14.43 \pm 0.06 ^c | 26.89 \pm 2.2 ^c |
| Lentils | Hull | Fasted | 6.36 \pm 0.07 ^c | < 0.1 ^c | 0.33 \pm 0.4 ^d | 6.69 \pm 0.5 ^d |
| | | Fed | 7.2 \pm 0.06 ^d | < 0.1 ^c | 2.53 \pm 0.07 ^e | 9.73 \pm 0.6 ^e |
| | Cotyledon | Fasted | 30.6 \pm 1 ^e | 30.84 \pm 0.7 ^d | 10.56 \pm 0.5 ^f | 72 \pm 0.9 ^f |
| | | Fed | 30.6 \pm 2.3 ^e | 25.38 \pm 2.55 ^e | 13.23 \pm 1.66 ^c | 69.21 \pm 1.0 ^g |
| Field pea | Hull | Fasted | 8.38 \pm 0.1 ^a | 8.57 \pm 0.1 ^f | 0.78 \pm 0.59 ^g | 17.73 \pm 0.58 ^h |
| | | Fed | 7.45 \pm 1.35 ^a | 2.75 \pm 1.06 ^b | 0.96 \pm 0.75 ^g | 11.16 \pm 0.83 ⁱ |
| | Cotyledon | Fasted | 25.62 \pm 0.7 ^f | 17.03 \pm 1.29 ^g | 10.5 \pm 1.73 ^f | 53.15 \pm 1.3 ^j |
| | | Fed | 25.23 \pm 0.48 ^f | 20.78 \pm 2.46 ^h | 11.33 \pm 0.09 ^h | 57.34 \pm 1.5 ^k |

Means within each column followed by different superscript letters (^{a, b, c, d}) are significantly different (P < 0.05).

Table 11. Percentage Copper (Cu) bioaccessibility relative to total quantity present in sample, values reported as means of six measurements \pm standard deviation

| Pulse | Digestion state | % Cu Bioaccessibility | | | | |
|-----------|-----------------|-----------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|
| | | Gastric digestion | Intestinal digestion | Colonic fermentation | Total % Bioavailability | |
| Lupin | Hull | Fasted | < 0.1 ^a | < 0.1 ^a | < 0.1 ^a | < 0.1 ^a |
| | | Fed | < 0.1 ^a | < 0.1 ^a | < 0.1 ^a | < 0.1 ^a |
| | Cotyledon | Fasted | 60.62 \pm 3.73 ^b | < 0.1 ^a | < 0.1 ^a | 60.62 \pm 3.7 ^b |
| | | Fed | 54.74 \pm 9.8 ^b | 1.97 \pm 1.92 ^b | < 0.1 ^a | 56.71 \pm 2.2 ^b |
| Lentils | Hull | Fasted | 29.3 \pm 1.45 ^c | 20.61 \pm 4.35 ^c | < 0.1 ^a | 49.91 \pm 1.0 ^c |
| | | Fed | 29.3 \pm 1.29 ^c | 27.97 \pm 1.93 ^d | < 0.1 ^a | 57.27 \pm 1.1 ^b |
| | Cotyledon | Fasted | 40.84 \pm 2.34 ^d | 27.5 \pm 2.31 ^d | < 0.1 ^a | 68.34 \pm 1.6 ^d |
| | | Fed | 43.62 \pm 0.49 ^d | 24.04 \pm 0.08 ^c | < 0.1 ^a | 67.66 \pm 0.8 ^d |
| Field pea | Hull | Fasted | 51.87 \pm 0.18 ^f | 15.87 \pm 0.54 ^e | < 0.1 ^a | 67.74 \pm 1.8 ^d |
| | | Fed | 48.06 \pm 3.18 ^f | 10.26 \pm 1.09 ^g | < 0.1 ^a | 58.32 \pm 0.07 ^b |
| | Cotyledon | Fasted | 45.76 \pm 2.28 ^d | 22.44 \pm 0.71 ^f | 5.7 \pm 0.35 ^b | 73.9 \pm 1.7 ^e |
| | | Fed | 47.57 \pm 2.81 ^d | 28.46 \pm 1.45 ^d | 4.05 \pm 0.36 ^c | 80.08 \pm 2.0 ^f |

Means within each column followed by different superscript letters (^{a, b, c, d}) are significantly different (P < 0.05).

*denotes no detectable levels of Cu present in the original sample