



Mineral nutrient elements and their bioaccessibility in hulled organic and conventional lentils (*Lens culinaris*) sold in the UK

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ABSTRACT

Lentils (*Lens culinaris*) are an accessible and cheap legume. Here, we report the findings from hulled organic and conventional products for their macro and micronutrient elements (NEs), phytic acid levels, bioaccessibility, and contribution to adult dietary reference values (DRVs). Raw and cooked samples were analysed for NEs using ICP-MS. SBET (Simplified Bioaccessibility Extraction Test) was used to assess the bioaccessibility of NEs, and phytic acid was analysed using the Megazyme method. Raw lentils were rich in macro (P, Mg, K and Ca) and micro (Fe, Zn, Mn and Cu) NEs. Mg, Zn and Cu were significantly higher in organic lentils, whereas K, Mo and Se were significantly higher in conventional lentils. Cooking significantly changed P, Mg, K, Cu, Fe, Zn, Mn and Mo concentrations. Phytic acid concentrations ranged from 0.272 to 0.471 g 100 g⁻¹. The bioaccessibility of Ca, Mg and K was >80 %, whereas it was >50 % for Fe, Cu, Mn and Zn. A serving size (80 g) of lentils contributed >25 % DRV of P, Fe, Cu, Mn and Mo. This highlights that including lentils as a regular dietary component is an affordable and accessible way to meet nutritional needs and prevent deficiencies.

1. Introduction

Micronutrients are vitamins and minerals required by the human body in small amounts. Micronutrient malnutrition, also known as hidden hunger, is a major global health issue, with over two billion people suffering from various deficiencies (WHO, 2022). These can lead to poor development in early childhood, exacerbation of disease, blindness as well as higher morbidity and mortality rates (Choukri et al., 2020). Some of the most prevalent mineral deficiencies include iron and zinc (60 % and 30 % of the human population, respectively) (Thavarajah et al., 2011). A quarter of the global population is affected by Fe-deficiency anaemia, for which WHO recommends the consumption of a diverse range of foods rich in iron, folate, Vitamin B12 and Vitamin A, as well as the use of recommended supplements by healthcare professionals (WHO, 2023). One way of reducing micronutrient deficiency is by including pulses in the diet as they are not only rich in protein (21–25 %) (Singh, 2017) but also various vitamins and minerals like iron (73–90 mg kg⁻¹), zinc (44–54 mg kg⁻¹) and selenium (425–673 µg kg⁻¹) (Thavarajah et al., 2011).

Lentils (*Lens culinaris*) are one of the most important pulse crops grown globally (Hefnawy, 2011; Wang et al., 2009). They are accessible,

cheap, high-caloric and nutrient-dense (Choukri et al., 2020; Alghamdi et al., 2014; Hefnawy, 2011). Compared to other pulses, lentils are quick to cook, requiring minimal preparation and processing (Alghamdi et al., 2014; Thavarajah et al., 2011). Lentils are grown on 6.1 million hectares globally, with an annual production of 6.3 million tons (Choukri et al., 2020), and consumed in over 100 countries worldwide (Thavarajah et al., 2011). From 1994 to 2019, global lentil production increased by 39 % or over 100 % in yield (Paffarini et al., 2021). Given that lentils are legumes, they can fix atmospheric nitrogen through *Rhizobium* symbiotic root bacteria and, therefore, are less dependent on nitrogen fertilisation than non-legume crops. Furthermore, lentils are environment-resistant and have low cultivation requirements, making them especially suitable for organic cultivation (Carbonaro et al., 2015).

Over the past decades, organic food production has increased considerably, from 200,000 producers globally in 1999 to over 2.7 million in 2016 (Gonzalez et al., 2019). The use of synthetic pesticides, herbicides and fertilisers is restricted in organic agriculture. Instead, farmers use animal and green manure for their crops. Furthermore, organic farming avoids synthetic chemicals, hormones, antibiotics and genetic engineering (Forman et al., 2012). In the UK, food can be labelled as "organic" if at least 95 % of its agricultural ingredients are

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organic and the remains are permitted within organic regulations (GOV. UK, 2022). The rising trend in consuming organic products is driven by a desire for improved personal health, animal welfare and environmental protection (Gonzalez et al., 2019). There is a perception among consumers that organic products possess healthier properties than their conventionally produced counterparts, including increased nutritional quality (Herencia et al., 2011). There are known health benefits to choosing organically over conventionally grown food, e.g. reduced exposure to pesticides, hormones and antibiotics (Gonzalez et al., 2019). However, the academic literature concerning the nutritional properties of organic versus conventional food is limited and divided (Forman et al., 2012). For instance, Hunter et al. (2011) compared the micro-nutrient composition of different plant foods produced by organic and conventional methods and reported that organic food had higher mineral contents in more cases than conventional foods. Bernacchia et al. (2016), on the other hand, found hardly any differences in nutrient contents of organic versus conventional food. Various researchers emphasise the need to investigate further to what extent a scientific basis exists for claims made about organic products, particularly their nutritional quality (Carbonaro et al., 2015).

Some of the published literature on NEs in lentils is based on their raw, uncooked state (Alghamdi et al., 2014). However, cooking generally results in some nutrient losses due to minerals leaching into the cooking water (Wang et al., 2009; Hefnawy, 2011). Huma et al. (2008) found that the mineral contents of legumes were reduced by 19–39 % post-cooking. The bioaccessibility of micronutrient elements (NEs) in cooked products can be influenced by antinutrients such as phytic acid because they can reduce the uptake of nutrient elements like Fe and Zn (Elliott et al., 2022). Cooking has also been found to reduce antinutrients (e.g. phytic acid) in pulses significantly (Wang et al., 2009) and therefore has the potential to enhance the bioaccessibility of these minerals.

A range of *in vitro* methods are available for quantifying bioaccessibility in foods, the majority of which are based on the application of specific combinations of pH, electrolytes, buffers and enzymes to simulate the physiological conditions found at different stages in the human digestive tract (Dima et al., 2020). The INFOGEST method (Brodkorb et al., 2019) was proposed as a standardised method; however, such Physiologically-Based Extraction Tests (PBET) can be laborious, and they sometimes suffer from enzyme-related interferences, including enzyme-metal complexation (Santos et al., 2018). The Simple Bioaccessibility Extraction test (SBET) is an easy and rapid alternative which does not require enzymes (Juhász et al., 2009). Instead, an acidified glycine solution is used to extract NEs from samples. The method has been extensively applied to evaluate the bioaccessibility of metals in soils (Wragg and Cave, 2003), but it has also been used to quantify mineral nutrient bioaccessibility in foods, including pulses (Santos et al., 2018), honeys (Oliveira et al., 2019) and corn, pepper, eggplants and spinach (Zhou et al., 2021).

The objectives of this study were (1) to compare the nutrient element (NE) profiles of hulled organic and conventional, raw and cooked lentils, (2) to determine phytic acid and bioaccessibility of NEs in cooked lentils, and (3) to establish what extent lentils contribute to daily NE requirements in adults.

2. Materials and methods

2.1. Sample collection and processing

Six packets of red (hulled) lentils were purchased from different UK retailers. Half of them have been certified by the Soil Association (Soil Association, 2023) as organic (purchased in M&S, Waitrose and Just Natural/Suma), and the rest were conventionally produced (purchased from ASDA, Coop, and Tesco). This sample size was chosen as only three lentil products were available as certified organic across retailers. Soil Association Certification Limited is the UK's largest organic certification body, and certified products legally comply with EU Organic

Regulations and their additional higher standards.

The total time between sample acquisition and analysis was around two weeks; all samples were refrigerated until the analysis. After stirring and mixing each lentil pack to create more uniform, homogenous samples, 100 g of lentils were measured from each packet. These were rinsed with de-ionised water (DI) five times to remove any residues from the production stages. No metal equipment was utilised as this could impact the mineral content in the samples. After this, samples were each split into two halves. The first half was used to analyse NEs, representing raw/uncooked samples.

The remaining half was cooked with DI water. The cooking process involved adding the lentil sample with five times the amount of DI water into a glass beaker – as per cooking instructions on the packaging (e.g. 50 g of lentils and 250 mL of water). This was brought to a slow boil whilst continuously stirring the beaker contents for 10 min. At that point, the lentils were cooked, and the water was almost fully absorbed, with only a few drops to drain after the cooking. The drained lentils were placed into an oven along with the washed raw lentil samples and left to dry at 40°C for 5 days until no further changes in the weight of the samples occurred. The dried lentil samples were ground up using an agate ball mill until they reached a powdered consistency. After this, triplicate samples were drawn from raw and cooked individual lentil products (36 samples) for NE analysis.

2.2. NE analysis

For total elemental analysis (Menon et al., 2024; Menon et al., 2021), approximately 0.2 g (dry weight) of the powdered lentil sample was microwave-digested in 6 mL HNO₃ (Primar grade, Fisher Scientific, UK) in perfluoro-alkoxy (PFA) vessels (Multiwave; Anton Paar GmbH, St. Albans, UK). The digested samples were diluted to 20 mL total volume in Milli-Q water (18.2 MΩ cm). This was then subsampled and further diluted 1-in-10 in Milli-Q water. Then, multi-element analysis of diluted solutions was undertaken by ICP-MS (Thermo-Fisher Scientific iCAP-Q and iCAP-TQ; Thermo Fisher Scientific, Bremen, Germany; LoD values presented, and additional ICP-MS settings and calibrations are provided in Supplementary Material 1; see also Menon et al., 2024). In this method, samples are introduced (flow rate 1.2 mL min⁻¹) from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a perfluoro-alkoxy (PFA) Microflow PFA-ST nebuliser (Thermo Fisher Scientific, Bremen, Germany). The iCAP-Q employs a collision cell that is charged with He gas and uses kinetic energy discrimination (KED) to remove polyatomic interferences. Peak dwell times are 100 mS for most elements, with 150 scans per sample. Internal standards, used to correct for instrumental drift, are introduced to the sample stream on a separate line (equal flow rate) via the ASXpress unit. Internal standards typically include Sc (10 µg L⁻¹), Ge (10 µg L⁻¹), Rh (5 µg L⁻¹), and Ir (5 µg L⁻¹). The matrices used for internal standards, calibration standards and sample diluents is 2 % Primar grade HNO₃ (Fisher Scientific, UK) with 4 % methanol (to enhance ionisation of some elements). Calibration standards typically include (i) a multi-element solution with Ag, Al, As, Ba, Be, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Sr, Ti, Tl, U, V and Zn, in the range 0 – 100 µg L⁻¹ (0, 20, 40, 100 µg L⁻¹) (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA); (ii) a bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, France) with Ca, Mg, Na and K in the range 0–30 mg L⁻¹ and (iii) a mixed phosphorus, boron and sulphur standard made in-house from salt solutions (KH₂PO₄, K₂SO₄ and H₃BO₃). Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific), utilising external cross-calibration between pulse-counting and analogue detector modes when required.

Apart from the calibration standards, we also used triplicates of certified reference material, NIST SRM 1568b rice flour, as an additional quality control and assurance measure. It helps to calibrate and verify the accuracy of specific measurements performed using ICP-MS. The

certified reference material is provided with a Certificate of Analysis and a Materials Safety Data Sheet. The analytical data obtained for the certified reference material from ICP-MS was compared against the Certificate of Analysis and a Materials Safety Data Sheet provided with the NIST standard to calculate the recovery (%). In our experiment, the average recovery for each of the analysed nutrients was the following: 92 % for Mg, 108 % for P, 109 % for K, 111 % for Ca, 103 % for Mn, 90 % for Fe, 129 % for Cu, 100 % for Zn, 96 % for Se and 100 % for Mo.

2.2.1. Bioaccessibility using SBET (Simplified Bioaccessibility Extraction Test)

The bioaccessibility of major and trace elements was measured using the Simplified Bioaccessibility Extraction Test (SBET), as outlined in Santos et al. (2018), where 1 g of cooked lentil sample was mixed with 40 mL of pre-prepared extraction solution containing 0.4 M glycine (Fisher, Analytical Grade) adjusted to pH 1.5 with ~5% (v/v) HCl (ACROS, Analytical Grade). The mixture was shaken for 2 h at 200 rpm in an incubator at 37°C (note that the extraction solution was also pre-warmed to this temperature before use), then centrifuged for 10 mins at 3000 rcf. The final extract was collected by passing the supernatant through a 0.22 µm Millex PES syringe filter. A 1 mL subsample of the extract was diluted with 9 mL deionised water before analysis by the same ICP-MS method described above for the aqueous digest samples (see Suppl. Material 1 for ICP-MS calibration details). All samples were prepared and analysed in triplicate. Blank solutions (containing the extraction solution only) were also prepared and analysed in triplicate, and the sample results were corrected against these. The bioaccessibility of each NE was calculated using Eq. 1, given below.

$$\text{Bioaccessibility}(\%) = \left(\frac{\text{mean mineral concentration in SBET sample}}{\text{total mean mineral concentration}} \right) \times 100 \quad (1)$$

2.2.2. Phytic acid (phytate) concentrations

Phytic acid analysis was carried out for the cooked samples using the Megazyme (2019) K-PHYT Assay Kit method, which is briefly outlined here. Initial extracts were obtained by mixing 1 g of cooked lentil sample with 20 mL of 0.66 mol L⁻¹ HCl in a glass beaker. The beakers were then covered with foil and left to mix overnight on an orbital shaker (set to 200 rpm). Afterwards, 1 mL of the extract was centrifuged at 16,100 rcf for 10 mins, and 0.5 mL of the resulting supernatant was neutralised with 0.6 mL of 0.75 mol L⁻¹ NaOH (Fisher, Analytical Grade) solution. A 0.05 mL subsample of the extract was then subjected to enzymatic dephosphorylation reaction by sequential incubation with phytase and alkaline phosphatase in the presence of buffers (at pH 5.5 and pH 10.4, respectively) to break down phytic acid into phosphate. A second subsample was incubated with the same buffers but without the enzymes. Finally, the two incubated subsamples were analysed colourimetrically on a Skalar San++ Continuous Flow Analyser, using the molybdenum blue method (British Standards Institute, 2004) to obtain both the enzyme-reacted and unreacted phosphorus (PO₄-P) concentrations in the extract. Samples were measured against a linear calibration curve with standards at 0.10, 0.20, 0.40, 0.60, 0.80 and 1.00 mg L⁻¹ PO₄-P (See Suppl. Material 1 for calibration curve). Reference standards, blanks and drift correction standards were included with every 10 samples. Diluted phytic acid concentration was calculated as in Eq. 2, using an adjustment factor of 28.2 % as the proportion of phytic acid represented by the liberated phosphorus.

$$\text{Phytic acid (g 100g}^{-1}) = \left(\frac{\text{Pin enzyme reacted sample} - \text{Pin unreacted sample}}{0.282} \right) \quad (2)$$

2.3. Statistical analysis

Using RStudio Version 2023.06.1+524, the analysis focused on macro (K, Ca, P, and Mg) and micro (Cu, Fe, Zn Mn, Se and Mn) NEs. The dataset was first checked for normality and the homogeneity of variance (homoscedasticity). Subsequently, a two-way ANOVA was used to analyse the effect of growing methods (i.e. organic v conventional) and processing (raw v cooked) and their interaction on NE concentration (Table 1). The bioaccessibility and phytic acid content were statistically analysed using Welch's two-sample T-test. Furthermore, a simple linear regression test was applied to the data to determine any association between phytic acid concentrations and bioaccessibility.

2.4. Contribution to daily nutrient requirements

After the mean NE concentrations had been determined for each lentil sample, they were compared to their dietary reference value (DRV) provided by the European Food Safety Authority (EFSA, 2019). Specifically, this study investigates how much one serving size of cooked lentils (80 g, according to package instructions) contributes to the daily requirements of various essential NEs, i.e. Cu, Zn, Mo, K, Mg, Fe, P, Mn, Se and Ca. The DRV is based on adequate intake (AI) or population reference intake (PRI). AI is the average nutrient level assumed to be adequate for the population's needs. It is based on observations or experiments and used when data is insufficient to calculate an average requirement. If PRI data was available, this metric was preferred in nutrient contribution calculations. PRI refers to the nutrient intake which is likely to meet the needs of almost all healthy individuals in a population (EFSA, 2019). The DRV refers to the daily nutrient requirements for adults over 18. Sex-specific differences in recommended nutrient intakes were indicated for the relevant NE where necessary. Notably, the recommended DRV for Zn depends on the daily phytic acid intake (LPI). The EFSA gives recommendations based on different phytic acid intake levels (300, 600, 900, 1200 mg day⁻¹). Given that the average UK adult phytic acid intake is around 809 mg day⁻¹ (Amir-abdollahian and Ash, 2010), the LPI of 900 mg day⁻¹ scenario was used for nutrient contribution calculations. See Supplementary Material 3 for the results breakdown.

The mean NEs concentration per lentil serving (80 g) was calculated using the average NE obtained from the chemical analysis of the cooked

Table 1

Statistical significance of growing condition, processing technique, and their interaction for each nutrient element (NE). Values were obtained through two-way ANOVA in RStudio. P-values < 0.05 indicate statistical significance (see bold values).

NE	Growing Condition (conventional v organic)	Processing Technique (raw v cooked)	Interaction between Growing Condition and Processing Technique
Ca	p < 0.0001 (F = 45.9)	p = 0.600 (F = 0.31)	p = 0.500 (F = 0.480)
Mg	p = 0.008 (F = 8.05)	p < 0.0001 (F = 64.5)	p = 0.600 (F = 0.310)
P	p = 0.07 (F = 3.42)	p < 0.0001 (F = 71.2)	p = 0.500 (F = 0.510)
K	p = 0.03 (F = 5.35)	p < 0.0001 (F = 454)	p = 0.100 (F = 2.69)
Se	p < 0.0001 (F = 1290)	p = 0.600 (F = 0.230)	p = 0.800 (F = 0.0900)
Mo	p < 0.0001 (F = 253)	p < 0.0001 (F = 71.9)	p < 0.0001 (F = 32.8)
Cu	p < 0.0001 (F = 21.2)	p = 0.0006 (F = 14.5)	p = 0.03 (F = 5.04)
Mn	p = 0.200 (F = 2.06)	p = 0.005 (F = 9.26)	p = 0.8 (F = 0.0700)
Zn	p < 0.0001 (F = 178)	p < 0.0001 (F = 24.3)	p = 0.04 (F = 4.64)
Fe	p = 0.100 (F = 2.88)	p < 0.0001 (F = 21.9)	p = 0.8 (F = 0.0800)

samples. Afterwards, DRV contribution per serving was calculated for each nutrient as follows:

$$DRV\ contribution(\%) = \left(\frac{100}{DRV\ of\ NE} \right) \times NE\ per\ serving \quad (3)$$

3. Results and discussion

3.1. NE concentrations in organic and conventional lentils

A comparison of the mean concentrations of the macro (Ca, Mg, P and K) and micro NEs (Mo, Se, Cu, Mn, Zn and Fe) in organically and conventionally produced lentils is shown in Fig. 1a & b (see [Supplementary material 2](#) for raw data). The concentration of major nutrients followed the trend of $K > P > Mg$ and Ca, whereas it was $Fe > Zn > Mn > Cu > Mo > Se$ for micronutrients. Significantly higher concentrations of Ca (25.3 %; $p < 0.0001$), Mg (6.62 %; $p < 0.008$), Cu (12.8 %; $p < 0.0001$) and Zn (24.3 %; $p < 0.0001$) were found in organic lentils compared to conventional ones ([Table 1](#)). However, significantly higher concentrations of K (5.53 %; $p < 0.03$), Se (95.4 %; $p < 0.0001$) and Mo (78.5 %; $p < 0.0001$) were found in conventional lentils compared to those organically produced.

[Tziouvalekas et al. \(2022\)](#) compared five different lentil genotypes grown under conventional and organic farming. They found mean Fe concentrations of $121\ mg\ kg^{-1}$ in raw, organic lentils and $154\ mg\ kg^{-1}$ in raw, conventional lentils. These values are higher than those found in this study, i.e. $63.6\ mg\ kg^{-1}$ in raw, organic lentils and $61.2\ mg\ kg^{-1}$ in raw, conventional lentils, and portray an opposite trend to organic lentils having higher Fe concentrations. These variations could be attributed to the crops' geographic location, local soil characteristics, climatic conditions, the maturity at harvest or post-harvest storage ([Forman et al., 2012](#)). Furthermore, [Tziouvalekas et al. \(2022\)](#) found Cu concentrations in raw, organic lentils to be around $8.8\ mg\ kg^{-1}$ and $8.9\ mg\ kg^{-1}$ in raw, conventional lentils. This is somewhat consistent with this study's results, i.e. $10.2\ mg\ kg^{-1}$ in raw, organic lentils and $8.4\ mg\ kg^{-1}$ in raw, conventional lentils.

In another study comparing NEs in organically and conventionally produced green lentils, [Arslanbas and Baydan \(2013\)](#) found higher Zn and Cu concentrations in their conventional samples ($p < 0.01$). They reported mean concentrations of $29.4\ mg\ kg^{-1}$ for Zn and $7.5\ mg\ kg^{-1}$ for Cu in organic lentils compared to $36.8\ mg\ kg^{-1}$ for Zn and $7.8\ mg\ kg^{-1}$ for Cu in conventional ones. These results disagree with the findings of this study, in which both Zn and Cu concentrations were

shown to be significantly higher in the organic samples, with mean concentrations of $42.8\ mg\ kg^{-1}$ (Zn) and $8.65\ mg\ kg^{-1}$ (Cu), compared with $30.5\ mg\ kg^{-1}$ (Zn) $8.03\ mg\ kg^{-1}$ (Cu) in the conventional ones. However, it is important to note that deviations in data from different studies are expected due to variations in soil type, water and nutrient management, cultivar and processing involved (hulled v whole).

[Hunter et al. \(2011\)](#) investigated studies that compare micronutrient concentrations in organic and conventionally produced legumes. They found that 56 % of the comparisons in the literature reported higher micronutrient concentrations in organic rather than conventional legumes, 38 % reported higher levels in conventional legumes, and 6 % found no differences ($n=183$ comparisons). These findings agree with the results of this study in the sense that a significant difference in nutrient element concentration was found for the majority of the analysed nutrients (7 out of 10), and the majority of those nutrients (4 out of 7) were higher in organic lentils. However, that is not a strong argument in favour of organic lentils. There were still several nutrient elements (Fe, P, Mn) where no significant differences in concentrations between organic and conventional lentil samples were found. In the instances where significant differences were found, conventional lentils had higher concentrations of Mo, K and Se. This circles back to the issue of whether an organic label equates to a healthier product by providing essential NEs. The academic literature disagrees with this, although only a few studies have been conducted thus far ([Carbonaro et al., 2015](#); [Forman et al., 2012](#)). However, the findings of this study indicate that consumers may not reap any clear nutritional benefits by opting for organic lentils over conventional ones since some NEs were higher in organic lentils, whereas others were higher in conventional lentils. There is still an argument that buying organic produce is healthier for the consumer for other reasons (e.g., free from pesticides or other chemicals).

3.2. Effect of cooking on NEs and phytic acid

In [Fig. 2a-d](#), the effect of processing (cooking) on NE retention (in $mg\ kg^{-1}$) was compared (see [Supplementary material 2](#) for raw data). In general, cooking reduced the concentration of most NEs studied, such as K ($p < 0.0001$), P ($p < 0.0001$), Mg ($p < 0.0001$), Zn ($p < 0.0001$), Cu ($p = 0.0006$) and Mo ($p < 0.0001$), whereas Fe ($p < 0.0001$) and Mn ($p = 0.005$) exhibited an increase (see also [Table 1](#)). No statistically significant effect of cooking was found for Ca or Se. The loss of K was slightly higher in conventional (42 %) than in organic (38 %) lentils ($p < 0.0001$). The P and Mg losses were smaller than K. P and Mg were

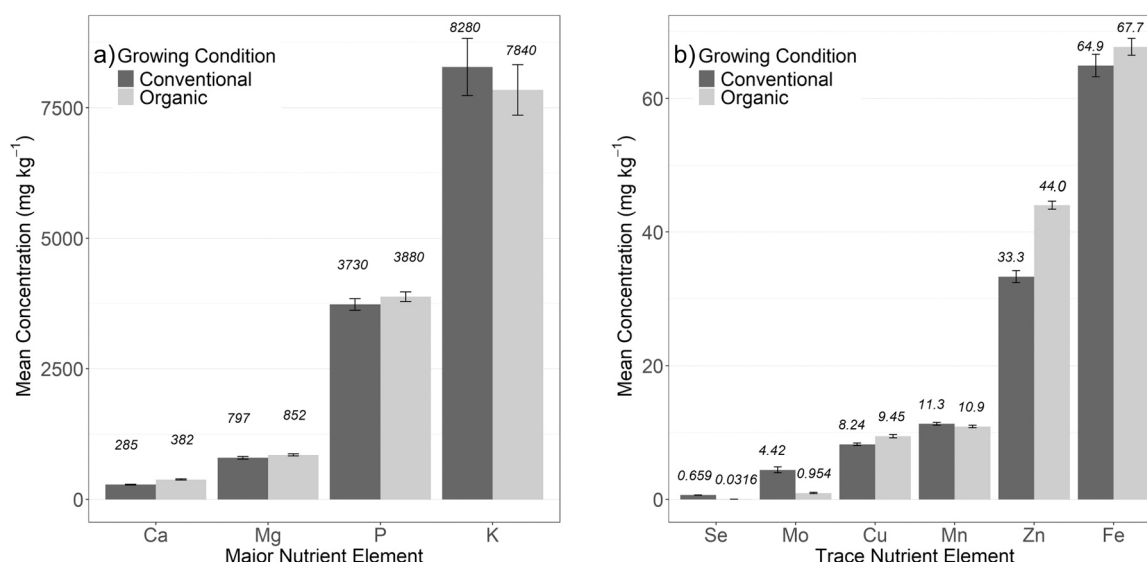


Fig. 1. a & b. The mean macro and micronutrient elements (NEs) in conventional and organic lentils. The error bars represent standard deviation (SD) of the mean.

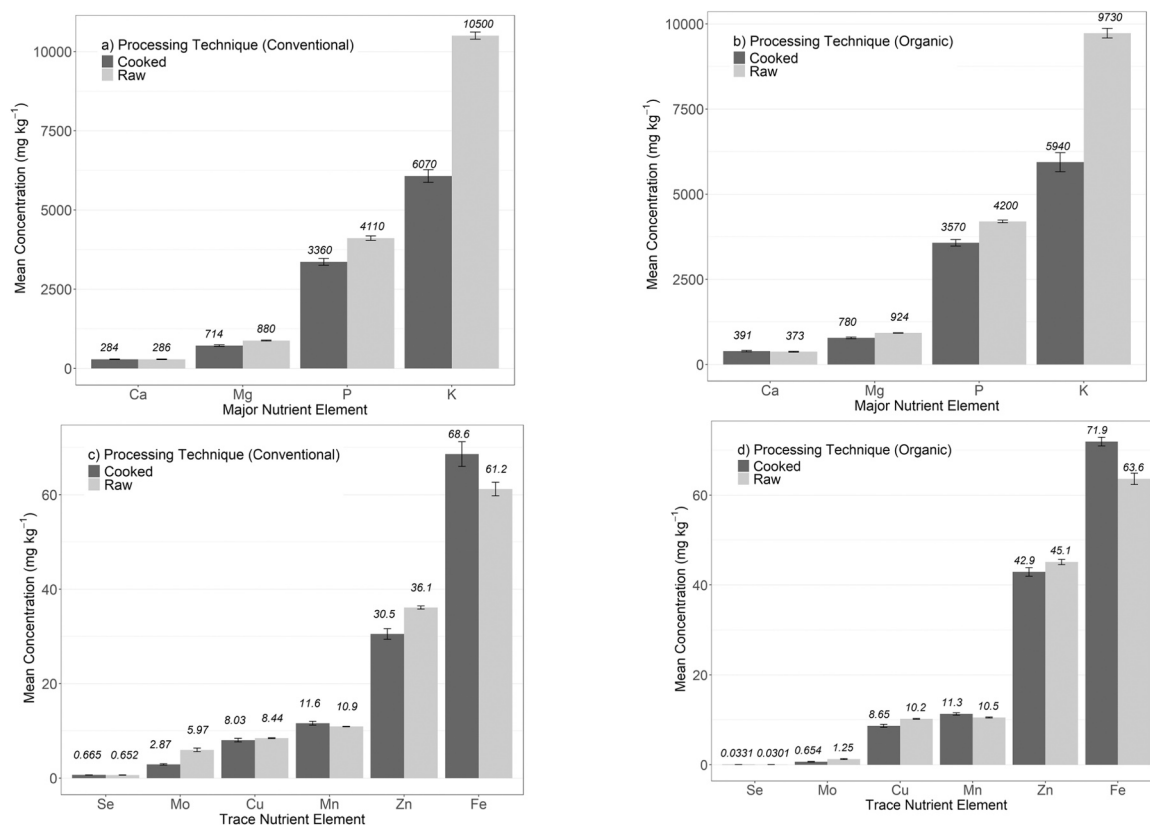


Fig. 2. a - d. The changes in macro (a-b) and micronutrient elements (NEs) (c-d) for both conventional and organic after cooking. Error bars represent the standard deviations (SD).

reduced by 18 % in conventional lentils and by 15 % in organic ($p < 0.0001$). Fe was slightly increased (12–13 %) after cooking for both types of lentils (conventional: $p = 0.02$, organic: $p < 0.0001$). A slight increase in Mn was also found in organic (8 %) and conventional lentils (5 %) after cooking, although this increase was only statistically significant in organic lentils ($p = 0.01$).

Wang et al. (2009) compared several lentil varieties and found that cooking them in distilled water significantly affected all the analysed minerals. It significantly increased Ca, Cu and Mn concentrations, while also resulting in significant Fe, K, Mg, P and Zn losses. These results show the same pattern as the present study for Mn, K, Mg, P and Zn, but differ for Cu (which decreased post-cooking in this study) and Fe (which increased in this study). It is noteworthy that Wang et al. (2009) strained and then froze their cooked samples before further analysis, whereas here, the cooking water was absorbed almost entirely so that no straining was required before drying the samples.

Hefnawy (2011) also studied the effect of different cooking methods on the various mineral contents of lentil seeds. Their study, which used distilled water to process their lentils, matched the present study by showing a post-cooking reduction in K, Mg, P, Cu, and Zn. However, in contrast to this study, they observed a decrease in Fe and Mn. Such differences could be due to the methods used (e.g., rinsing), lentil characteristics, cultivar types, etc. The nutrient losses during the cooking process can be attributed to minerals leaching from lentils into the cooking water. Huma et al. (2008), for instance, found that the mineral content of lentils was significantly reduced (by 33 %) post-cooking. On the other hand, the process of cooking also has been found to significantly reduce antinutrients like phytic acid and tannins in pulses (Wang et al., 2009). This could, in turn, enhance the bioavailability of some minerals that would have otherwise been impacted by those antinutrients.

Mean phytic acid concentrations in the cooked lentils from the 6

different retailers ranged from $0.272 - 0.471 \text{ g } 100 \text{ g}^{-1}$. These results match Thavarajah et al. (2009), which found phytic acid levels of $0.25 - 0.44 \text{ g } 100 \text{ g}^{-1}$, but were slightly lower than the reported values of $0.77 \text{ g } 100 \text{ g}^{-1}$ in Wang et al. (2009) and $0.67 - 0.88 \text{ g } 100 \text{ g}^{-1}$ in Thavarajah et al. (2010). When averaged by product type, phytic acid concentrations in the organic ($0.419 \text{ g } 100 \text{ g}^{-1}$) and the conventional ($0.378 \text{ g } 100 \text{ g}^{-1}$) lentils showed no significant difference in a two-sample t-test ($p = 0.5$).

Phytic acid is an antinutrient for humans as it binds to essential micronutrients like Fe and Zn, decreasing their bioavailability. Therefore, high phytic acid levels in food can become an issue, especially considering that mineral micronutrient deficiency is a major global health issue – particularly for people dependent upon cereals and legumes (Thavarajah et al., 2010). Whilst phytic acid does provide some health benefits for humans when micronutrient requirements are met, diets should generally aim to be low in phytic acid (Konietzny and Greiner, 2003). The phytic acid levels detected in lentils in this study ($0.272 - 0.471 \text{ g } 100 \text{ g}^{-1}$) were consistently lower than mean concentrations of other legumes like kidney bean ($1.1 - 1.7 \text{ g } 100 \text{ g}^{-1}$), pea ($0.22 - 0.82 \text{ g } 100 \text{ g}^{-1}$), chickpea ($0.49 - 0.61 \text{ g } 100 \text{ g}^{-1}$) or soybean ($1.0 - 1.5 \text{ g } 100 \text{ g}^{-1}$) (Thavarajah et al., 2009). This could make hulled red lentils the superior legume for optimised low phytic acid intake and enhanced mineral uptake.

In this study, lentils were washed and boiled. However, several other (pre)processing techniques can produce differing results. Sharif et al. (2014) found that soaking lentils before cooking reduced cooking time by 54.2 % and decreased phytic acid levels by 51.9 %. Microwave roasting led to a reduction of phytic acid levels by 45.4 %. However, both pre-cooking treatments resulted in NE losses, too, e.g. 44.0 % for Zn and 7.8 % for Cu. Furthermore, Wang et al. (2009) found that dehulling lentils resulted in significant increases in phytic acid levels, as well as significant reduction in Fe, Cu, Ca, Mg and Mn. Margier et al. (2018)

compared boiled and canned lentils. They found that canning consistently led to lower NE levels, e.g. 20 mg kg⁻¹ Fe and 2.1 mg kg⁻¹ Cu in cooked lentils compared with 15 mg kg⁻¹ Fe and 1.8 mg kg⁻¹ Cu in canned lentils.

Moreover, Hefnawy (2011) recommends microwave cooking for lentils as they found smaller mineral nutrient losses in lentils cooked by microwaving compared to boiling and autoclaving. For instance, Cu levels in lentils varied from 9.4 mg kg⁻¹ for microwave cooking to 8.1 mg kg⁻¹ for autoclaving and 7.3 mg kg⁻¹ for boiling. There are various ways in which lentils are being prepared domestically; therefore, it is essential that different studies are conducted on the many processing methods.

3.3. Bioaccessibility of NEs

The bioaccessibility of the analysed major and trace minerals from the cooked lentil samples can be found in Fig. 3a & b below. The bioaccessibility of the nutrient elements does not differ significantly between organic and conventional lentils ($p = 0.9$). The major NEs (Ca, Mg and K) all have a bioaccessibility of >80 %, whereas the trace NEs (Fe, Cu, Mn and Zn) all have a bioaccessibility of >50 %. Suliburska and Krejpcio (2014), measuring the bioaccessibility of cooked green lentils using gastrointestinal (pepsin and pancreatin) digestion, obtained similar values for Zn (at 92.2 %), but lower values for Ca, Fe and Mg (at 52.0 %, 32.6 %, 39.2 %, respectively). Ramírez-Ojeda et al. (2018), using a similar 2-stage digestion method, reported lower lentil bioaccessibilities for Ca (31.5 %), Cu (43.9 %) and Mg (46.5 %), but their results for Fe (50.0 %) and Zn (73.1 %) were more consistent with those obtained in this study. Sahuquillo et al. (2003), using a different 2-stage method (with a lower pH for the intestinal stage) obtained lower bioaccessibilities for Ca (28.8–46.6 %), Fe (11.5–15.6 %) and Zn (53.6–71.3 %), but the pattern of most bioaccessible element to least bioaccessible (i.e. Zn > Ca > Fe) was consistent with the present study. Zhang et al. (2018) applied a 3-stage digestion procedure (incorporating gastric and intestinal digestion, followed by colonic fermentation) to quantify the bioaccessibility of green lentil cotyledons and hulls separately (though the former is more far more nutrient-rich than the latter, so comparisons have only been made with the cotyledon data). They reported higher bioaccessibilities for P (51.8 %), but slightly lower bioaccessibilities for Fe (42.3 %), Cu (67.7 %) and Mn (72.0 %), and much lower values for Ca (52.7 %), Mg (60.1 %), K (58.3 %) and Zn (27.1 %). The result for Zn is strikingly different not only from this study, but from the other cited studies, a disparity which may be due to the less common colonic fermentation method adopted by Zhang et al. (2018).

Differences between studies are expected given the wide variety of methods applied, but these comparisons further indicate that SBET tends to overestimate bioaccessibility when compared with PBET methods (Mingot et al., 2011). This may be due to the lower pH (1.5) used in SBET, which may have the dual effect of liberating less labile metals

while also largely eliminating the complexation effects observed in PBET methods (Santos et al., 2018).

A level of discrepancy between total mineral content and the amount of minerals absorbed during the digestive process is expected. It can be attributed to many factors, such as antinutrients like phytic acid or physical barriers like cell walls. The effect of cooking can be two-fold. On the one hand, processing the lentils can break down antinutrients and, therefore, enhance overall bioaccessibility; conversely, it can lead to minerals leaching into the processing liquid (Rousseau et al., 2019). This study shows that whilst most of the analysed NEs uphold a bioaccessibility of at least >50 % post-cooking, Se, Mo and P portray relatively low absorption levels. There is no evidence of organic lentils containing significantly more bioaccessible nutrient elements than conventional lentils ($p = 0.9$), highlighting that consuming non-organic lentils does not compromise nutrient absorption and is just as appropriate as consuming organic lentils.

The relationships between the cooked lentil phytic acid content and the bioaccessibility (%) of Fe and Zn were also investigated using simple linear regression, but no significant trend was found for either element.

3.4. Contribution to nutrient requirements

Fig. 4 (see also Suppl. Material 3) displays the major and trace minerals' contribution to the dietary reference value (DRV) set by the EFSA – for organic and conventional lentils, respectively. The NEs' contribution is based on one serving size of cooked, dry lentils, i.e. 80 g (or ca. 120 g in cooked lentils with additional water weight). In some instances, DRV varies by sex; this was accounted for in the percentage contributions (e.g. see "Fe_F" and "Fe_M" in Fig. 4).

One serving size of cooked, organic lentils contains 76.9 % of the recommended Mo intake, whereas a portion of conventional lentils exceeded the DRV by contributing 354 % of the recommended daily intake. Cu has different recommendations based on sex; a serving of organic lentils contributes 53.1 % to the DRV for women and 43.1 % for men, whereas a conventional lentil portion contains 49.2 % of the DRV for women and 40.3 % for men. One serving size of organic lentils contributes 51.9 % to the recommended daily intake of P, and conventional lentils contribute 48.8 %. Furthermore, regarding Fe, pre-menopausal women have a higher DRV, to which a portion of organic lentils contributes about 35.9 %, and conventional lentils contribute 34.2 %. For men and post-menopausal women, a serving size of organic lentils contains 52.3 % of their recommended Fe intake, and conventional lentils contain 49.8 %. The DRV for Zn also differs by sex – a serving size of organic lentils contributes 31.2 % to the recommended intake for women and 24.5 % to that of men, whereas a portion of conventional lentils contains 22.2 % of the DRV for women and 17.4 % of men.

Moreover, a serving of organic and conventional lentils covers the daily Mn needs almost equally (30.2 % and 31.1 %). Regarding Mg, one portion of organic lentils contributes 20.8 % to the DRV for women and

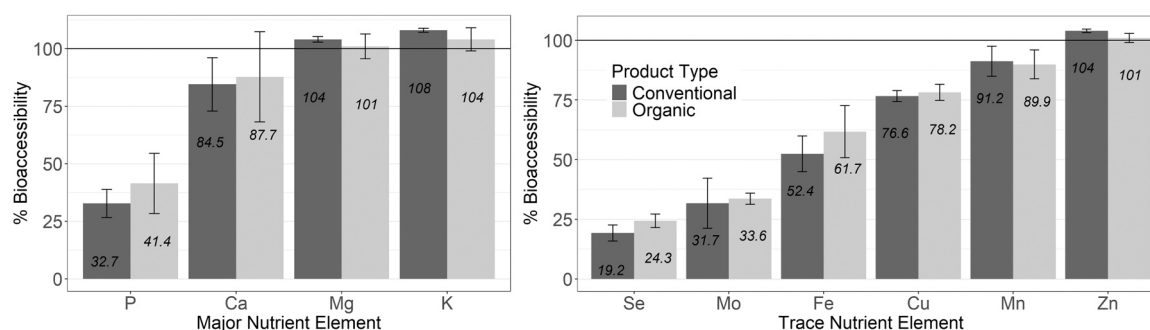


Fig. 3. a & b: Bioaccessibility of major (a) and micro (b) nutrients of cooked conventional and organic lentil samples. The error bars represent standard deviation (SD) of the mean.

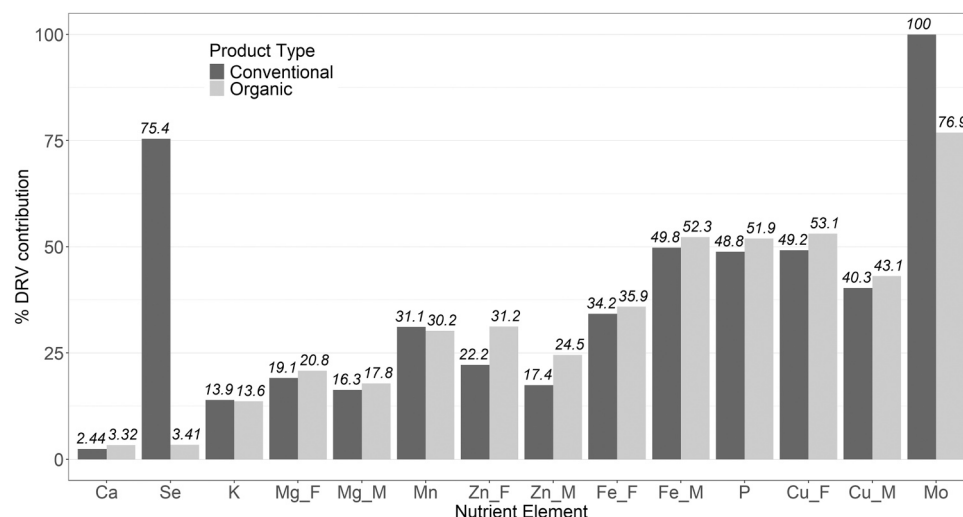


Fig. 4. : The contribution of analysed nutrient elements to the daily dietary reference value (DRV) for an adult based on one serving size (80 g) of cooked lentils. Some nutrients are presented for adult males (M) and females (F) separately.

17.8 % for men, and conventional lentils contain 19.1 % of the recommended intake for women and 16.3 % of that of men. A serving size of organic and conventional lentils covers about 13.6 % and 13.9 % respectively of the DRV for K. For Se, on the other hand, a portion of organic lentils only contributes 3.41 % to the recommended daily intake, whereas conventional lentils contribute 75.4 % based on this study. Lastly, a serving size of organic and conventional lentils contains similar amounts of the DRV for Ca (3.32 and 2.44 %).

Significantly higher concentrations of Cu, Zn, Ca and Mg were found in organic lentils. In contrast, K, Se and Mo were found in significantly higher conventional lentils. There is no statistically significant difference in Fe, P or Mn concentrations between organic and conventional lentil samples, so different DRV contributions of those minerals cannot be attributed to the samples' product type.

It is important to mention that when it comes to major and trace mineral concentrations, 'more' is not necessarily better. There are tolerable upper intake levels that should not be exceeded to avoid health issues. EFSA, 2019 provides those upper intake levels (UL) that indicate the maximum chronic daily nutrient intake where risks of adverse health effects are still unlikely. None of the NE concentrations in a serving size of lentils in this study neared that UL, even if several servings were to be consumed. So, given that overconsuming any of the major or trace minerals dealt with in this study is not a risk, higher levels of minerals can be viewed as favourable.

It is also necessary to address the fact that the % contribution of a portion of lentils to the daily recommended nutrient intakes has different implications and significances depending on how prevalent lentils are as a staple food for different populations. In countries like India or Brazil, where per capita pulse consumption stood at 15.8 and 16.2 kg, respectively, in 2018–2020, the nutritional contributions of pulses like lentils will have a bigger impact on the overall diet and health of populations compared to countries like the United States or China, where average pulses consumption was at 4.9 kg/capita and 1.5 kg/capita in 2018–2020 (OCED, 2021).

Including lentils as a regular component in people's diets is a healthy choice to meet nutritional needs and prevent deficiencies. This study shows that just one serving size of cooked lentils contains around a quarter of the recommended daily intake for Fe (for women), Zn and Mn, and around half of the recommended daily intake for Fe (for men), Cu and P. Aside from that, lentils are a good source of protein and dietary fibre (Singh, 2017). These nutritional benefits are affordable, especially compared to foods high in Fe, Zn, and protein, such as animal-based proteins. Also, lentil products are cheaper than any other meat

products available in major retailers in the UK. For example, 1 kg of beef is at least £10 in a mid-range UK supermarket, whereas 1 kg of lentils costs around £2.50. This means that even if higher quantities of lentils are required to equate to the NE content of beef, lentils still end up being cheaper to the consumer. Lentils are already widely and regularly consumed in many parts of the world, but they are mostly a staple food in South and West Asia, Latin America and parts of Africa. Consumer preferences in the Global North might deter some from routinely incorporating lentils into their diet, although legume consumption is projected to increase in upcoming years due to an expanding market for plant-based proteins and growing sustainability concerns (Cusworth et al., 2021).

4. Conclusion

This study investigated the nutrient element content in hulled organic and conventional lentils. Furthermore, the study explored the effect of cooking on NEs, phytic acid levels and nutrient bioaccessibility in cooked lentils. It also established to what extent a serving of cooked lentils contributed to adults' daily recommended nutrient intake. NEs such as Ca, Mg, Zn, and Cu were found to be higher in organic lentils than the conventional ones, whereas the opposite was true for K, Mo and Se. Cooking reduced the concentration of K, P, Mg, Zn, Cu and Mo, whereas Fe and Mn exhibited an increase.

Mean phytic acid concentrations in cooked lentils ranged from 0.272 to 0.471 g 100 g⁻¹, and there was no significant difference in phytic acid concentrations between organic and conventional lentils. Cooked lentils retained a bioaccessibility of >80 % for the major nutrient elements Ca, Mg and K and a bioaccessibility of >50 % for the trace nutrient elements Fe, Cu, Mn and Zn. Bioaccessibility levels did not differ significantly between organic and conventional lentils. Our findings showed that one serving (80 g) of lentil could substantially contribute to the DRV for adults as follows: >75 % Mo, ~50 % of P, >40 % of Cu, >30 % of Fe, ~30 % of Mn, >15 % of Zn and Mg as well as ~14 % of K.

One limitation of this study is the limited sample size due to the general lack of availability of organic lentils in UK retailers. A bigger sample size could have increased the statistical robustness and representativeness of the study results. Future studies could include a comparison of organic and conventional cultivation practices under the same soil and climatic conditions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jfca.2024.106372](https://doi.org/10.1016/j.jfca.2024.106372).

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