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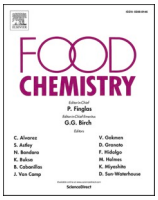

Chemical composition and biological activities of whole and dehulled hemp (*Cannabis sativa* L.) seeds

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ABSTRACT

This study aimed to determine a complete chemical composition of eight different varieties of whole hemp seeds and eight samples of commercial dehulled hemp seeds. We also evaluated the phenolic profiles and antioxidant, cytotoxic, and antimicrobial properties of hydromethanolic seed extracts. Whole hemp seeds contain much more fibre than dehulled hemp seeds, which contain more fat and protein. Sucrose and raffinose were the most abundant soluble sugars, and citric and oxalic acids were the most abundant organic acids. In the hydromethanolic hemp seed extracts, we detected the phenolic acids ferulic acid-hexoside and syringic acid. Whole hemp seed extracts exhibited better antioxidant activity than dehulled hemp seed extracts, especially in the TBARS assay. Cytotoxic activity against NCI-H460 cells was also observed. The dehulled hemp seed extracts displayed antibacterial activity, especially against *Bacillus cereus*, *Listeria monocytogenes*, and *Enterococcus faecalis*, and antifungal activity to a lesser extent.

1. Introduction

Hemp (*Cannabis sativa* L., Cannabaceae) is a widespread herbaceous plant native to Central Asia. It is thought that hemp was cultivated in China 8500 years ago, making it one of the first crops. The versatility of this plant has driven its cultivation. Indeed, hemp is utilised in industrial, medicinal and food sectors. For example, hemp fibre, obtained from the plant stems (i.e., phloem), is traditionally used in the shipbuilding industry and for other purposes. Moreover, female flowers exhibit pharmacological activity, and seeds are used mainly as food (Small, 2015).

From the nutrition point of view, hemp seeds contain large amounts of nutrients such as fibre (27–36 g/100 g), fat (25–35 g/100 g), and protein (21–28 g/100 g). Concerning fatty acids, hemp seeds contain significant amounts of linoleic acid, which accounts for more than half of total fatty acids. The remaining fatty acid content is comprised of α-linolenic acid (16–19%), oleic acid (12–17%), palmitic acid (5–8%), γ-linolenic acid (1–3%), and some other minor fatty acids (Callaway, 2004; House, Neufeld, & Leson, 2010; Vonapartis, Aubin, Seguin, Mustafa, & Charron, 2015; Alonso-Esteban et al., 2020).

Hemp is known for its psychotropic and medicinal effects and complex phytochemistry. A variety of compounds, including phenolic compounds (mainly flavonoids, stilbenoids, and lignanamides), terpenoids, alkaloids, and cannabinoids, which are the most distinctive compounds, are synthesised by its secondary metabolism (Flores-Sánchez & Verpoorte, 2008). Cannabinoids are almost exclusively produced in glandular trichomes, located in the bracts of future female flowers. Other plant parts and seeds also contain cannabinoids; however, the content is significantly lower than in the bracts (Small and Naraine, 2016). The best-known cannabinoid is Δ^9-tetrahydrocannabinol (Δ^9-THC), which has psychoactive activity and medicinal properties. In the European Union, approved cultivated hemp varieties contain a Δ^9-THC concentration of $<0.2\%$ (European Parliament & Council of the European Union, 2013).

Nowadays, there is an increased consumption of hemp seeds and derivative food products, especially among vegans. While whole hemp

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seeds can be consumed as food (e.g., roasted seed snacks), they are primarily used as raw material for development of other products such as hemp seed flour, oil, and protein. Hemp protein and hemp flour are by-products obtained from the oil extraction and can be used as an alternative to soy ingredients (Zając et al., 2019). On the other hand, dehulled hemp seeds are commonly marketed as a functional food due to their claimed functional properties (Alonso-Esteban et al., 2020).

The objective of this study was to determine the chemical composition and biological properties of different hemp seed varieties and commercial dehulled hemp seeds. Towards this goal, we analysed the proximate composition, soluble sugars, organic acids, and phenolic compounds and evaluated the antioxidant, cytotoxic, and antimicrobial properties of whole and dehulled hemp seeds.

2. Material and methods

2.1. Plant material

Eight varieties of whole hemp seeds were supplied by "Cáñamo Bajo Aragón" from their crops, located in the province of Teruel, Spain. 'Bialobrzeskie', 'Carmagnola', 'Fedora 17', 'Felina 32', 'KC Dora', 'Kompolti', 'Santhica 27', and 'Tiborszallasi' were the studied varieties, which are included in the Plant Variety Database of the European Commission (2021). Eight commercial samples of dehulled hemp seeds were purchased in different Spanish markets. The variety of these seeds was unknown, as well as their origin. Therefore, two lots of four different widely marketed brands were chosen in order to obtain a broad and representative range of this food product. The samples were cleaned when necessary and stored in a desiccator. Before analysis, all seed samples were reduced to a fine powder (0.8 mm) with a grinder.

2.2. Proximate composition and energy value determination

Moisture was determined by desiccation at 103 ± 2 °C to constant weight, according to AOAC 984.25 (Horwitz, 2000). Protein content was determined by the Kjeldahl method, according to AOAC 920.87 (Horwitz, 2000), and total nitrogen content was converted to protein using the nitrogen-protein conversion factor 5.3. Fat content was determined by Soxhlet extraction with petroleum ether as the solvent, according to AOAC 920.39 (Horwitz, 2000). Available carbohydrate content was determined by the anthrone colorimetric method, according to Osborne and Voogt (1986). Before performing the assay, samples are subjected to starch hydrolysis with perchloric acid 52% (v/v) monitored spectrophotometrically at 630 nm. The fibre content was determined as neutral detergent fibre according to Van Soest and Wine (1967). Total mineral content was determined as ash by incineration at 550 ± 15 °C, according to AOAC 923.03 (Horwitz, 2000). The results of these determinations were expressed as g/100 g of fresh weight (fw).

Energy values were calculated using the following conversion factors: 4 kcal/g for proteins and carbohydrates, 9 kcal/g for fat, and 2 kcal/g for fibre (European Parliament & Council of the European Union, 2011). The results of these calculations are expressed in kcal/100 g fw.

Hemp seeds were classified according to Regulation (EC) No 1924/2006 on nutrition and health claims made on foods (European Parliament & Council of the European Union, 2006).

2.3. Analysis of soluble sugars

Soluble sugars were analysed according to Pinela et al. (2016). Powdered samples were spiked with the internal standard melezitose (25 mg/mL) and extracted with ethanol/water 80:20 (v/v) at 80 °C. The analysis was performed in a high-performance liquid chromatography (HPLC) system coupled to a refraction index (RI) detector. Chromatographic separation was achieved with a Eurospher 100–5 NH_2 column (4.6 × 250 mm, 5 µm, Knauer). The mobile phase was acetonitrile/water 70:30 (v/v). Soluble sugars were identified by chromatographic comparisons with authentic standards and quantified based on the internal standard concentration. The results are expressed in g/100 g fw.

2.4. Analysis of organic acids

Organic acids were determined by ultrafast liquid chromatography (UFLC) coupled to photodiode array detector (PDA) after extraction with *meta*-phosphoric acid 4.5% (w/v), according to a procedure previously described by Pereira, Barros, Carvalho, and Ferreira (2013). Chromatographic separation was achieved on a SphereClone (Phenomenex) reverse phase C18 column (250 × 4.6 mm, 5 µm). Elution was performed with sulphuric acid 3.6 mM. Detection was carried out with a PDA, measuring the absorption at 215 and 245 nm (for ascorbic acid). Organic acids were quantified by comparing the peak areas with calibration curves obtained from commercial standards. The results are expressed in mg/100 g fw.

2.5. Analysis of phenolic compounds

Phenolic compounds were analysed in hemp seed hydromethanolic extracts obtained by solid–liquid extraction as previously described by Pereira, Barros, Carvalho, and Ferreira (2011). Dry extracts (~10 mg) were dissolved in 2 mL of methanol/water 20:80 (v/v) and filtered through 0.22 µm disposable filter disks. The analysis was carried out in a Dionex Ultimate 3000 UPLC system (Thermo Scientific, San Jose, CA, USA) equipped with a diode array detector (DAD, 280 and 370 nm) coupled to an electrospray ionisation mass spectrometer (ESI-MS) detector. The system and analytical procedures were previously described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). MS detection was performed in negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an ESI source. Phenolic compounds were identified based on their chromatographic behaviour, UV–Vis and mass spectra by comparing the collected data with standard compounds (when available) and data reported in the literature. A calibration curve based on the UV–Vis signal for each available phenolic standard was constructed for quantitative analysis. The results are expressed in mg/g of dry extract.

2.6. Determination of biological activities

Antioxidant, cytotoxic, and antimicrobial activities were evaluated in the same extracts used for the phenolic compound analyses. The antioxidant activity of the extracts (at concentrations from 0.156 to 40 mg/mL) was evaluated *in vitro* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity, reducing power, β-carotene bleaching inhibition, and thiobarbituric acid reactive substances (TBARS) formation inhibition assays, as described previously by Pinela et al. (2012). Results are presented as half maximal effective concentration (EC_{50}) values (µg/mL). The sulforhodamine B assay was performed to evaluate the cytotoxic activity of the hydromethanolic extracts (at concentrations from 0.125 to 4 mg/mL) against the MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma) human tumour cell lines, and PLP2 non-tumour primary cells, as previously described by Pires et al. (2018). The authors previously described the procedures and the origin of cell lines. Cytotoxic and hepatotoxic activity results are presented as half-maximal growth inhibition concentration (GI_{50}) values (µg/mL). For antimicrobial activity analysis, hydromethanolic extracts (at concentrations from 0.005 to 5 mg/mL) were tested against *Bacillus cereus* (food isolate), *Staphylococcus aureus* (ATCC 11632), *Listeria monocytogenes* (NCTC 7973), and *Enterococcus faecalis* (ATCC 19433) (Gram-positive bacteria), and *Escherichia coli* (ATCC 35218) and *Salmonella enterica* serovar Typhimurium (ATCC 13311) (Gram-negative bacteria) (Soković, Glamočlija, Marin, Brkić, & van Griensven, 2010). Additionally, antifungal activity was evaluated against *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC

12066), *Aspergillus niger* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 36839), and *Penicillium verrucosum* var. *cyclopium* (food isolate) (Soković & van Griensven, 2006). The authors previously described the procedures and the origin of the microorganisms. As positive controls for the antibacterial activity assay, streptomycin and ampicillin (Sigma-Aldrich) were used. Alternatively, ketoconazole and bifonazole were used as the positive controls for the antifungal assays. The results are presented as minimum inhibitory (MIC) and minimum bactericidal (MBC) or minimum fungicidal (MFC) concentrations (mg/mL).

2.7. Statistical analysis

The experiments were carried out in triplicate, and the results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA), using the SPSS Statistics software (IBM SPSS Statistics for Mac, Version 21.0. Armonk, NY, IBM Corp.), was applied to detect statistically significant differences (p-value < 0.05) between whole and dehulled hemp seed samples. The homogeneity of variance was assessed using 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. Proximate composition

Proximate composition results of whole and dehulled hemp seeds are summarised in Tables 1 and 2, respectively. The moisture in hemp seeds was low, ranging from 4.53 to 7.06 g/100 g in whole and 4.50 to 6.56 g/100 g in dehulled seeds. In general, the whole hemp seeds results are consistent with previous studies (Callaway, 2004; House, Neufeld, & Leson, 2010; Vonapartis, Aubin, Seguin, Mustafa, & Charron, 2015). Vonapartis et al. (2015) reported a lower moisture content value of 1.13 g/100 g in the 'Alyssa' variety, which was not evaluated in this study. Another study by Zając et al. (2019) reported a moisture content of 6.8 g/100 g for the 'Bialobrzeskie' variety, which is slightly higher than our result of 4.53 g/100 g. Environmental features could explain these differences, but storage conditions too, particularly temperature. The moisture of dehulled hemp seeds seems consistent with previous works (Callaway & Pate, 2009; House et al., 2010; USDA, 2019; Zając et al., 2019), but there are slight differences in other nutrients.

The neutral detergent fibre was the most abundant fraction of the whole hemp seeds. The fibre content was greater than 30 g/100 g in all eight varieties analysed, with values higher than 40 g/100 g in the 'Kompolti' and 'Tiborszallasi' varieties. The fibre content is difficult to compare with literature because the analytical method influences the fibre fractions determined. For example, Callaway (2004) reported a fibre content of 27.6 g/100 g in 'Finola'. While this value is out of the fibre content range reported herein, the employed methodology is unknown, and we did not analyse this variety. Vonapartis et al. (2015) determined neutral detergent fibre, as we did, and their results are similar to those here presented. In their study, all the analysed varieties contained more than 30 g/100 g, but none were greater than 40 g/100 g. It should be pointed out that Vonapartis et al. (2015) analysed the 'Finola' variety and reported a value that was over 30 g/100 g, which is greater than previously reported by Callaway (2004). Vonapartis et al. (2015) also reported acid detergent fibre values lower than those for neutral detergent fibre. House et al. (2010) presented results consistent with Vonapartis et al. (2015) and herein. Besides, Vonapartis et al. (2015) reported that cellulose, hemicellulose, and lignin (i.e., components of insoluble fibre) contents were around 15, 7.5 and 10.5 g/100 g, respectively, which would make the insoluble fibre content greater than 30 g/100 g. Lastly, Zając et al. (2019) reported a total fibre content of 42.83 g/100 g, a result greater than our maximum result. House et al. (2010) determined neutral detergent fibre, and their average value was 7.8 g/100 g. However, this value was overestimated because one sample had a high neutral detergent fibre content, 18.1 g/100 g, but the rest of the samples displayed lower values, some of which were within our range. USDA (2019) reported a fibre content of 4.0 g/100 g, close to the average value of the present study, 4.57 g/100 g. According to European regulations (European Parliament & Council of the European Union, 2006), food can use the nutrition claim "source of fibre" if it contains at least 3 g/100 g and can claim "high fibre" if it contains 6 g/100 g or more. In this sense, whole hemp seeds could be labelled as "high fibre content", and dehulled seeds as "source of fibre".

Fat content ranged from 29.1 to 32.66 g/100 g in whole hemp seeds,

Table 1
Proximate composition, energy value and its distribution by nutrient, soluble sugars and organic acids of whole hemp seeds. The results are presented as the mean ± standard deviation (n = 3).

	'Bialobrzeskie'	'Carmagnola'	'Fedora 17′	'Felina 32′	'KC Dora'	'Kompolti'	'Santhica 27′	'Tiborszallasi'
Proximate composition (g/100 g)								
Moisture	4.53 ± 0.06^{f}	$5.2 \pm 0.2^{a,b,c,d,e,f}$	5.67 ± 0.03^{e}	6.72 ± 0.02^{b}	6.04 ± 0.04^{c}	7.06 ± 0.01^{a}	$5.94 \pm 0.04^{c,d}$	$5.73 \pm 0.05^{d,e}$
Protein	23.0 ± 0.5^{a}	$21.9 \pm 0.3^{a,b}$	$19.8 \pm 0.8^{c,d}$	18.3 ± 0.8^{d}	$21.2 \pm 0.5^{b,c}$	18.8 ± 0.2^{d}	18.3 ± 0.9^{d}	$22.3 \pm 0.6^{a,b}$
Fat	32.66 ± 0.01^{a}	32.3 ± 0.2^{a}	$31.3 \pm 0.3^{a,b,c}$	$29.8 \pm 0.9^{c,d}$	$31.5 \pm 0.2^{a,b}$	31.7 ± 0.2^{a}	$29.9 \pm 0.9^{b,c,d}$	29.1 ± 0.8^{d}
ACH	10.28 ± 0.04^{a}	9.1 ± 0.5^{b}	$9.3 \pm 0.2^{a,b}$	$9.4 \pm 0.5^{a,b}$	8.7 ± 0.5^{b}	$9.4 \pm 0.4^{a,b}$	10.4 ± 0.3^{a}	$9.6 \pm 0.5^{a,b}$
NDF	32.5 ± 0.5^{d}	$39.5 \pm 0.2^{a,b,c}$	36.6 ± 0.3^{c}	$38 \pm 2^{a,b,c}$	$37 \pm 2^{b,c}$	40.4 ± 0.8^{a}	$37.44 \pm 0.02^{a,b,c}$	$40.0 \pm 1.2^{a,b}$
TMC	4.56 ± 0.08^{f}	$5.46 \pm 0.02^{b,c}$	4.84 ± 0.01^{e}	5.1 ± 0.1^{d}	$5.29 \pm 0.07^{c,d}$	6.32 ± 0.09^{a}	4.2 ± 0.1^{g}	5.69 ± 0.08^{b}
Energy value (kcal/100 g) and its distribution by nutrient (%)								
Energy value	492 ± 2^{a}	$494 \pm 5^{a,b}$	$471 \pm 4^{a,b}$	$456 \pm 10^{a,b}$	$476 \pm 3^{a,b}$	479 ± 2^{b}	$459 \pm 9^{a,b}$	$469 \pm 8^{a,b}$
Protein	18.7 ± 0.3^{a}	$21.9 \pm 0.3^{a,b}$	$16.8 \pm 0.6^{b,c}$	16 ± 1^{c}	$17.8 \pm 0.4^{a,b}$	15.7 ± 0.2^{c}	16.0 ± 0.6^{c}	19.0 ± 0.4^{a}
Fat	59.8 ± 0.2^{a}	58.8 ± 0.3^{a}	59.8 ± 0.6^{a}	58.9 ± 0.6^{a}	59.4 ± 0.6^{a}	59.6 ± 0.3^{a}	58.7 ± 0.8^{a}	55.8 ± 0.6^{b}
ACH	$8.36 \pm 0.04^{a,b}$	$7.4 \pm 0.4^{c,d}$	$7.9 \pm 0.1^{b,c,d}$	$8.3 \pm 0.4^{a,b,c}$	7.3 ± 0.4^{d}	$7.9 \pm 0.4^{b,c,d}$	9.0 ± 0.4^{a}	$8.2 \pm 0.3^{a,b,c,d}$
NDF	13.2 ± 0.2^{d}	$16.0 \pm 0.1^{a,b,c}$	$15.6 \pm 0.2^{b,c}$	$16.8 \pm 0.5^{a,b,c}$	15.5 ± 0.9^{c}	$16.9 \pm 0.3^{a,b}$	$16.3 \pm 0.3^{a,b,c}$	17.1 ± 0.7^{a}
Soluble sugars (g/100 g)								
Fructose	0.60 ± 0.01^{a}	0.113 ± 0.002^{b}	nd	0.113 ± 0.003^{b}	nd	nd	nd	nd
Glucose	0.78 ± 0.01^{a}	0.232 ± 0.006^{b}	nd	0.121 ± 0.003^{c}	nd	nd	nd	nd
Sucrose	1.69 ± 0.03^{h}	2.99 ± 0.03^{d}	3.62 ± 0.04^{b}	2.62 ± 0.03^{e}	2.32 ± 0.06^{g}	2.47 ± 0.02^{f}	3.87 ± 0.05^{a}	3.16 ± 0.01^{c}
Raffinose	0.65 ± 0.01^{d}	0.548 ± 0.009^{e}	0.99 ± 0.04^{a}	0.97 ± 0.03^{a}	0.82 ± 0.02^{b}	$0.81 \pm 0.02^{b,c}$	0.752 ± 0.004^{c}	0.49 ± 0.02^{f}
Total	$3.73 \pm 0.07^{c,d}$	3.88 ± 0.01^{b}	4.61 ± 0.01^{a}	$3.82 \pm 0.05^{b,c}$	3.14 ± 0.08^{f}	3.28 ± 0.05^{e}	4.62 ± 0.05^{a}	3.64 ± 0.03^{d}
Organic acids (mg/100 g)								
Oxalic acid	128 ± 5^{c}	94 ± 2^{d}	87.1 ± 0.8^{d}	51 ± 4^{f}	64 ± 1^{e}	18.5 ± 0.3^{g}	155.0 ± 0.2^{b}	191 ± 2^{a}
Citric acid	213 ± 2^{b}	85 ± 3^{e}	186 ± 4^{c}	119 ± 7^{d}	218 ± 10^{b}	128 ± 6^{d}	353 ± 4^{a}	186 ± 8^{c}
Fumaric acid	tr	tr	tr	tr	tr	tr	tr	tr

ACH: available carbohydrates; NDF: neutral detergent fibre; TMC: total mineral content; nd: not detected; tr: traces. Different letters in the same line indicate significant differences ($p < 0.001$) between samples.

Table 2
Proximate composition, energy value and its distribution by nutrient, soluble sugars and organic acids of dehulled hemp seeds. The results are presented as the mean ± standard deviation (n = 3).

	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
Proximate composition (g/100 g)								
Moisture	$5.6 \pm 0.3^{a,b,c,d,e}$	6.25 ± 0.03^{b}	6.56 ± 0.02^{a}	4.96 ± 0.04^{d}	6.07 ± 0.02^{c}	4.50 ± 0.02^{e}	$5.48 \pm 0.09^{b,c,d,e}$	4.60 ± 0.02^{e}
Protein	28.4 ± 0.3^{a}	28.3 ± 0.2^{a}	24.6 ± 0.2^{c}	24.85 ± 0.09^{c}	$25.4 \pm 0.1^{b,c}$	$25.3 \pm 0.2^{b,c}$	25.6 ± 0.5^{b}	$25.0 \pm 0.2^{b,c}$
Fat	49.6 ± 0.4^{e}	50.2 ± 0.3^{e}	52.6 ± 0.3^{c}	51.58 ± 0.04^{d}	$52.1 \pm 0.4^{c,d}$	$51.7 \pm 0.2^{c,d}$	55.0 ± 0.5^{a}	53.77 ± 0.02^{b}
ACH	$4.7 \pm 0.2^{c,d}$	$4.5 \pm 0.3^{d,e}$	$5.46 \pm 0.09^{a,b}$	$5.2 \pm 0.2^{b,c}$	$5.2 \pm 0.1^{b,c}$	5.9 ± 0.1^{a}	4.0 ± 0.2^{e}	5.31 ± 0.08^{b}
NDF	4.8 ± 0.2^{c}	6.0 ± 0.2^{a}	$5.1 \pm 0.3^{b,c}$	$5.9 \pm 0.4^{a,b}$	$4.4 \pm 0.4^{c,d}$	3.9 ± 0.2^{d}	$3.6 \pm 0.3^{d,e}$	2.92 ± 0.06^{e}
TMC	$5.79 \pm 0.03^{a,b}$	5.96 ± 0.06^{a}	4.94 ± 0.05^{d}	$5.1 \pm 0.1^{b,d}$	$5.45 \pm 0.08^{b,c}$	$5.08 \pm 0.03^{c,d}$	$4.99 \pm 0.02^{c,d}$	$5.3 \pm 0.2^{a,c,d}$
Energy value (kcal/100 g) and its distribution by nutrient (%)								
Energy value	589 ± 4^{e}	$595 \pm 3^{d,e}$	$604 \pm 3^{b,c}$	$596.2 \pm 0.4^{c,d,e}$	$600 \pm 4^{c,d}$	$598 \pm 2^{c,d}$	621 ± 5^{a}	611.1 ± 0.9^{b}
Protein	19.3 ± 0.2^{a}	19.04 ± 0.02^{a}	16.3 ± 0.2^{b}	16.67 ± 0.05^{b}	16.9 ± 0.1^{b}	16.9 ± 0.1^{b}	16.5 ± 0.3^{b}	16.4 ± 0.1^{b}
Fat	75.9 ± 0.1^{c}	76.0 ± 0.2^{c}	$78.39 \pm 0.02^{a,b}$	77.86 ± 0.08^{b}	$78.1 \pm 0.2^{a,b}$	77.9 ± 0.1^{b}	$79.7 \pm 0.4^{a,b,c}$	79.2 ± 0.1^{a}
ACH	$3.2 \pm 0.2^{c,d}$	3.0 ± 0.2^{d}	$3.62 \pm 0.06^{a,b}$	$3.5 \pm 0.2^{b,c}$	$3.49 \pm 0.08^{b,c}$	3.92 ± 0.09^{a}	2.6 ± 0.1^{e}	$3.47 \pm 0.05^{b,c}$
NDF	1.62 ± 0.06^{b}	2.01 ± 0.06^{a}	1.69 ± 0.09^{b}	2.0 ± 0.2^{a}	$1.5 \pm 0.1^{b,c}$	$1.29 \pm 0.07^{c,d}$	$1.2 \pm 0.1^{d,e}$	0.95 ± 0.02^{e}
Soluble sugars (g/100 g)								
Fructose	nd	nd	nd	nd	nd	nd	nd	nd
Glucose	nd	nd	1.01 ± 0.02^{a}	0.209 ± 0.004^{e}	0.851 ± 0.005^{b}	0.64 ± 0.01^{c}	0.249 ± 0.002^{d}	0.241 ± 0.003^{d}
Sucrose	2.75 ± 0.01^{e}	2.74 ± 0.03^{e}	3.34 ± 0.07^{b}	4.58 ± 0.04^{a}	3.00 ± 0.05^{d}	4.54 ± 0.04^{a}	$3.07 \pm 0.03^{c,d}$	3.14 ± 0.05^{c}
Raffinose	2.46 ± 0.07^{a}	2.26 ± 0.01^{a}	$0.321 \pm 0.006^{d,e}$	0.485 ± 0.007^{b}	0.257 ± 0.001^{e}	0.468 ± 0.006^{b}	0.433 ± 0.005^{c}	0.305 ± 0.004^{d}
Total	$5.21 \pm 0.08^{b\ d}$	5.00 ± 0.02^{c}	4.7 ± 0.1^{d}	5.28 ± 0.05^{b}	4.11 ± 0.06^{e}	5.65 ± 0.06^{a}	3.75 ± 0.04^{f}	3.68 ± 0.05^{f}
Organic acids (mg/100 g)								
Oxalic acid	$89 \pm 3^{d,e}$	81.7 ± 0.7^{e}	134 ± 7^{a}	91.6 ± 0.3^{d}	101 ± 3^{c}	90.9 ± 0.2^{d}	113.8 ± 0.3^{b}	116 ± 2^{b}
Citric acid	260 ± 2^{c}	$223 \pm 9^{d,e}$	303 ± 4^{b}	$218 \pm 2^{d,e}$	$207 \pm 6^{e,f}$	330 ± 10^{a}	192 ± 7^{f}	225 ± 3^{d}
Fumaric acid	tr	tr	1.5 ± 0.1^{c}	2.65 ± 0.03^{b}	0.678 ± 0.007^{d}	tr	0.60 ± 0.01^{d}	3.489 ± 0.02^{a}

ACH: available carbohydrates; NDF: neutral detergent fibre; TMC: total mineral content; nd: not detected; tr: traces. Different letters in the same line indicate significant differences ($p < 0.001$) between samples.

which correspond to the 'Bialobrzeskie' and 'Tiborszallasi' varieties. The fat content was higher in dehulled seeds, with close to or even higher than 50 g/100 g. Vonapartis et al. (2015) reported fat contents slightly lower than ours in whole hemp seeds, while Callaway (2004) published higher results than present values. The data of House et al. (2010) approach the results of the present study, as their average value was 30.4 g/100 g and the average value in this work was 31.02 g/100 g. The values in this study also seem consistent with Aluko (2017), who indicated a fat content of 30 g/100 g. Zając et al. (2019) reported a fat content for 'Bialobrzeskie' of 30.69 g/100 g, lower than the 32.66 g/100 g herein quantified, but still within the range of values. For dehulled hemp seeds, Zając et al. (2019) reported 51.17 g/100 g of fat, while those of the present study yielded an average value of 52.07 g/100 g.

Protein content was also higher in dehulled hemp seeds compared to whole seeds. On average, the protein content in dehulled seeds was 26 g/100 g, while it was 20.4 g/100 g in the whole ones. 'Bialobrzeskie' was the variety with the highest protein content, 23.0 g/100 g. While fibre content is difficult to compare because of the methodology, problems comparing protein content come from the nitrogen-protein conversion factor (sometimes different or unknown). The protein content reported by Callaway (2004) is 24.8 g/100 g in the 'Finola' variety, and the conversion factor is not specified. Vonapartis et al. (2015) used the conversion factor 6.25, yielding a result that was higher than that of Callaway (2004) for the same variety. The same happens with the results of House et al. (2010), causing many of the values to higher than those in the present work, because they also used the conversion factor of 6.25. The data of Aluko (2017) and Zając et al. (2019) are also slightly higher than ours. It is plausible that these studies also used the conversion factor of 6.25 too. As with whole hemp seeds, the protein content of dehulled seeds is lower than previously reported by other authors (Callaway & Pate, 2009; House et al., 2010; Zając et al., 2019). USDA (2019) protein content, 31.56 g/100 g, was calculated with the same conversion factor used in this study (5.3), leading to values entering the range presented here (24.6–28.4 g/100 g). Nutrition claims about proteins are related to their percentage related to energy value. The claim "source of protein" requires at least 12% of the energy value provided by protein, and 20% for "high protein content". Therefore, both whole and dehulled hemp seeds, could use the nutrition claim "source of protein" (European Parliament & Council of the European Union, 2006).

Whole hemp seeds contained about 9.5 g/100 g of available carbohydrates, and dehulled seeds contained 5.03 g/100 g on average. There is not available carbohydrates data in whole hemp seeds from previous studies, while concerning dehulled seeds, very few data are published about available carbohydrates, being estimated by difference, so this is the first study reporting analytical data about available carbohydrates in hemp seeds.

Total mineral content was similar in both sample types, ranging from 4.2 to 6.32 g/100 g in whole hemp seeds and 4.94 to 5.96 g/100 g in dehulled seeds. Total mineral content seems to be consistent with previous works (Callaway, 2004; House et al., 2010; Vonapartis et al., 2015). Zając et al. (2019) reported a much higher result for the 'Bialobrzeskie' variety, 10.0 g/100 g, more than twice the result described here for the same variety, 4.56 g/100 g, and more than all ranges reported by the other authors. Total mineral content previously reported was slightly higher than 6 g/100 g, close to our maximum value, 5.96 g/100 g. Zając et al. (2019) reported a total mineral content of 17.5 g/100 g, much higher than the values of this work and other authors.

3.2. Energy value

Energy value results and its distribution by nutrient of whole and dehulled hemp seeds are summarised in Tables 1 and 2, respectively. The energy value of whole hemp seeds ranged from 456 to 494 kcal/100 g, and more than half (58.8% on average) corresponded to fat, which is the most energetic nutrient. The energy value of dehulled seeds ranged from 589 to 621 kcal/100 g. This augmented energy value is due to their increased fat content, which in some cases accounted for 75 to 79% of the total energy. Additionally, the protein contribution to the energy value is remarkable, close to 17.2% in both cases. This result justifies the need to use an appropriate nitrogen-protein conversion factor for seeds to avoid less accurate estimates.

Callaway (2004) reported an energy value of 506 kcal/100 g for whole hemp seeds. This value is slightly higher than that here reported because of the higher fat content of the 'Finola' variety used in that study. House et al. (2010) also reported a higher hemp seed energy value, but the authors used calorimetry and did not include conversion factors in their calculations. USDA (2019) reports 533 kcal/100 g for dehulled hemp seeds. This reduced value compared to ours is because

that study used lower conversion factors for fat and protein, the most abundant nutrients in this type of seeds. All these results can be very useful in completing food composition databases and for labelling purposes.

3.3. Soluble sugars

The results of soluble sugars content of whole and dehulled hemp seeds are presented in Table 1, and Table 2. Sucrose was the most abundant soluble sugar in whole hemp seeds. Its content ranged from 1.69 g/100 g in the 'Bialobrzeskie' variety to 3.87 g/100 g in 'Santhica 27′. We also detected raffinose at concentrations lower than 1 g/100 g in all varieties analysed. Fructose and glucose were also detected in the 'Bialobrzeskie', 'Carmagnola', and 'Felina 32′ varieties. The 'Bialobrzeskie' variety had the highest concentrations of these two monosaccharides, with 0.60 g/100 g of fructose and 0.78 g/100 g of glucose. The total soluble sugar content of whole hemp seeds ranged from 3.14 to 4.62 g/100 g. Despite the lack of fructose and glucose, the 'Fedora 17′ and 'Santhica 27′ varieties had the highest total soluble sugar content values because of their high sucrose content.

Total soluble sugars in dehulled hemp seeds ranged from 3.68 and 5.65 g/100 g. They contained sucrose and raffinose and most of them also contained glucose up to 1 g/100 g, but fructose was not detected in any of the samples. Sucrose was the most abundant, with values ranging from 2.74 to 4.58 g/100 g. The raffinose content was lower than 0.5 g/100 g in most samples, but two of them (Brand 1) exceeded 2 g/100 g. Glucose was not detected in the high raffinose-containing samples.

Information about soluble sugars in hemp seeds is scarce. Recently, Schultz et al. (2020) reported the soluble sugars content of dehulled hemp seeds. As in the present work, sucrose and raffinose were the predominant sugars in dehulled seeds, and fructose and glucose were not present in all the analysed samples. Moreover, the observed sucrose content in their samples ranged from 1.5 to 3.8 g/100 g, which is slightly higher than the results here presented. Furthermore, they detected raffinose concentrations of<0.5 g/100 g, an observation, except the two samples with elevated raffinose levels, consistent with the data presented herein.

3.4. Organic acids

The organic acid results from whole and dehulled hemp seeds are shown in Tables 1 and 2, respectively. Citric acid was the most abundant organic acid in most samples of whole hemp seeds analysed with values ranging from 85 to 353 mg/100 g. The minimum content corresponded to the 'Carmagnola' variety, one of the two varieties with a citric acid content lower than oxalic acid, which had a content of 94 mg/100 g. The other variety was 'Tiborszallasi', which had a citric acid content of 186 mg/100 g and an oxalic acid content of 191 mg/100 g. Thus, the content of these two organic acids was quite similar in these two varieties. The maximum citric acid content corresponded to 'Santhica 27′, which was the only variety with more than 300 mg/100 g. In whole hemp seeds, oxalic acid concentrations ranged from 18.5 to 191 mg/100 g. The maximum value was detected in the 'Tiborszallasi' variety, and the 'Kompolti' variety had the lowest levels, roughly one-tenth the maximum. It is important to point out that the differences in oxalic acid content were notable, with statistically significant differences detected between nearly all the samples. The only exception was when we compared the 'Carmagnola' and 'KC Dora' varieties. Only trace amounts of fumaric acid were detected in whole hemp seeds.

In dehulled hemp seeds, citric acid content ranged from 192 to 330 mg/100 g, making it the most abundant organic acid in this seed type. Oxalic acid ranged from 81.7 to 134 mg/100 g. Fumaric acid was detected at trace levels in three samples and quantified in the other five. In general, the content was low, with the highest content being 3.489 mg/100 g. To the best of the authors' knowledge, this is the first time the organic acid content of hemp seeds has been reported.

3.5. Phenolic compounds

Table 3 presents the identification and quantification of phenolic compounds in the hydromethanolic extracts of hemp seeds, and Table 4 shows the results from whole and dehulled hemp seeds. It was determined that all the hydromethanolic extracts obtained from the eight varieties of whole hemp seeds contained ferulic acid-hexoside, in a range between 0.266 and 0.54 mg/g extract, corresponding to 'Bialobrzeskie' and 'Tiborszallasi', respectively. Syringic acid was also detected in half of the varieties analysed, with the 'Kompolti' variety containing the highest concentrations of this phenolic acid, 0.72 mg/g extract. Concerning total phenolic acid concentrations, these values ranged from 0.266 to 1.20 mg/g extract. The lower value observed in the 'Bialobrzeskie' variety was due to reduced ferulic acid-hexoside concentrations and the lack of syringic acid. On the other hand, the elevated total phenolic acid levels observed in the 'Kompolti' variety was because this variety had the highest syringic acid content and augmented levels of ferulic acid-hexoside, 0.48 mg/g extract.

All hydromethanolic dehulled hemp seed extracts contained ferulic acid-hexoside and syringic acid. The former ranged from 0.371 to 0.619 mg/g extract, and the latter from 0.29 to 0.63 mg/g extract. The ferulic acid-hexoside range was slightly higher in the hydromethanolic dehulled hemp seed extracts than whole seeds. The maximum syringic acid content was higher in the hydromethanolic whole seed extracts. Total phenolic acids in hydromethanolic dehulled seed extracts ranged from 0.66 to 1.25 mg/g. The maximum values obtained in hydromethanolic extracts of whole and dehulled hemp seeds were similar. However, the minimum value in the dehulled samples was greater due to the presence of syringic acid in the extracts.

A previous study showed that ferulic acid is a minor compound in hemp seeds (Iraki et al., 2019). Additionally, Multari et al. (2016) reported higher levels of the free form of this compound than the bound one in hemp flour. The present results seem consistent with this result since we observed ferulic acid-hexoside. Interestingly, Irakli et al. (2019) did not detect syringic acid in their samples, but Multari et al. (2016) found it in hemp flour. The presence of these phenolic acids is attractive since these compounds have been shown to exhibit antioxidant properties (Cheemanapalli, Mopuri, Ramanjaneyulu, Anuradha, & Kuman, 2018; Kumar & Pruthi, 2014).

3.6. Biological activities

The results of the antioxidant activity of the hydromethanolic extracts of whole and dehulled hemp seeds are presented in Table 5. The higher antioxidant activities are characterised by lower EC_{50} values. The lowest EC_{50} values were detected in the TBARS assay, which required 0.31 to 0.75 mg/mL of whole seed extract to provide 50% of the activity. This assay measures the extract's capacity to inhibit the formation of malondialdehyde and other low molecular-weight end-products generated from the *ex vivo* decomposition of certain lipid peroxidation products (Alonso et al., 2009). We opted to employ porcine brain cells because they are rich in polyunsaturated fatty acids and an established model for lipid peroxidation studies. The EC_{50} values obtained in the β-carotene bleaching inhibition capacity assay were slightly higher, ranging from 0.38 to 1.2 mg/mL. The reducing power (EC_{50} 1.21–2.35 mg/mL) and the DPPH scavenging activity (EC_{50} 2.5–9.2 mg/mL) were low.

The hydromethanolic dehulled hemp seed extracts were less active than those obtained from whole seeds. The TBARS and β-carotene bleaching inhibition capacity assay results exhibited broad EC_{50} ranges of 0.75 to 2.4 mg/mL and 0.43 to 2.3 mg/mL, respectively. These seed extracts appeared to lack significant reducing power (EC_{50} 2.3–5.3 mg/mL) and DPPH scavenging activity (EC_{50} 19.1–35.1 mg/mL).

Interestingly, Hong et al. (2015) measured the DPPH scavenging activity of dehulled hemp seeds, and the results were similar to the EC_{50} values of whole seeds in the present work. However, they carried out

Table 3
Phenolic acids identified in the hemp seed hydromethanolic extracts. The retention time (Rt), wavelength of maximum absorption (λ_{max}) in the UV–Vis region, mass spectral data, and quantification parameters are presented.

Compound	Rt (min)	λ_{max} (nm)	Pseudomolecular ion $[M-H]^-$ (*m/z*)	MS^2 fragments (*m/z*)*	Standard	Calibration curve	LOD (μg/mL)	LOQ (μg/mL)
Unknown	5.7	253, 280	318	301 (20), 300 (100), 282 (5), 257 (8), 172 (6), 128 (5)	–	–	–	–
Ferulic acid-hexoside	6.5	290	355	193 (100)	Ferulic acid	y = 633126x – 185,462 (R^2 = 0.9990)	0.20	1.01
Syringic acid	12.6	280	197	153 (12), 121 (100)	Syringic acid	y = 376056x – 141,329 (R^2 = 0.9995)	0.23	0.72

LOD: limit of detection; LOQ: limit of quantification.
* The relative abundance of each fragment ion is presented within parentheses.

Table 4
Phenolic acids content (mg/g of dry extract) in the whole and dehulled hemp seed hydromethanolic extracts. The results are presented as the mean ± standard deviation (n = 3).

	Whole hemp seeds							
	'Bialobrzeskie'	'Carmagnola'	'Fedora 17'	'Felina 32'	'KC Dora'	'Kompolti'	'Santhica 27'	'Tiborszallasi'
Ferulic acid-hexoside	0.266 ± 0.004^{d}	0.39 ± 0.01^{f}	0.47 ± 0.02^{b}	0.33 ± 0.01^{e}	0.38 ± 0.01^{d}	0.48 ± 0.02^{b}	0.43 ± 0.02^{c}	0.54 ± 0.02^{a}
Syringic acid	nd	nd	nd	0.431 ± 0.001^{b}	nd	0.72 ± 0.04^{a}	0.68 ± 0.05^{a}	0.35 ± 0.01^{c}
TPA	0.266 ± 0.004^{g}	0.39 ± 0.01^{f}	0.47 ± 0.02^{e}	0.76 ± 0.02^{d}	0.38 ± 0.01^{f}	1.20 ± 0.06^{a}	1.11 ± 0.06^{b}	0.89 ± 0.03^{c}
	Dehulled 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**
Ferulic acid-hexoside	0.58 ± 0.03^{b}	0.619 ± 0.002^{a}	0.41 ± 0.03^{d}	0.41 ± 0.01^{d}	0.371 ± 0.002^{e}	0.46 ± 0.01^{c}	0.46 ± 0.02^{c}	$0.39 \pm 0.02^{d,e}$
Syringic acid	0.61 ± 0.04^{a}	0.63 ± 0.01^{a}	0.30 ± 0.02^{e}	$0.32 \pm 0.02^{d,e}$	0.29 ± 0.01^{e}	$0.35 \pm 0.02^{c,d}$	0.50 ± 0.03^{b}	0.37 ± 0.03^{c}
TPA	1.19 ± 0.07^{a}	1.25 ± 0.02^{a}	$0.71 \pm 0.05^{d,e}$	$0.73 \pm 0.03^{d,e}$	0.66 ± 0.01^{e}	0.81 ± 0.03^{c}	0.96 ± 0.04^{b}	$0.77 \pm 0.04^{c,d}$

TPA: total phenolic acids; nd: not detected. Different letters in the same line indicate significant differences ($p < 0.001$) between samples.

Table 5
Antioxidant, cytotoxic, and hepatotoxic activity of the whole and dehulled hemp seed hydromethanolic extracts. The results are presented as the mean ± standard deviation (n = 3).

	Whole hemp seeds								Positive control
	'Bialobrzeskie'	'Carmagnola'	'Fedora 17'	'Felina 32'	'KC Dora'	'Kompolti'	'Santhica 27'	'Tiborszallasi'	
Antioxidant activity (EC_{50}, mg/mL)									Trolox
DPPH SA	9.2 ± 0.3^{a}	7.6 ± 0.4^{b}	3.6 ± 0.1^{f}	3.9 ± 0.1^{e}	4.7 ± 0.1^{d}	2.5 ± 0.1^{g}	5.9 ± 0.2^{c}	4.0 ± 0.2^{e}	0.042 ± 0.001
RP	2.05 ± 0.06^{b}	1.58 ± 0.03^{e}	1.64 ± 0.04^{d}	1.21 ± 0.02^{h}	1.25 ± 0.01^{g}	1.31 ± 0.03^{f}	2.35 ± 0.04^{a}	1.89 ± 0.05^{c}	0.041 ± 0.003
β-Carotene BI	0.86 ± 0.06^{c}	$1.2 \pm 0.2^{a,b}$	0.38 ± 0.01^{e}	0.7 ± 0.1^{c}	1.15 ± 0.08^{a}	0.47 ± 0.05^{d}	0.49 ± 0.02^{d}	1.01 ± 0.06^{b}	0.018 ± 0.001
TBARS FI	$0.40 \pm 0.04^{d,e}$	0.38 ± 0.04^{e}	0.74 ± 0.05^{a}	0.51 ± 0.01^{b}	0.31 ± 0.01^{f}	$0.48 \pm 0.03^{b,c}$	0.75 ± 0.01^{a}	$0.44 \pm 0.01^{c,d}$	0.023 ± 0.001
Cytotoxic activity (GI_{50}, μg/mL)									Ellipticine
HepG2	179 ± 9^{c}	204 ± 13^{b}	>400	>400	203 ± 8^{b}	334 ± 11^{a}	>400	>400	13 ± 1
NCI-H460	47 ± 3^{d}	99 ± 4^{c}	>400	274 ± 19^{a}	121 ± 6^{b}	280 ± 13^{a}	>400	>400	8.0 ± 0.2
HeLa	72 ± 4^{d}	292 ± 6^{b}	>400	>400	227 ± 11^{c}	303 ± 8^{a}	>400	>400	4.75 ± 0.05
MCF-7	92 ± 4^{a}	215 ± 13^{b}	>400	>400	216 ± 11^{b}	302 ± 15^{a}	>400	>400	3.7 ± 0.2
Hepatotoxic activity (GI_{50}, μg/mL)									Ellipticine
PLP2	271 ± 19^{b}	323 ± 6^{a}	>400	>400	>400	>400	>400	>400	8.6 ± 0.1
	Dehulled 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**	
Antioxidant activity (EC_{50}, mg/mL)									Trolox
DPPH SA	22.1 ± 0.3^{d}	19.8 ± 0.2^{e}	33.7 ± 0.3^{b}	30.1 ± 0.1^{c}	35.1 ± 0.7^{a}	19.1 ± 0.5^{f}	30 ± 2^{c}	$21 \pm 1^{d,e}$	0.042 ± 0.001
RP	5.3 ± 0.2^{a}	5.2 ± 0.2^{a}	2.8 ± 0.2^{c}	2.46 ± 0.08^{d}	2.3 ± 0.3^{d}	3.6 ± 0.2^{b}	2.4 ± 0.2^{d}	2.4 ± 0.2^{d}	0.041 ± 0.003
β-Carotene BI	1.20 ± 0.02^{b}	0.43 ± 0.06^{e}	2.128 ± 0.004^{a}	1.09 ± 0.03^{c}	2.3 ± 0.2^{a}	0.75 ± 0.02^{d}	$1.4 \pm 0.2^{b,c}$	0.87 ± 0.09^{d}	0.018 ± 0.001
TBARS FI	2.14 ± 0.02^{b}	2.40 ± 0.04^{a}	$1.0 \pm 0.1^{d,e}$	1.04 ± 0.05^{d}	0.75 ± 0.06^{f}	1.25 ± 0.08^{c}	0.92 ± 0.06^{e}	1.20 ± 0.03^{c}	0.023 ± 0.001
Cytotoxic activity (GI_{50}, μg/mL)									Ellipticine
HepG2	>400	>400	142 ± 4^{a}	131 ± 10^{b}	114 ± 8^{c}	>400	>400	>400	13 ± 1
NCI-H460	>400	>400	77 ± 6^{d}	70 ± 2^{d}	95 ± 3^{c}	>400	172 ± 12^{a}	160 ± 13^{b}	8.0 ± 0.2
HeLa	>400	>400	122 ± 5^{c}	99 ± 4^{d}	129 ± 7^{b}	>400	318 ± 4^{a}	>400	4.75 ± 0.05
MCF-7	>400	>400	153 ± 8^{c}	127 ± 9^{d}	224 ± 20^{b}	>400	>400	251 ± 23^{a}	3.7 ± 0.2
Hepatotoxic activity (GI_{50}, μg/mL)									Ellipticine
PLP2	>400	>400	>400	>400	>400	>400	>400	>400	8.6 ± 0.1

DPPH SA: DPPH scavenging activity; RP: reducing power; β-carotene BI: β-carotene bleaching inhibition; TBARS FI: TBARS formation inhibition. Different letters in the same line indicate significant differences ($p < 0.001$) between samples.

extractions with ethanol and supercritical CO_2, which could account for the difference. Chen et al. (2012) analysed the DPPH scavenging activity of dehulled hemp seeds and hemp hulls but not evaluate whole hemp seeds. The authors concluded that the observed activity was due to phenolic compounds. In the present study, it seems that the presence of hull in the seed favoured the antioxidant activity, probably due to the phytochemical constituents it contains, but further work is needed to find possible correlations.

The cytotoxic activities of the hydromethanolic extracts of whole and dehulled hemp seeds against MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma) cell lines and the hepatotoxic activity against PLP2 (liver primary culture) are summarised in Table 5. Lower GI_{50} values correspond to higher cytotoxic activities in these assays, and GI_{50} values of more than 400 μg/mL were considered inactive.

Four varieties of the whole hemp seed extracts were active against the four tumour cell lines tested, and another extract was only active against NCI-H460 (Table 5). The 'Bialobrzeskie' variety was found to be the most active, with GI_{50} values of less than 100 μg/mL against MCF-7, NCI-H460 and HeLa cells, and an GI_{50} of 179 μg/mL against HepG2 cells. The cytotoxic activity was especially marked against NCI-H460 cells because there were five active extracts with low GI_{50} values. Two varieties, 'Bialobrzeskie' and 'Carmagnola', were active against the PLP2 hepatic line; however, the GI_{50} values were about 300 μg/mL, which does not suggest marked hepatotoxicity.

Not all the hydromethanolic extracts of dehulled hemp seeds displayed cytotoxic activity (Table 5). Three were active against the four tumour cell lines, and two were active against two cell lines. Similar to

Table 6
Antibacterial and antifungal activity of the whole and dehulled hemp seed hydromethanolic extracts.

	Whole hemp seeds									
	'Bialobrzeskie'	'Carmagnola'	'Fedora 17′	'Felina 32′	'KC Dora'	'Kompolti'	'Santhica 27′	'Tiborszallasi'	PC1	PC2
Antibacterial activity (MIC/MBC, mg/mL)										
B. cereus	0.037/0.075	0.02/0.037	0.075/0.15	0.02/0.037	0.01/0.02	0.15/0.3	0.15/0.3	0.01/0.018	0.1/0.2	0.25/0.40
S. aureus	0.075/0.15	0.075/0.15	0.3/0.6	0.15/0.3	0.037/0.075	0.6/0.9	0.3/0.6	0.15/0.3	0.04/0.1	0.25/0.45
L. monocytogenes	0.15/0.3	0.15/0.3	0.9/1.2	0.3/0.6	0.3/0.6	0.6/1.2	0.45/0.6	0.3/0.6	0.2/0.3	0.4/0.5
E. faecalis	0.075/0.15	0.15/0.3	0.3/0.6	0.075/0.15	0.075/0.15	0.2/0.6	0.15/0.3	0.018/0.037	0.2/0.3	0.25/0.5
E. coli	0.15/0.3	0.15/0.3	0.9/1.2	0.3/0.6	0.3/0.6	0.9/1.2	0.6/0.9	0.3/0.6	0.2/0.3	0.4/0.5
S. Typhimurium	0.2/0.3	0.15/0.3	0.9/1.2	0.3/0.6	0.15/0.3	0.6/0.9	0.6/0.9	0.15/0.3	0.2/0.3	0.75/1.2
Antifungal activity (MIC/MFC, mg/mL)										
A. fumigatus	0.60/1.2	1.2/1.8	0.60/1.2	0.6/1.2	0.6/1.2	0.3/0.6	0.3/0.60	0.3/0.6	0.25/0.5	0.15/0.2
A. ochraceus	0.45/0.6	0.6/1.2	0.15/0.3	0.3/0.6	0.15/0.3	0.15/0.3	0.075/0.15	0.15/0.3	0.2/0.5	0.1/0.2
A. niger	0.9/1.8	1.2/2.4	0.3/0.6	0.45/1.2	0.3/0.6	0.9/1.2	0.3/0.6	0.6/1.2	0.2/0.5	0.15/0.2
P. ochrochloron	0.6/1.2	0.6/1.2	0.15/0.3	0.2/0.3	0.075/0.15	0.45/1.2	0.3/0.6	0.2/0.6	0.2/0.5	0.2/0.25
P. funiculosum	0.6/1.2	0.6/1.2	0.3/0.6	0.3/0.6	0.075/0.15	0.3/0.6	0.2/0.3	0.2/0.6	2.5/3.5	0.2/0.25
P. verrucosum var. *cyclopium*	0.45/0.6	0.9/1.8	0.15/0.3	0.45/1.2	0.15/0.3	0.3/0.6	0.2/0.3	0.45/0.6	0.2/0.3	0.1/0.2
	Dehulled 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**	**PC1**	**PC2**
Antibacterial activity (MIC/MBC, mg/mL)										
B. cereus	0.075/0.15	0.1/0.15	0.01/0.018	0.075/0.15	0.02/0.037	0.075/0.15	0.075/0.15	0.075/0.15	0.1/0.2	0.25/0.4
S. aureus	0.3/0.6	0.3/0.6	0.15/0.3	0.15/0.3	0.05/0.15	0.3/0.6	0.3/0.6	0.15/0.3	0.04/0.1	0.25/0.45
L. monocytogenes	0.15/0.3	0.2/0.6	0.1/0.3	0.15/0.3	0.15/0.3	0.2/0.3	0.15/0.3	0.15/0.3	0.2/0.3	0.4/0.5
E. faecalis	0.15/0.3	0.2/0.3	0.037/0.075	0.10/0.15	0.075/0.15	0.1/0.15	0.075/0.15	0.037/0.075	0.2/0.3	0.25/0.5
E. coli	0.3/0.6	0.3/0.6	0.15/0.3	0.30/0.6	0.075/0.15	0.3/0.6	0.3/0.6	0.2/0.3	0.2/0.3	0.4/0.5
S. Typhimurium	0.3/0.6	0.3/0.6	0.075/0.15	0.075/0.15	0.05/0.15	0.2/0.3	0.15/0.3	0.2/0.3	0.2/0.3	0.75/1.2
Antifungal activity (MIC/MFC, mg/mL)										
A. fumigatus	0.6/1.2	0.6/1.2	0.6/1.2	0.6/1.2	0.601.2	0.3/0.6	1.2/1.8	1.2/2.4	0.25/0.5	0.15/0.2
A. ochraceus	0.3/0.6	0.2/0.6	0.2/0.3	0.45/0.9	0.3/0.9	0.3/0.6	0.6/1.2	0.6/1.2	0.2/0.5	0.1/0.2
A. niger	0.6/1.8	0.45/0.9	0.6/1.2	1.2/1.8	0.45/0.9	0.6/0.9	1.2/2.4	1.2/2.4	0.2/0.5	0.15/0.2
P. ochrochloron	0.45/0.6	0.3/0.6	0.6/1.2	0.6/0.9	0.45/0.9	0.6/0.9	1.2/1.8	1.2/1.8	0.2/0.5	0.2/0.25
P. funiculosum	0.6/1.2	0.6/1.2	0.45/0.9	0.6/1.2	0.45/0.9	0.45/0.9	0.6/1.8	1.2/2.4	2.5/3.5	0.2/0.25
P. verrucosum var. *cyclopium*	0.6/1.2	0.6/1.2	0.6/1.2	0.6/1.2	0.6/1.2	0.6/1.2	1.2/1.8	1.2/2.4	0.2/0.3	0.1/0.2

MIC: minimum inhibitory concentration; MBC: minimal bactericidal concentration. Streptomycin and ampicillin (PC1 and PC2, respectively) were used as positive controls for the antibacterial activity assays, ketoconazole and bifonazole (PC1 and PC2, respectively) were used as the positive controls for the antifungal activity assays.

whole hemp seeds, the extracts displayed significant cytotoxic activity against NCI-H460. This result is the line with more active extracts against it, and the GI_{50} values were lower than against the other tumour cell lines. None of the hydromethanolic dehulled hemp seed extracts displayed hepatotoxic activity, as evidenced by GI_{50} values of greater than 400 μg/mL.

Chen et al. (2013) identified cannabisin B as a compound with activity against HepG2. We did not detect this compound in the analysed samples. However, some hemp seed extracts displayed activity against this cell line, indicating that the extracts contain other active phytochemicals. Logarušić et al. (2019) demonstrated the activity of hemp protein hydrolysates against HeLa, but some of the crude extracts were also active against it. Moccia et al. (2019) studied the cytotoxic activity of hemp seed extracts against different human colorectal cell lines, Caco-2 and HT-29, and showed that they did not interfere with growth.

A summary of the antimicrobial and antifungal activity results with hydromethanolic whole and dehulled hemp seed extracts against the bacteria and fungi tested are presented in Table 6. Marked antibacterial activity against *B. cereus* was confirmed since almost all the hydromethanolic whole hemp seed extracts had lower MIC and MBC values than the positive controls (MIC 0.1 mg/mL and MBC 0.2 mg/mL). Two of the extracts were more active than ampicillin (MIC 0.25 mg/mL and MBC 0.40 mg/mL). It is worth mentioning that the activity against *E. faecalis* also stood out. However, in this case, six extracts were more active than the two positive controls (MIC 0.2 mg/mL and MBC 0.3 mg/mL), and 'Kompolti' variety had a MIC value lower than ampicillin (MIC 0.25 mg/mL and MBC 0.5 mg/mL) and was equivalent to streptomycin. The most active extracts were from the 'Bialobrzeskie' and 'Carmagnola' varieties, with MIC and MBC values lower than the two positive controls, except against *S. aureus*, for which the MIC and MBC values were only lower than ampicillin (MIC 0.25 mg/mL and MBC 0.45 mg/mL). Notably, the hydromethanolic extract of the 'KC Dora' variety displayed substantial antibacterial activity against *B. cereus*, *S. aureus*, *E. faecalis*, and *S.* Typhimurium, with MIC and MBC values lower than the positive controls.

The antifungal activity of hydromethanolic extracts of whole hemp seeds against the *Aspergillus* tested was not remarkable. However, the extract obtained from 'Santhica 27′ produced MIC and MFC values lower than the two positive controls (MIC 0.15 and MFC 0.02 mg/mL). The antifungal activity against the *Penicillium* tested was similar, mainly against *P. funiculosum*. The most active extract was from the 'KC Dora' variety, which had MIC and MFC values against *P. ochrochloron* and *P. funiculosum* lower than the two positive controls (MIC 0.2 mg/mL and MFC 0.25 mg/mL). Additionally, the MIC and MFC values of the 'KC Dora' variety extract against *P. verrucosum* var. *cyclopium* were lower than those of ketoconazole (MIC 0.2 mg/mL and MFC 0.3 mg/mL).

The antibacterial activity of hydromethanolic extracts of dehulled hemp seeds was better than that of whole seeds, which may suggest the involvement of lipophilic constituents (either as bioactives or facilitators of the interaction of antibacterial compounds with the cell membrane). All extracts showed lower MIC and MBC values than the two positive controls against *B. cereus*, *E. faecalis*, and *L. monocytogenes*, except for one of the extracts that had a higher MBC value. In addition, six of these extracts were more active than the two positive controls against *S.* Typhimurium and three against *E. coli*. On the contrary, the antifungal activity of the hydromethanolic extracts of dehulled hemp seeds was less effective than whole hemp seeds. None of the extracts had MIC and MFC values lower than those of the two positive controls.

Frassinetti, Gabriele, Moccia, Longo, and Di Giogia (2020) tested the antimicrobial activity of ethanolic extracts of hemp seeds. Their results against *S. aureus*, *E. faecalis*, *E. coli* and *S.* Typhimurium were slightly higher than in this study, but the strains differed. They also tested the extracts against *Enterobacter aerogenes*, but the activity was not remarkable. Sokmen, Jones, and Erturk (1999) reported that the methanolic extracts of hemp seeds did not inhibit the growth of *B. cereus*, *S. aureus*, *E. coli* or the yeast *Candida albicans*. However, we found that hydromethanolic extracts were active against these bacteria, especially *B. cereus*.

4. Conclusions

A comprehensive composition study on whole and dehulled hemp seeds is presented. Both contain interesting amounts of fat and protein, but whole seeds exhibit higher fibre content than dehulled seeds. Despite the attenuated fibre content of dehulled seeds, they could use the nutrition claim "source of fibre", while whole seeds could use the "high fibre" claim. Additionally, both could use the "source of protein" claim. The primary organic acids detected in the hemp seeds were citric acid and oxalic acid. Moreover, ferulic acid-hexoside and syringic acid were the most abundant phenolic acids detected in the hydromethanolic hemp seed extracts. Interestingly, the antioxidant activity of the hydromethanolic extracts of whole seeds was better than that of dehulled seeds. This activity was particularly evident in the β-carotene bleaching inhibition and especially the TBARS assays. We observed that some extracts displayed notable cytotoxic activity against NCI-H460 and that dehulled seed extracts exhibited antibacterial activity against *B. cereus*, *L. monocytogenes*, and *E. faecalis*. In general, the antibacterial activity of the hydromethanolic seed extracts was better than the antifungal activity. However, future studies are needed to establish possible correlations between compounds and bioactivities. Finally, the nutritional composition and potential bioactivities make hemp seeds a potentially highly beneficial food.

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.

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