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Mineral elements and related antinutrients, in whole and hulled hemp (*Cannabis sativa* L.) seeds

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ABSTRACT

Hemp (*Cannabis sativa* L.) seeds have been traditionally used as food and their consumption is increasing nowadays. They have a remarkable nutritional value, but scarce information is available about mineral elements and their bioavailability. The objectives of this study were to determine the mineral element and phytate contents of eight different varieties of whole hemp seeds and commercial hulled hemp seeds. Phosphorus was the most abundant mineral element with higher content in hulled seeds (1.1 g/100 g) than whole seeds, as well as potassium, magnesium, and zinc. Calcium, manganese, and copper contents were higher in whole seeds. Iron content (8 mg/100 g) was similar in whole and hulled seeds; while sodium was below 5 mg/100 g. Phytate was abundant in hemp (especially hulled) seeds (4 g/100 g), and could compromise iron and zinc absorption (phytates/Fe and phytates/Zn molar ratios above 20 and 15, respectively), as well as involve poor phosphorous absorption. These seeds represent a valuable contribution to manganese (>5 mg/100 g) and copper (>1.4 mg/100 g) intake, approaching 100% and 50%, respectively, of daily NRVs, with a serving size of 30 g. Hemp seeds are an apparently excellent source of minerals, although phytates content should be taken into account to properly interpret nutritional claims.

1. Introduction

Hemp (*Cannabis sativa* L.) is a widespread herbaceous plant native from Central Asia that belongs to the Cannabaceae family (Small, 2015). This plant could be considered as one of the first crops because it is thought that it was cultivated in China 8500 years ago (Schultes and Hofmann, 1980). Hemp has been traditionally cultivated with industrial, medicinal and food purposes. Its fibre was obtained from stems, specifically from the phloem, and it has been widely used, especially in the shipbuilding industry. Female flowers were useful because of their pharmacological properties, and seeds were used mainly as food (Small, 2015).

Hemp phytochemistry is quite complex and cannabinoids are its most distinctive compounds (Flores-Sánchez and Verpoorte, 2008). They are produced almost entirely in glandular trichomes, which are commonly located in the bracts of female flowers. Nevertheless, other parts of the plant, such as seeds, could contain a small quantity of cannabinoids (Small and Naraine, 2016), which is subject of regulation in different countries of the world. The most important one is Δ^9-tetrahydrocannabinol (Δ^9-THC), due to its psychoactive properties, and only varieties with low Δ^9-THC content can be cultivated (European Parliament and Council of the European Union, 2013; Agriculture Improvement Act, 2018).

Nowadays, the consumption of hemp seeds and derivative products is increasing. Whole hemp seeds are used as a raw material, but could be consumed as food as well, and hulled hemp seeds are commonly marketed as functional food (Alonso-Esteban et al. 2020). Hemp seeds contain large amounts of fibre, fat, and protein, at 27–40, 25–35, and 18–28 g/100 g, respectively. They contain significant amounts of linoleic acid, which represents 50% of total fatty acids, 16–19% of α-linolenic acid, 12–17% of oleic acid, and other minor fatty acids, being remarkable the presence of γ-linolenic acid (Alonso-Esteban et al., 2020, 2022; Callaway, 2004; House et al., 2010; Vonapartis et al., 2015). In addition to nutrients, hemp seeds contain bioactive compounds which confer a biological potential, such as antioxidant, antimicrobial and cytotoxic properties (Alonso-Esteban et al., 2022). Chen et al. (2012, 2013) attribute some of these bioactive properties to phenolic compounds, the main being lignanamides, such as cannabisins (Irakli et al. 2019).

The studies about mineral elements in hemp seeds are scarce, but

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Callaway (2004) and Mihoc et al. (2012) reported an interesting content of some of them, such as phosphorus, potassium, magnesium, calcium, iron, zinc, manganese, and copper, which are essential dietary elements for mammals and are involved in many physiological processes (EFSA, 2017). Mineral element bioavailability could be compromised by different anti-nutrient components in foods, such as phytates and oxalates, which are present in hemp seeds (Romero-Aguilera et al., 2017; Alonso-Esteban et al., 2022). Phytic acid is the main storage form of phosphorus in seeds and it can form insoluble complexes with some cations, such as calcium, magnesium, iron, and zinc (Romero-Aguilera et al., 2017). The chelating capacity of phytates is especially damaging in the case of iron and zinc, and their deficiencies constitute a significant public health problem (Dahdouh et al., 2019). The absorption of calcium of plant origin is impaired by the presence of oxalic acid, which promotes the formation of insoluble calcium oxalates (Guil et al., 1996).

The objectives of this study were to determine the mineral element content of different varieties of whole hemp seeds and commercial hulled hemp seeds, as well as the phytate content, which is closely related to the mineral element bioaccessibility.

2. Material and methods

2.1. Plant materials

Eight different varieties of whole hemp seeds were supplied by "Cáñamo Bajo Aragón" from their own crops, located in the province of Teruel (Spain). The varieties were 'Bialobrzeskie', 'Carmagnola', 'Fedora 17', 'Felina 32', 'KC Dora', 'Kompolti', 'Santhica 27', and 'Tiborszallasi' all of them included in the Plant Variety Database of the European Commission (2021). Commercial hulled hemp seeds from four different brands (two lots of each brand) were bought in different Spanish markets. Samples were cleaned if necessary, and then stored in a desiccator. Before analysis, the seeds were reduced to fine powder (20 mesh) with a coffee grinder.

2.2. Mineral element extraction

The mineral elements were extracted from ashes, which were previously obtained by the incineration of 0.5 g of plant material at 550 ºC according to AOAC m923.03 (Horwitz, 2000), by the addition of 2 ml of HNO_3 35% (*v*/*v*) and 2 ml of HCl 19% (*v*/*v*) (Merck, Germany). The resulting liquid extracts were filtered through ash-free filter paper for quantitative analysis (Albet DP 145, Germany) and diluted to 25 ml with distilled water (Torija-Isasa, 1980; García-Herrera et al., 2014). These extracts were used for all mineral element analysis.

2.3. Atomic absorption spectroscopy for mineral element analysis

Sodium, potassium, magnesium, calcium, manganese, iron, copper, and zinc were analysed by flame atomic absorption spectroscopy (AAS), according to Torija-Isasa (1980) and García-Herrera et al. (2014), with several modifications. For sodium and potassium analysis, a 1:10 dilution was carried out by adding 2 ml of CsCl 1% (*w*/*v*) and 7 ml of distilled water to 1 ml of extract. A 1:10 dilution was done for magnesium and calcium analysis too, as 2 ml of $LaCl_3$ ~9% (*p*/*v*) and 7 ml of distilled water were added to 1 ml of extract. The analyses of manganese, iron, copper, and zinc were carried out directly from the extracts. The analyses were performed with an AAnalyst 200 AA spectrometer (PerkinElmer, USA), using an oxidising air/acetylene flame. The calibration curves and the measuring conditions are summarised in Table 1. The analyses were carried out in triplicate and the results were expressed as mg/100 g (fw).

2.4. Phosphorus analysis

Phosphorus was analysed by UV/visible spectroscopy using the molybdenum blue method (de la Fuente et al., 2003; de la Fuente and Juárez, 1995). Due to the high content of phosphorus, the extracts were diluted 1:50 with distilled water. In 96-well plates, 150 µl of the diluted extract, 60 µl of $HClO_4$ 65% (*v*/*v*), 60 µl of 2,4-diaminophenol 50.7 mM with $Na_2S_2O_5$ 1.1 M, and 30 µl of $Mo_7O_{24}(NH_4)_6$ 67.2 mM were added. The absorbance was measured at 750 nm and the results were expressed as mg/100 g (fw). The calibration curve was built with KH_2PO_4 from 1.2 to 4 µg P/ml. The analyses were carried out in triplicate. The methodology was validated for this kind of samples, according to AOAC guidelines (Horwitz, 2002). The analyses were carried out in triplicate and the results were expressed as mg/100 g (fw).

Table 1
Analytical conditions and calibration curves for Na, K, Mg, Ca, Mn, Fe, Cu, and Zn analysis by Atomic Absorption Spectrometry.

Mineral element	Wavelength (nm)	Slit width (mm)	Concentration range (ppm)	Calibration curve	r^2 (%)
Na	589.5	1.8/0.6	0.1–0.5	y = 1.6673x + 0.0014	99.61
K	766.5	2.7/ 0.45	2–50	y = 0.0162x + 0.0010	99.95
Mg	285.2	2.7/1.5	2–50	y = 0.0273x - 0.0174	99.86
Ca	422.7	2.7/0.6	2–10	y = 0.0091x + 0.0034	99.68
Mn	279.5	1.8/0.6	0.1–0.5	y = 0.0388x - 0.0007	98.58
Fe	248.3	1.8/ 1.35	0.2–5	y = 0.0144x - 0.0011	99.91
Cu	324.8	2.7/0.8	0.1–1	y = 0.0437x + 0.0013	99.85
Zn	213.9	2.7/1.8	0.1–5	y = 0.1104x + 0.0159	99.50

2.5. Phytate analysis

Phytates were determined by an indirect iron (III) complexometry with sulfosalicylic acid like an indicator of the titration endpoint, according to Romero-Aguilera et al. (2017). The extraction was carried out under magnetic agitation for 90 min from 2 g of sample with 40 ml of HCl 0.4 M, which contained Na_2SO_4 5% (*p*/*v*). The resulting suspension was centrifuged at 1890*g* during 8 min, and the supernatant was filtered and made up to 50 ml with the extraction solution. After that, 20 ml of the extraction solution, 20 ml of $FeCl_3$ 0.02 M (in HCl 0.16 M), and 20 ml of sulfosalicylic acid 20% (*p*/*v*) were added to 25 ml of the extract, and the mixture was made up to 100 ml with distilled water. Blank was prepared in the same way, but 25 ml of the extraction solution were used instead of the extract. The solutions were heated for 15 min in a boiling water bath and then they were cooled in an ice bath. One aliquot of 20 ml was pipetted together with 200 ml of distilled water and 1.5 g of glycine were added. This solution was heated at 70 °C and titrated with EDTA 10 mM while still warm until the solution turned yellow. All the volumes obtained from the titration of the samples and standards were subtracted from the blank value, obtaining the amount of EDTA equivalent to the complexing activity of.

the phytates in the sample. For quantification purposes, a calibration curve was built using a commercial phytic acid standard. The analyses were performed in triplicate and the results were expressed as g of phytic acid equivalents (PAE)/100 g (fw).

2.6. Statistical analysis

The experiments were carried out in triplicate and the results were expressed as mean ± standard deviation (SD). The SPSS Statistics software (IBM SPSS Statistics for Mac, Version 21.0. Armonk, NY, IBM Corp.) was used to analyse differences among samples of unhusked and husked hemp seeds separately by applying the one-way analysis of variance (ANOVA). The homogeneity of variance was tested by the Levene's test. All dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

3. Results and discussion

3.1. Mineral element content

The results of mineral element analysis of both whole and hulled hemp seeds are shown in Table 2. Regarding macroelements, sodium was the least abundant, with an average value of 2.75 and 1.41 mg/100 g in whole and hulled hemp seeds, respectively. All the samples contained less than 5 mg/100 g. On the other hand, phosphorus was most abundant one, with an average value of 871.2 and 1099.5 mg/100 g in whole and hulled hemp seeds, respectively. Phosphorus content was lower in whole hemp seeds, being the maximum value 928.1 mg/100 g, which corresponded to 'Tiborszallasi' variety. Potassium content was also high in hemp seeds, especially in hulled seeds, which contained on average 919.5 mg/100 g, while the average content in whole hemp seeds was 569.6 mg/100 g. Potassium ranges were wide, from 311.5 to 713.6 mg/100 g in whole hemp seeds and from 778.8 to 1067.7 mg/100 g in hulled hemp seeds. Magnesium content was also higher in hulled hemp seeds, in which ranged from 482.3 to 934.2 mg/100 g, with an average value of 696.9 mg/100 g. However, the range in whole hemp seeds was very narrow and there were not statistically significant differences between the eight analysed varieties. The average magnesium content in whole hemp seeds was 383.4 mg/100 g. Calcium content, on the contrary, was higher in whole seeds, with an average value of 175.6 mg/100 g, being half in hulled hemp seeds (81.55 mg/100 g as average).

In respect to microelements, most of them were more abundant in whole seeds. Iron was the major microelement in whole hemp seeds, with an average content of 8.04 mg/100 g, closely followed by zinc and manganese, whose average contents were 7.94 and 7.48 mg/100 g, respectively. Zinc was the most abundant microelement in hulled hemp seeds, with an average content of 9.81 mg/100 g. Iron content in hulled hemp seeds were slightly lower than in whole seeds, 7.83 mg/100 g, while manganese content was much lower, 5.18 mg/100 g. Copper content was lower than the other microelements, on average, 2.30 and 1.48 mg/100 g in whole and hulled hemp seeds, respectively.

Among the analysed varieties, 'Carmagnola' variety stood out because it had the maximum values of sodium, calcium, manganese, iron, and zinc, and the minimum values of magnesium and phosphorus, and the 'Kompolti' variety stood out because it had the maximum values of potassium and copper, and the minimum values of calcium, manganese, and iron. Regarding hulled hemp seeds, there were a wide variation among the different brands.

As it has been said previously, few studies on mineral elements in whole hemp seeds are available, so it is difficult to compare these results with previous studies. Callaway (2004) reported the contents of mineral elements of the 'Finola' variety, which was not analysed in this study, and Mihoc et al. (2012) studied five different Romanian varieties that were not analysed in this work either. The sodium content reported by Callaway (2004) was slightly higher, 12 mg/100 g, while all the varieties analysed in this work had sodium content lower than 5 mg/100 g (Table 2), but its content was much lower than the other macroelements in both cases. That study also reported higher potassium content (859 mg/100 g), closer to hulled hemp seeds than to whole seeds. Mihoc et al. (2012) obtained a wide range for this mineral element, from 569.3 to 1889.7 mg/100 g, on average. Two of those varieties had potassium

Table 2
Mineral elements content (mg/100 g) and Ca/P molar ratio in whole hemp seeds.

	Whole hemp seeds 'Bialobrzeskie'	'Carmagnola'	'Fedora 17'	'Felina 32'	'KC Dora'	'Kompolti'	'Santhica 27'	'Tiborszallasi'	Average ± SD
Na	2.96 ± 0.17^{c}	4.16 ± 0.08^{a}	2.78 ± 0.13^{c}	3.67 ± 0.07^{b}	2.99 ± 0.12^{c}	1.45 ± 0.21^{d}	1.30 ± 0.07^{d}	2.70 ± 0.26^{c}	2.75 ± 0.98
K	311.5 ± 11.1^{d}	$616.7 \pm 53.2^{a,b,c,d}$	$709.3 \pm 45.9^{a,b,c,d}$	551.9 ± 5.0^{b}	$656.4 \pm 18.6^{a,b}$	713.6 ± 13.9^{a}	$582.0 \pm 32.1^{a,b,c,d}$	415.1 ± 13.2^{c}	569.6 ± 141.9
Mg	381.8 ± 8.0	394.9 ± 38.1	410.9 ± 23.3	367.1 ± 21.2	365.9 ± 12.2	375.5 ± 24.2	360.8 ± 35.5	410.6 ± 15.2	383.4 ± 19.9
Ca	$205.1 \pm 2.9^{a,b}$	$211.9 \pm 10.4^{a,d}$	$189.0 \pm 11.9^{a,b,c}$	$181.7 \pm 7.2^{b,c,d}$	$161.3 \pm 2.2^{d,e}$	137.3 ± 6.6^{e}	146.3 ± 13.7^{e}	172.0 ± 9.0^{c}	175.6 ± 26.6
P	835.4 ± 23.7^{b}	810.3 ± 47.8^{c}	$876.4 \pm 25.1^{a,b,c}$	$870.4 \pm 21.6^{a,b,c}$	$874.5 \pm 43.1^{a,b,c}$	$880.5 \pm 16.0^{a,b,c}$	$893.8 \pm 22.9^{a,b}$	928.1 ± 5.3^{a}	871.2 ± 35.6
Mn	$8.81 \pm 0.26^{a,c}$	$9.71 \pm 0.68^{a,b}$	$6.47 \pm 0.39^{b,d}$	$7.41 \pm 0.06^{b,c}$	$7.34 \pm 0.09^{b,c}$	$5.55 \pm 0.38^{b,d}$	6.07 ± 0.27^{b}	$8.46 \pm 0.14^{a,d}$	7.48 ± 1.44
Fe	10.11 ± 0.43^{a}	10.65 ± 0.62^{a}	$6.45 \pm 0.14^{b,c}$	7.72 ± 0.66^{b}	$6.39 \pm 0.39^{b,c}$	6.05 ± 0.55^{c}	$7.26 \pm 0.58^{b,c}$	9.70 ± 0.16^{a}	8.04 ± 1.84
Cu	1.77 ± 0.07^{c}	2.20 ± 0.09^{b}	2.76 ± 0.23^{a}	2.67 ± 0.16^{a}	1.63 ± 0.11^{c}	2.82 ± 0.01^{a}	2.79 ± 0.28^{a}	1.76 ± 0.07^{c}	2.30 ± 0.53
Zn	$8.81 \pm 0.26^{a,c}$	$9.71 \pm 0.68^{a,b}$	$6.69 \pm 0.26^{b,d}$	7.02 ± 0.21^{b}	$7.11 \pm 0.08^{b,c}$	$7.84 \pm 0.36^{a,b}$	$7.88 \pm 0.34^{a,b}$	$8.46 \pm 0.14^{a,d}$	7.94 ± 1.02
Ca/P	0.19	0.20	0.17	0.16	0.14	0.12	0.13	0.14	0.16 ± 0.03
	Hulled hemp seeds Brand 1 lot 1	Brand 1 lot 2	Brand 2 lot 1	Brand 2 lot 2	Brand 3 lot 1	Brand 3 lot 2	Brand 4 lot 1	Brand 4 lot 2	Average ± SD
Na	$1.33 \pm 0.11^{c,d}$	$1.43 \pm 0.23^{b,c}$	$1.33 \pm 0.07^{c,d}$	0.97 ± 0.15^{d}	$1.13 \pm 0.07^{c,d}$	$1.79 \pm 0.05^{a,b}$	$1.43 \pm 0.03^{b,c}$	1.86 ± 0.22^{a}	1.41 ± 0.30
K	$792.5 \pm 46.4^{d,e}$	1067.7 ± 22.4^{a}	$990.4 \pm 42.3^{a,b}$	$899.9 \pm 51.6^{b,c,d}$	$991.6 \pm 40.5^{a,b}$	$866.9 \pm 42.6^{c,d,e}$	$968.4 \pm 32.0^{a,b,c}$	778.8 ± 48.0^{e}	919.5 ± 102.6
Mg	$518.8 \pm 11.6^{c,d}$	934.2 ± 19.8^{a}	786.5 ± 39.1^{b}	578.3 ± 21.7^{c}	$868.4 \pm 75.5^{a,b}$	482.3 ± 12.1^{d}	821.0 ± 23.0^{b}	585.4 ± 9.0^{c}	696.9 ± 174.7
Ca	$87.86 \pm 7.45^{a,b}$	91.99 ± 2.02^{a}	$76.73 \pm 4.10^{b,c}$	$74.06 \pm 5.81^{b,c}$	94.00 ± 6.05^{a}	65.01 ± 2.47^{c}	$81.71 \pm 4.21^{a,b}$	$81.00 \pm 7.07^{a,b}$	81.55 ± 9.69
P	$1156.1 \pm 87.5^{a,b}$	$1145.3 \pm 26.3^{a,b}$	$1122.8 \pm 39.7^{a,b}$	1003.9 ± 87.6^{b}	$1114.0 \pm 67.9^{a,b}$	1201.7 ± 96.9^{a}	$1042.5 \pm 35.0^{a,b}$	1009.3 ± 26.0^{b}	1099.5 ± 72.7
Mn	9.14 ± 0.22^{a}	$5.48 \pm 0.07^{b,c}$	5.15 ± 0.10^{c}	3.53 ± 0.11^{f}	5.90 ± 0.15^{b}	$3.74 \pm 0.22^{e,f}$	4.49 ± 0.11^{d}	4.00 ± 0.21^{e}	5.18 ± 1.81
Fe	10.89 ± 0.27^{a}	9.60 ± 0.58^{a}	$6.57 \pm 0.41^{b,c}$	5.10 ± 0.45^{c}	9.78 ± 0.71^{a}	5.87 ± 0.47^{c}	$6.83 \pm 0.39^{b,c}$	7.33 ± 0.54^{b}	7.83 ± 1.97
Cu	$1.89 \pm 0.18^{a,b}$	$1.81 \pm 0.09^{a,c}$	$1.30 \pm 0.01^{a,b}$	1.62 ± 0.04^{a}	$1.45 \pm 0.05^{a,b}$	$1.45 \pm 0.13^{a,b}$	$1.31 \pm 0.04^{b,c}$	1.02 ± 0.08^{b}	1.48 ± 0.29
Zn	$9.14 \pm 0.22^{a,b}$	9.45 ± 0.12^{a}	$11.20 \pm 0.43^{a,b}$	8.18 ± 0.11^{b}	$12.74 \pm 0.96^{a,b}$	$8.78 \pm 0.16^{a,b}$	$10.27 \pm 0.34^{a,b}$	$8.74 \pm 0.01^{a,b}$	9.81 ± 1.5
Ca/P	0.06	0.06	0.05	0.06	0.07	0.04	0.06	0.06	0.06 ± 0.01

SD: standard deviation; For each line, different letters indicate statistically significant differences between samples ($p < 0.05$)

contents within the range of this study (311.5–713.6 mg/100 g, Table 2), but the other three had much higher content. Calcium values reported by Mihoc et al. (2012) were close to those of this work (137.3–211.9 mg/100 g, Table 2), except one of the varieties, which had a content three times higher than the maximum value. The magnesium content range reported by Mihoc et al. (2012) included all the values obtained in this work, and Callaway (2004) indicated a higher value, 483 mg/100 g, but close to the maximum obtained in this work (410.9 mg/100 g, Table 2). According to Callaway (2004), phosphorus was the most abundant mineral element, but the value was higher, 1160 mg/100 g, closer to phosphorus content in hulled hemp seeds, which were higher than 1000 mg/100 g in every sample (Table 2). Iron content reported by Callaway (2004) was slightly higher than those of this work, 14 mg/100 g, but the values reported by Mihoc et al. (2012) were higher than 160 mg/100 g, 20 times the average value in whole hemp seeds (8.04 mg/100 g, Table 2). Manganese, copper, and zinc content data were consistent to those reported by Callaway (2004) and Mihoc et al. (2012).

The only literature source of mineral element content data in hulled hemp seeds is the FoodData Central (USDA, 2019). Sodium, magnesium, calcium, iron, manganese, copper, and zinc values included in the database were close to those of this work, while potassium and phosphorus values, 1200 and 1650 mg/100 g, respectively, were higher than the maximum values obtained in this work (1067.7 mg/100 g of potassium and 1156.1 mg/100 g of phosphorus, Table 2).

The mineral element content in hemp seeds is nutritionally interesting. Considering the nutrient reference values (NRVs) of mineral elements established by the European Union (European Parliament and Council of the European Union, 2011), Fig. 1a represents the percentage that 100 g of whole and hulled hemp seeds contribute to those NRVs, while Fig. 1b represents the contribution percentages of 30 g, which is the serving size. As it can be seen in Fig. 1a, and according to European Union regulation on nutritional claims made in foods, both whole and hulled hemp seeds could be considered high in magnesium, phosphorus, manganese, copper, and zinc because their contributions were higher than 30% of NRVs (European Parliament and Council of the European Union, 2011). The contribution of whole hemp seeds to potassium NRV was slightly lower than 30%, so they could be considered as a source of potassium (more than 15%), while hulled hemp seeds could be considered, high in potassium. Whole, but not hulled hemp seeds, could be considered a source of calcium. Hemp seeds could be considered a sodium-free food, as whole and hulled hemp seeds contained less than 5 mg/100 g (European Parliament and Council of the European Union, 2006).

Notwithstanding the foregoing, the real contribution of hemp seeds to NRVs is lower because their serving size is approximately 30 g. As it can be seen in Fig. 1b, whole hemp seeds could be considered a source of potassium and calcium, but the contribution to NRVs of 30 g would be only 9% and 7%, respectively. Hulled hemp seeds could be considered high in potassium and the contribution is lower than 14%. However, the contribution to manganese and copper NRVs of a serving size were higher than 40% in both whole and hulled hemp seeds. The contribution to manganese NRV stood out because the consumption of 30 g of whole hemp seeds would exceed an intake of 100% NRV.

In this view, the interpretation of the claims can be confusing for the consumer due to the fact that they are linked to the presence of these compounds in 100 g of seeds without taking into account the serving size.

3.2. Phytate content and mineral element bioavailability

Phytate content, expressed as g PAE/100 g, is shown in Table 3. The average phytate content in whole hemp seeds was 2.80 g/100 g, being 'Carmagnola' and 'Tiborszallasi' the varieties with the lowest phytate content, 2.66 mg/100 g, and 'Fedora 17' the variety with the highest, 3.08 g/100 g. The phytate content was higher in hulled hemp seeds, 4.00 g/100 g on average. Mattila et al. (2018) reported a phytic acid content of 3.5 g/100 g, which was higher than the phytate contents of this work. Their result was expressed in dry matter, so it would be lower if expressed in fresh weight and closer to the maximum valued obtained in this work, which was higher than 3 g/100 g. Schultz et al. (2020) analysed different varieties and their average value was 2.67 g/100 g, very close to the average value obtained in this work (2.80 g/100 g, Table 3). It was not possible to find previous studies about phytate content in hulled hemp seeds.

Based on phytate and phosphorus content, phosphorus from phytates (28.2% of phytic acid molecular mass) and the proportion of phosphorus from phytates with respect to total phosphorus content were calculated (Table 3). The phosphorus from phytate calculation is indicative, as different phytates have a different number of phosphate groups and they are expressed in this work as PAE. So, the proportion of phosphorus from phytates with respect to total phosphorus content is also indicative, but it provides information about phosphorus bioavailability. In whole hemp seeds, the proportion of phosphorus from phytates with respect to total phosphorus content was higher than 90%, and it was even higher in hulled hemp seeds. These results indicate that almost all of the phosphorus is found as phytates in hemp seeds, so its bioavailability is supposed to be low.

From phytate, iron, and zinc contents, the phytates/zinc and phytates/zinc molar ratios were calculated, according to Dahdouh et al. (2019), and included in Table 3. It is considered that iron bioavailability is affected by a phytates/iron molar ratio above 1 (Dahdouh et al., 2019), and whole and hulled hemp seeds showed values higher than 20 and 30, respectively. In the case of zinc, a phytates/zinc molar ratio above 15 is considered to compromise its bioavailability (Dahdouh et al., 2019). All the samples analysed had phytates/zinc molar ratios much higher than 15, even above 60.

The fractional absorption of phosphorus is higher than that of calcium and it is recommended a calcium/phosphorus molar ratio should range between 1.4 and 1.9 for a suitable equilibrium and absorption of

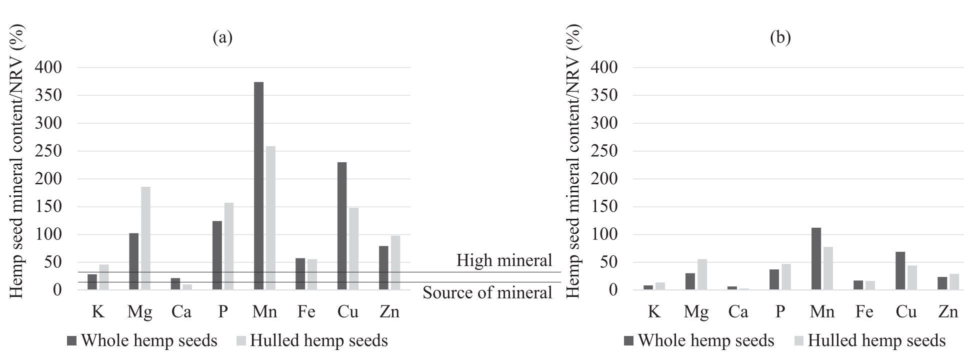

Fig. 1. Contribution (%) to the nutrient reference values (NRV) of 100 g (a) and 30 g (habitual serving size) (b) of whole and hulled hemp seeds. In (a), lines represent the minimum level that allows nutritional claims in food labelling, according to EU Regulations No 1924/2006, and No 1169/2011.

Table 3
Phytate content (g equivalents of phytic acid (PAE)/100 g), P from phytates (g/100 g), proportion of P from phytates with respect to total P content (%), and phytates/Fe and phytates/Zn molar ratios.

	Whole hemp seeds 'Bialobrzeskie'	'Carmagnola'	'Fedora 17'	'Felina 32'	'KC Dora'	'Kompolti'	'Santhica 27'	'Tiborszallasi'	Average ± SD
Phytates	$2.77 \pm 0.02^{c,d}$	2.66 ± 0.01^{d}	3.08 ± 0.09^{a}	$2.90 \pm 0.05^{b,c}$	$2.98 \pm 0.01^{a,b}$	2.68 ± 0.09^{d}	2.67 ± 0.11^{d}	2.66 ± 0.01^{d}	2.80 ± 0.17
$P_{phytates}$	0.779	0.750	0.869	0.816	0.839	0.754	0.751	0.749	0.788 ± 0.047
$P_{phytates}/P_{total}$	93.22	92.57	99.10	93.72	95.99	85.58	84.05	80.69	90.61 ± 6.42
Phytates/Fe	23.14	21.16	33.80	38.34	39.08	37.44	31.11	23.20	30.91 ± 7.44
Phytates/Zn	31.09	27.16	43.48	40.32	44.13	33.82	33.55	31.13	35.59 ± 6.28
	Hulled hemp seeds Brand 1 lot 1	Brand 1 lot 2	Brand 2 lot 1	Brand 2 lot 2	Brand 3 lot 1	Brand 3 lot 2	Brand 4 lot 1	Brand 4 lot 2	Average ± SD
Phytates	$4.03 \pm 0.03^{b,c,d}$	$4.12 \pm 0.02^{b,c}$	4.18 ± 0.09^{b}	3.73 ± 0.09^{d}	4.19 ± 0.12^{b}	4.62 ± 0.04^{a}	3.26 ± 0.05^{e}	$3.84 \pm 0.26^{c,d}$	4.00 ± 0.40
$P_{phytates}$	1.151	1.178	1.195	1.065	1.197	1.319	0.931	1.097	1.125 ± 0.112
$P_{phytates}/P_{total}$	99.57	102.81	106.39	106.11	107.41	109.79	89.28	108.69	102.30 ± 6.61
Phytates/Fe	31.30	36.33	53.86	54.42	36.22	66.53	40.38	44.35	45.42 ± 11.90
Phytates/Zn	43.68	43.22	36.98	45.14	32.55	52.13	31.43	43.50	41.08 ± 6.95

SD: standard deviation; For phytates lines, different letters indicate statistically significant differences between samples ($p < 0.05$)

both elements (EFSA, 2017). Considering the calcium and phosphorus contents shown in Table 2, the calcium/phosphorus molar ratio ranged from 0.12 to 0.20 in whole hemp seeds and from 0.04 to 0.07 in hulled hemp seeds. These values were very low, especially in hulled seeds, due to their higher phosphorus content and their lower calcium content. All values were much lower than the recommendation, so the calcium absorption could be compromised because of the high content of phosphorus. However, it has been previously said that phosphorus contained in hemp seeds is difficult to assimilate because it is in the phytate form, so the calcium/phosphorus molar ratio would not represent a real competition in terms of absorption.

Another antinutritional compound that could reduce the calcium bioaccessibility in plant foods is oxalic acid. It is said that an oxalic acid/calcium ratio higher than 2.25 could compromise calcium absorption (Mitjavila, 1990). Considering the calcium contents shown in Table 2 and the oxalic acid content previously reported by Alonso-Esteban et al. (2022), the oxalic acid/calcium ratio was lower than 2.25 in all samples, with average values of 0.56 and 1.27 in whole and hulled hemp seeds, respectively. So, the presence of oxalic acid in hemp seeds could not compromise calcium bioaccessibility. Oxalate/calcium molar ratio would be even lower due to the higher molecular weight of oxalates, so there would be no risk according to Israr et al. (2013) and Udomkun et al. (2019), who indicate that an oxalate/calcium molar ratio higher than 2 could be hazardous.

The impeachment of phosphorus, iron, zinc and calcium absorption due to their chemical forms and the presence of antinutrients, reveals that using just the numerical contents of nutrients to make nutritional or health claims in foods, is not a suitable tool for a proper information to consumers, since their contents may not be totally available for the human body.

The bioaccessibility of other mineral elements would not be compromised by phytates or oxalates. However, future studies concerning mineral element bioaccessibility and bioavailability in whole and hulled hemp seeds should be carried out in order to evaluate if they are a good dietetic source of mineral elements.

4. Conclusions

Hemp seeds had a very interesting mineral element content. They contained a low quantity of sodium. Phosphorus, potassium, magnesium, and zinc content was higher in hulled seeds, while calcium, manganese, and copper content was higher in whole seeds. The iron content was almost the same in both whole and hulled hemp seeds. Phytate content was high in hemp seeds, especially in hulled seeds, and most of phosphorus contained in hemp seeds was in phytate form, so its absorption is supposed to be low. The low phosphorus bioaccessibility would not compromise the calcium absorption, even though the calcium/phosphorus molar ratio indicated that the calcium absorption could be limited. The high phytate content could compromise iron and zinc absorption, as phytates/iron and phytates/zinc molar ratios were much higher than the recommendations. Hemp seeds could be an apparently excellent source of mineral elements, but the presence of phytates may reduce their nutritional quality. The number of health claims relating to mineral elements which could be used for hemp seeds is enormously high. The interpretation of some nutritional claims that can be made on foods labelling, could be confusing for the consumer since they are linked to the presence of these compounds in 100 g of seeds, without taking into account aspects related to habitual serving sizes, and bioaccessibility. Future bioavailability assays would clarify the role of hemp seeds as a dietary source of mineral elements.

CRediT authorship contribution statement

José Ignacio Alonso-Esteban: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing. **Esperanza Torija-Isasa**: Conceptualization, Investigation, Project administration, Resources, Supervision, Writing, Review. **María de Cortes Sánchez-Mata**: Conceptualization, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

Declarations of interest

None.

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