Journal of Agriculture and Food Research 16 (2024) 101084

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Chemical and functional characterization of the main bioactive molecules contained in hulled *Cannabis sativa* L. seeds for use as functional ingredients

S. Frazzini [a,*], M.C. Torresani [b], G. Roda [c], M. Dell'Anno [a], G. Ruffo [a], L. Rossi [a]

[a] Department of Veterinary Medicine and Animal Sciences – DIVAS, University of Milan, Via dell'Università 6, 26900, Lodi, Italy
[b] Biotecnologie B.T. Srl., Via Albert Einstein, 26900, Lodi, Italy
[c] Department of Pharmaceutical Sciences, Via Trentacoste 2, 201034, Milan, Italy

ARTICLE INFO

Keywords:
Cannabis sativa L.
Hemp seeds
Bioactive compounds
Functional properties
Bio accessibility

ABSTRACT

Functional nutrition is a key tool for preventing and improving health conditions both in humans and animals. Among the different functional ingredients, industrial hemp (*Cannabis sativa* L.) has received particular attention, with most studies extolling its high nutritional value, however it also has several functional benefits due to its content of the different bioactive molecules. This study thus comprehensively analyzes the chemical and functional aspects of hulled hemp seeds, and also assesses the retention of these functional properties after the digestive process in order to consider using hemp in animal nutrition. Bioactive molecules were identified through metabolomic analysis along with the determination of the total phenolic and total flavonoid content. The functional properties of the hemp seeds were also determined before and after digestion, which was simulated with a 3-step *in vitro* process. Specifically, the antioxidant activity was assessed using the 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) assay (ABTS), while the antimicrobial activity was assessed by the micro-dilution method. The analysis confirmed the high nutritional value of hemp seeds, represented mainly by crude lipids (52.02 ± 0.94% on a fresh matter basis) and crude protein (34.52 ± 0.38% on a fresh matter basis). The metabolomic characterization highlighted bioactive molecules belonging to the polyphenol and flavonoid families. These molecules could explain the antioxidant activity observed (2720.95 ± 85.58 μM Trolox equivalents) and the antimicrobial properties. Our results also highlighted those bioactive compounds also persisted after *in vitro* digestion. The polyphenol content increased after digestion from 172.30 ± 4.06 mg TEA/g of sample after the oral phase to 358.60 ± 14.04 mg TEA/g of sample after the intestinal phase. The same trend was also observed for flavonoids (from 4.39 ± 1.11 mg CE/g sample to 20.97 ± 1.40 mg CE/g sample). *In vitro* digestion significantly increased ($p < 0.01$) the radical scavenging capacity of the plants, suggesting an increase in the bioavailability of the bioactive compounds in the hulled hemp seeds after digestion. In conclusion, the high nutritional value of hemp combined with its functional nature suggest that hemp can be considered a viable alternative for the feed industry. It provides essential nutrients and simultaneously promotes the health status of animals, overall contributing to the reduction in antibiotics.

1. Introduction

Functional nutrition has emerged as a valuable approach to preventing and managing health issues in both humans and animals [1–3]. Antioxidant-rich foods form the cornerstone of functional nutrition [4], and hemp has been increasingly recognized for its potential health benefits. Hemp (*Cannabis sativa* L.) was initially used for medical purposes and later in industry [5]. After being banned in the 1970s, it was reintroduced to the consumer market in European, thanks to the establishment of delta-9-tetrahydrocannabinol (Δ⁹-THC) limits [6]. Hemp is now used in arable farming due to its agronomic qualities [7] for soil remediation according to nutritional ecology principles [8]. In addition to its agronomic resilience, the hemp plant can be used as a source of bioactive compounds for nutritional use, and the waste and by-products from industry can be used in biofuel production [9]. Today's society faces several challenges, such as overpopulation, global warming, and biodiversity losses [10]. A holistic and sustainable approach from a circular economy perspective has therefore become fundamental, promoting the responsible use of resources and waste reduction and fostering innovation for a more resilient and equitable

* Corresponding author.
E-mail address: sara.frazzini@unimi.it (S. Frazzini).

https://doi.org/10.1016/j.jafr.2024.101084
Received 16 November 2023; Received in revised form 18 February 2024; Accepted 28 February 2024
Available online 29 February 2024

future [11]. Industrial hemp crops have recently gained attention due to their sustainability potential, and given the many waste products derived from industrial hemp processing [12]. In fact, the size of the global market of industrial hemp which was estimated to be $4.74 billion in 2022 and is expected to grow at a compound annual growth rate (CAGR) of 17.1% from 2023 to 2030 [13]. Different parts of the hemp plant, such as roots, stems, seeds, and flowers, serve various industrial fields [14–16]. Overall, hemp seeds are widely recognized for their high nutritional value. They contain approximately 20–25% protein, which consists of essential amino acids (i.e., histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). They are thus characterized by a high biological value; 20–30% carbohydrate-rich insoluble fibre; and 25–35% lipids, which are represented by polyunsaturated fatty acids (PUFAs) [17]. In addition, hemp seeds are rich in natural antioxidants and other functional components, such as bioactive peptides, phenolic compounds, tocopherols, carotenoids, and phytosterols [18,19]. The functional aspects of hemp seeds are still poorly investigated. Hemp is rich in natural antioxidants and other functional components related to the benefits found in vivo after hemp-based food intake [20]. However, in animal production, these aspects are highly important [21] considering the urgent need for a reduction in antibiotics [22] due to the global concern regarding antimicrobial resistance [23,24]. In fact, from the perspective of animal livestock, which reflects the principles dictated by the One Health approach [25], it is increasingly important to introduce ingredients that are considered functional and promote health and prevent diseases [26]. The introduction of hemp seeds into the diets of laying hens has been shown not to have any adverse effects on egg-laying performance and, at the same time, led to an increase in linoleic acid and alpha-linolenic acid in the egg yolk [27–29]. Taubner and colleagues [30] highlighted that adding flaxseed and hemp seed (80 and 50 g/kg feed, respectively) to broiler feed increases digestive enzyme activities, positively affecting growth parameters, feed conversion and digestibility. Hemp is a good source of protein that is not degraded by the rumen and is therefore suitable for use in ruminant feed. The introduction of hemp seeds as a protein source into the diet of calves instead of a mixture of soybean meal and barley (1.0 kg of protein feed [i.e., 1.0 kg of hempseed cake vs. 0.5 kg of soybean meal and 0.5 kg of barley] animal^{-1} day^{-1}) led to a similar production and improved rumen function [31,32]. In a recent article, we highlighted the potential of hemp in the pet food sector as animal feed [33]. However, little is known about the individual bioactive components of hemp and the interactions that occur within this organism. Our aim in the present study was to, therefore, perform a comprehensive chemical and functional characterization of hulled hemp seeds in order to assess their nutritional potential and properties for use in animal nutrition. In addition, given the current lack of studies assessing whether the bioactive activities of hemp are retained even after the digestive process and, therefore, whether hemp provides health benefits, our second aim was to investigate whether these functional properties are also maintained after the digestive process.

2. Materials and methods

2.1. Chemical composition

The chemical analyses were performed on the samples following the "Official Methods of Analysis," according to AOAC [34], to determine the main nutritional components (ash, crude fiber, crude protein, ether extract).

2.2. Extraction procedure

For the evaluation of functional compounds, the following extraction procedure was employed. Five grams of *Cannabis sativa* L. ground seeds were defatted twice in 10 mL of n-hexane and placed in an ultrasonic bath for 5 min. Afterwards, the sample was centrifuged at 5000×*g* for 5 min at RT, after which the hexanoic phase was discarded. A total of 10 mL of HPLC-H2O and 10 mL of acetonitrile were added to the sample, which was manually agitated for 2 min. QuEChERS salts (Agilent, California, United States) were added to the sample. The sample was again manually agitated for 2 min, and then centrifuged at 5000×*g* for 5 min at room temperature (RT). After centrifugation, the supernatant was discarded, and 10 mL of methanol was added. The sample was manually agitated for 2 min and centrifuged at 5000×*g* for 5 min at RT. The supernatant was filtered through a 0.22 μm RC filter.

2.3. HPLC-Q-TOF-MS/MS metabolomic characterization

High performance liquid chromatography (HPLC) and mass spectrometric analyses (LC–MS^2) were performed according to the methods of Xiang and colleagues [35] through an HPLC (Sciex, Massachusetts, USA) instrument equipped with a photodiode array (PDA) detector (Sciex, Massachusetts, USA) and an autosampler (Sciex, Massachusetts, USA) coupled to a quadrupole time-of-flight mass spectrometer (Q-TOF-MS) (X500R QTOF, Sciex, Massachusetts, USA) with some modifications. Briefly, the analytical column used was a 100 mm × 3 mm Accucore C18 column (Thermo Fisher Scientific, Waltham, USA). Ten microliter of the sample was injected by an autosampler and eluted through the column with a binary mobile phase consisting of A (water containing 0.1% formic acid) and B (methanol containing 0.1% formic acid). A flow rate of 0.4 mL/min was used. A 35 min linear gradient was programmed as follows: 0–5 min, 0.5% A, 99.5% B; 5–25 min, 75% A, 25% B; 25–31 min, 5% A, 95% B; and 31–35 min, 95% A, 5% B. Compounds were identified by comparing retention time (RT) and UV spectral information with those in the library of Natural Compounds (Sciex, Massachusetts, USA) and confirmed by HPLC-Q-TOF-MS^2.

2.4. Evaluation of total polyphenol content (TPC)

TPC of *C. sativa* L. extracts, at different dilutions (100%; 50%; 25%; 12.5%; 6.25%; 3.12%), was evaluated by the Folin-Ciocalteu method, according to Attard [36]. TPC was determined spectrophotometrically at 630 nm (V-630 UV–vis, JASCO, Germany). Calibration curves were prepared in five 1:2 dilutions, from 960 μg/mL to 60 μg/mL, with tannic acid as the standard. Each sample and standard were run in triplicate. The TPC was expressed as Tannic Acid Equivalents (TAE) per mg of seeds (mg TAE/100g).

2.5. Evaluation of total flavonoid content (TFC)

TFC of *C. sativa* L. extract, at different dilutions (100%; 50%; 25%; 12.5%), was measured using the aluminum chloride (AlCl3) colorimetric method described by Herald, T.J [37]. Each sample and standard were run in triplicate. Calibration curves were prepared in 1:2 serial dilutions, from 250 μg/mL to 7 μg/mL, with catechin as the standard. TFC was expressed as mg catechin equivalent (CE) g−1 sample (mg CE/g).

2.6. Evaluation of antioxidant properties

2.6.1. Evaluation of total antioxidant capacity (TAC)

TAC of *C. sativa* L. extracts was assessed using an antioxidant assay kit (MAK334 Sigma-Aldrich, MO, USA) following the manufacturer's instructions. The TAC of the sample was calculated by the following formula:

$$TAC\ (\mu M) = \frac{(A_{570})_{sample} - (A_{570})_{blank}}{Slope\ (\mu M^{-1})} * n$$

where

$(A570)_{sample}$ = absorbance of the sample

$(A570)_{blank}$ = absorbance of the medium blank

n = sample dilution factor

2.6.2. Evaluation of ABTS radical scavenging activity

The scavenging activity of the hemp seeds was evaluated using an ABTS assay according to Hsu et al. [38]. Briefly, 10 μL of the sample was added to 1 mL of $ABTS^{\bullet+}$ working solution. The absorbance was recorded every 6 min for a total of 30 min of incubation in the dark, and all the determinations were performed in triplicate. The percentage inhibition of radical scavenging activity (PI%) was calculated according to Frazzini et al. [39].

2.7. Growth inhibition assay

A liquid culture-based growth inhibition assay with *Escherichia coli* O138 was performed to assess their ability to inhibit bacterial growth [40]. The growth inhibition assay was performed according to Frazzini et al. [41] on three different concentrations (1:4, 1:8; 1:16). In order to verify the replicability of the experiment using the same procedures, all assays were performed in technical quadruplicate and with three biological replicates. This included repeating the investigation starting from the sample extraction and repeating the test on different days.

2.8. Minimal inhibitory concentration (MIC)

MIC were determined through the broth microdilution method. Briefly, hemp seed extracts at different concentrations (20 mg/mL, 15 mg/mL, 10 mg/mL, 5 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.1 mg/mL, and 0 mg/mL) were prepared by diluting the dried extracts with Luria–Bertani (LB) broth. A total of 100 μL of the different concentrations were plated in a 96-well microplate, and 10 μL of an overnight culture of *E. coli* O138 in LB broth (approximately 10^6 CFU/mL) was inoculated in each well of the plate, except for the blank and negative control ($CTRL^-$) wells, and incubated at 37 °C for 20 h. The change in absorbance was determined after the microplates were read at 620 nm in a microplate reader spectrophotometer (Scan-Ready P-800, Life Real, Hangzhou, China). The following formula was used to estimate the inhibition rate:

$$Inhibition\ Rate\ (\%) = 100 * \left(\frac{ODCTRL^- - ODsample}{ODCTRL^- - ODBlank}\right)$$

The MIC was defined as the lowest extract concentration that did not produce turbidity compared to that of a positive control (0 mg of extract/mL) [42]. All the experiments were performed in technical triplicates and with two biological replicates.

2.9. In vitro digestion

In vitro digestibility was determined according to the methods of Minekus et al. [43] with some modifications. Briefly, 1 g of ground hulled *Cannabis sativa* L. seeds was weighed, and 20 mL of deionized water was added to 1 mL of α-amylase solution (150 mg of α-amylase [Sigma–Aldrich A3176–500KU] in 1 mL of $CaCl_2$ 1 M). The samples were then incubated at 37 °C for 30 min. The pH was then adjusted to 2 using HCl, and 2 mL of pepsin solution (100 mg of pepsin; Sigma–Aldrich P7000) in 2 mL of 0.1 M HCl was added to each sample. The samples were then incubated at 37 °C for 120 min. The pH was restored to neutral with NaOH and 2 mL of pancreatin/bile solution (200 mg of pancreatin (Sigma–Aldrich P1625) + 50 mg of bile (Sigma–Aldrich B8631) in 2 mL of 0.1 M NaCO3). Finally, the samples were incubated at 37 °C for 180 min. At the end of the *in vitro* digestion, a soluble fraction and an undigested fraction (UF) were obtained. The UF mixture was then collected in a filtration unit using a porcelain filtration funnel lined with pre-weighed filter paper (Whatman no. 54). The UF mixture and filter paper were dried overnight at 65 °C. The UF was used to calculate the *in vitro* digestibility (IVD) using the following equation:

$$IVD\ (\%) = \frac{(sample\ (DM) - sample\ UF\ (DM))}{(sample\ (DM) * 100)}$$

2.10. Determination of total phenolic and flavonoid contents after in vitro digestion

At the end of each digestive phase (oral, gastric, and intestinal), aliquots (2 mL) corresponding to the digested fraction were taken and frozen at −80 °C to stop the enzymatic activity and subsequently used to evaluate the TPC and TFC during *in vitro* digestion following the procedure described in Sections 2.4 and 2.5 below.

2.11. Bioaccessibility index

The bioaccessibility indices of phenolic and flavonoid compounds were calculated according to Santana Andrade and colleagues [44], based on the following formulas:

$$Phenolic\ bioaccesibility\ (\%) = \left(\frac{PC_2}{PC_1}\right) * 100$$

$$Flavonoids\ boaccesibility\ (\%) = \left(\frac{FC_2}{FC_1}\right) * 100$$

where PC_1: total phenolic content before *in vitro* digestion; PC_2: total phenolic content after *in vitro* digestion; FC_1: total flavonoid content before *in vitro* digestion; and FC_2: total flavonoid content after *in vitro* digestion.

2.12. Determination of antioxidant activity after in vitro digestion

The assessment of antioxidant activity following the different stages of digestion (oral, gastric, and intestinal) was performed by evaluating the ABTS radical scavenging activity according to Hsu et al. [38], as described in Section 2.6.

2.13. Determination of growth inhibition capacity against Escherichia coli O138 following simulation of the in vitro digestive process

To determine the growth inhibition capacity against *Escherichia coli* during and after digestion, the protocol of Pettersen and colleagues [45] was readjusted. Briefly, a culture of *E. coli* was prepared and allowed to grow overnight to obtain a concentration of 10^6 cells/ml. The *in vitro* digestion model of Minekus et al. [43], was performed as described in Section 2.9. Specifically, at the beginning of the digestive phase, 1 mL of cultured *Escherichia coli* was added to the sample to an initial concentration of 10^6 cells/ml per gram of sample. At the end of each digestive phase (oral, gastric, and intestinal), aliquots (2 mL) corresponding to the digested fraction were taken, and their absorbance was read by a spectrophotometer at a wavelength of 600 nm. The measured OD was then converted into the $\log_{10}$ of the number of cells/mL, considering 1 OD = 1 * 10^9 cells/mL [46]. All assays were performed in triplicate to verify the replicability of the experiments.

2.14. Statistical analysis

All the data were analysed using GraphPad Prism (version 9.0.0). The normality of the distribution of the data and residuals was evaluated by D'Agostino–Pearson tests. For the total polyphenol content, the data were analysed using one-way analysis of variance (ANOVA). Antioxidant activity data were analysed using one-way and two-way analysis of variance). For the growth inhibition assay, the data were analysed using two-way analysis of variance, which included the effects of treatment, time, and their interaction. Post hoc pairwise comparisons were performed using Bonferroni Sidak's test. The data are reported as the mean ± standard error, and differences were considered to be statistically

significant at $p \leq 0.05$.

3. Results

3.1. Chemical-nutritional composition

To determine the main nutritional components (ash, crude fibre, crude protein, ether extract), the chemical composition of hulled *C. sativa* L. seeds was analysed using the Official methods [34]. The results showed that hemp seeds, with a moisture equal to 6.75 ± 0.64%, had high amounts of crude lipids (52.02 ± 0.94% on a fresh matter basis) and crude proteins (34.52 ± 0.38% on a fresh matter basis), while the ash and fibre values were 3.61 ± 0.27% and 3.01 ± 0.10%, respectively, on a fresh matter basis.

3.2. Evaluation of the metabolomic profiles of the main molecules with bioactive properties

To identify the most commonly found bioactive molecules in hemp seeds, a metabolomic profiling analysis was carried out via HPLC-QTOF-MS/MS. This analysis revealed the presence of different molecules with bioactive properties in the hulled hemp seeds (Table 1). Most of the molecules found belonged to the class of phenols and flavonoids. The most common polyphenolic molecule found in the hemp seed extract was 4-hydroxybenzoic acid, with a peak area of 1628036. In addition to phenols and flavonoids, other molecules were revealed from different biochemical classes known for their bioactive characteristics. Our results showed high levels of linoleic acid, an essential fatty acid (EFA); geniposide, a natural component derived from the class of iridoid glycosides; and citric acid, an organic acid.

3.3. Evaluation of TPC and TFC

The TPC was evaluated in the extract of hulled hemp seeds using tannic acid as a reference standard. The results showed that the content was correlated with the dilution tested which, in the undiluted sample, was equal to 251.80 ± 6.34 mg TEA/g of sample (Fig. 1a). In addition to polyphenols, hemp seeds are known for their high content of flavonoids. This was also highlighted in our study, where the flavonoid content followed the same trend as that found in the polyphenol analysis, decreasing proportionally to the dilution. In our sample, the flavonoid content was thus equal to 71.16 ± 0.70 mg CE/g of sample (Fig. 1b).

3.4. Evaluation of antioxidant activity

The TAC was analysed, and the results revealed that the extract of the hulled hemp seeds had a TAC of 2720.95 ± 85.58 μM Trolox equivalent. In addition to TAC, the ability of the hemp seed extract to neutralize free radicals generated by the ABTS radical system was also evaluated. Our study revealed that, regardless of the dilution used, the oxidation process was completed after 30 min of incubation, during which the percentage inhibition of radical scavenging activity reached the maximum level (Fig. 2). After 30 min of incubation, the hulled hemp seeds had an ABTS radical scavenging activity, expressed as a percentage inhibition (PI%), equal to 60.00 ± 2.73 at a dilution of 1:2., As expected, the PI% decreases as the dilution factor increases until it reached a value of 7.06 ± 1.04 at a dilution of 1:32 (Fig. 3).

3.5. Evaluation of antimicrobial activity

3.5.1. Assessment of the growth inhibition against Escherichia coli O138

The growth inhibition activity of different dilutions (1:4, 1:8, 1:16) was evaluated against *Escherichia coli*, a gram-negative bacterium. The O138 strain of our strain library was isolated from *E. coli* and had been previously genetically characterized to verify the presence of two virulence factors: VT2e toxin and F18 adhesive fimbriae [40]. The results obtained showed that the 1:8 dilution was the optimal dilution for testing the growth inhibition of *E. coli*. Considering Log_{10} cells/mL, the absorbance of the hemp extracts (v/v) was lower than that of the bacteria during natural growth (Fig. 4b). On the other hand, this antimicrobial effect was not visible at lower and subsequent dilutions. The results (Fig. 4) highlighted that after 3 h of incubation, the addition of hemp extract to the *E. coli* liquid culture inhibited bacterial growth.

3.5.2. Evaluation of the MIC against Escherichia coli O138

The data obtained from the evaluation of growth inhibition against *E. coli* were confirmed by determining the MICs. As shown in Table 2, a concentration of 10 mg/ml, corresponding to an extract concentration of 1:8, was the lowest concentration at which an antimicrobial effect of the extract was observed against *E. coli* O138. The lowest inhibitory concentrations determined revealed an inhibition rate of 87% for the hemp seed extracts.

3.6. Evaluation of total polyphenols and flavonoids following in vitro digestion

The TPC and TFC were evaluated following the *in vitro* digestion process to assess the behavior of bioactive molecules following the digestive process. Our results, shown in Fig. 5a, revealed that at the end of the oral phase, there was partial degradation, with a consequent reduction in phenolic compounds (172.30 ± 4.06 mg TEA/g of sample). A significant ($p < 0.01$) increase in the TPC (501.50 ± 9.98 mg TEA/g of sample) was observed at the next stage compared to the initial content. Lastly, following the intestinal phase, the TPC was significantly lower than in the previous phase (358.60 ± 14.04 mg TEA/g of sample, $p < 0.01$). The same trend was also observed for flavonoids (Fig. 5b). As highlighted from the polyphenol analysis during the first phase of digestion, the release of flavonoids was minimal (4.39 ± 1.11 mg CE/g of sample). This value increased considerably during the gastric stage ($p < 0.01$) when the assay performed revealed that the TFC was equal to 48.74 ± 1.93 mg CE/g of sample, and the value was halved (20.97 ± 1.40 mg CE/g of sample, $p < 0.01$) throughout the intestinal phase.

Table 1
Chemical compounds identified after HPLC and mass spectrometric analysis.

Biochemical class	Compound identified	Formula	Area	RT (min)	$[M-H]^-$ (*m/z*, Da)	*m/z* (Da) of main fragments (relative intensity, %), MS/MS
Essential fatty acid	Linoleic acid	$C_{18}H_{32}O_2$	93659855	20.67	279.2320	261.21 (10); 279.23 (95)
Flavonoids	Glucosylvitexin	$C_{27}H_{30}O_{15}$	298389	13.08	593.1511	293.04 (100); 413.08 (80); 593.15 (15)
	Vitexin-2-*O*-rhamnoside	$C_{27}H_{30}O_{14}$	789316	13.04	577.1557	293.04 (100); 311.05 (20); 413.08 (70); 457.11 (10); 577.15 (10)
Iridoid glycoside	Geniposidic acid	$C_{16}H_{22}O_{10}$	597106	7.14	373.1137	59.01 (70); 96.96 (20); 211.06 (100)
Organic acid	Citric acid	$C_6H_8O_7$	191313429	2.38	191.0194	67.01 (80); 85.02 (35); 87.00 (100); 111.00 (90)
Phenols	4-Hydroxybenzoic acid	$C_7H_6O_3$	1628036	13.35	137.0244	65.03 (30); 93.03 (100)
	p-Coumaric acid	$C_9H_8O_3$	1507500	8.13	163.0401	93.03 (50); 117.03 (20); 119.05 (100)
	Isoferulic acid	$C_{10}H_{10}O_4$	11288204	9.12	193.0505	134.03 (100)
	Pinoresinol Diglucoside	$C_{32}H_{42}O_{16}$	128565	11.02	681.2394	151.04 (10); 357.13 (1009

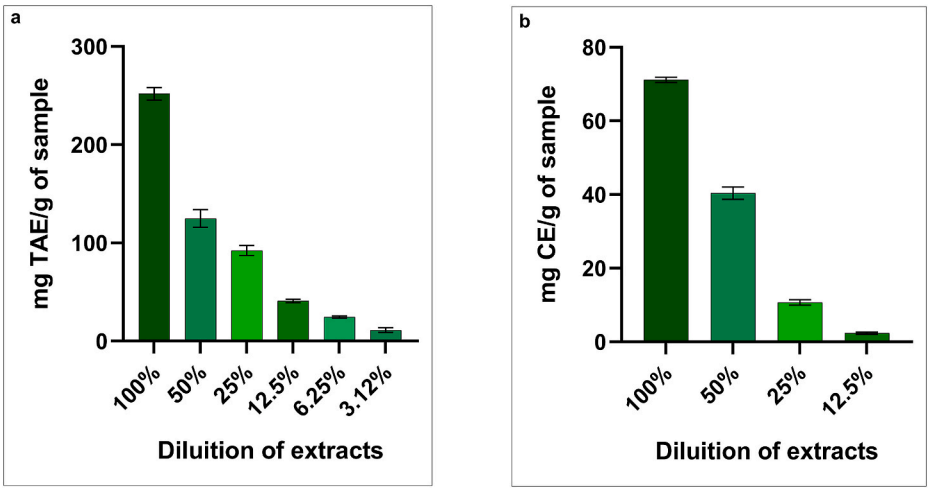

Fig. 1. Principal bioactive compounds in the hulled hemp seed extracts. (a) Total phenolic content (TPC). (b) Total Flavonoid Content (TFC). TAE: tannic acid equivalent; CE: catechin equivalent. The data are shown as the means ± standard deviations (SDs) (n = 3).

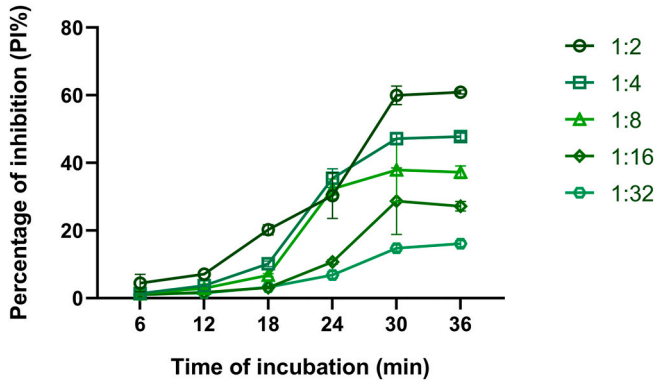

Fig. 2. Percentage inhibition (PI%) of radical scavenging activity at five different dilutions (1:2; 1:4; 1:8; 1:16; 1:32) of hulled hemp seed extract in relation to incubation time used to perform the ABTS assay. The data are shown as the means ± standard deviations (SDs) (n = 3).

3.7. Evaluation of the bioaccessibility indices of phenolic and flavonoid compounds

After evaluating the presence of bioactive compounds following the digestive process, the bioavailability of these compounds were assessed. The results showed that after the digestive process, the phenolic compounds had a bioavailability of 142.39%. In contrast to phenolic compounds, molecules belonging to the flavonoid class were less bioavailable, with a bioavailability index of 29.47%.

3.8. Evaluation of functional activity after in vitro digestion

3.8.1. Evaluation of antioxidant activity after in vitro digestion

Regarding the antioxidant activity analysed following the digestive process, the antioxidant activity (Fig. 6), expressed as a PI%, increased as the digestion time progressed from 39.97 ± 0.71% after the oral phase to 50.28 ± 0.62% at the end of the intestinal phase ($p < 0.01$).

3.8.2. Evaluation of antimicrobial activity after in vitro digestion

The antimicrobial capacity of the hemp seeds was also maintained during the digestion process. In fact, as shown in Fig. 7, during the gastric and intestinal phases, the sample simulating the ingestion of hemp seeds was shown to significantly ($p < 0.01$) inhibit the growth of *Escherichia coli* by 12.57% and 14.94%, respectively. In addition, the antimicrobial activity of the hemp seeds significantly ($p < 0.01$) increased between the first and second phases of digestion, from 7.16 ± 0.07 $\log_{10}$ cell/mL to 6.67 ± 0.01 $\log_{10}$ cell/mL. This trend, although less obvious, was maintained between the following two phases of digestion. In fact, during the last phase of digestion (the intestinal phase), the $\log_{10}$ cell/mL ratio was equal to 6.57 ± 0.10 ($p = 0.023$).

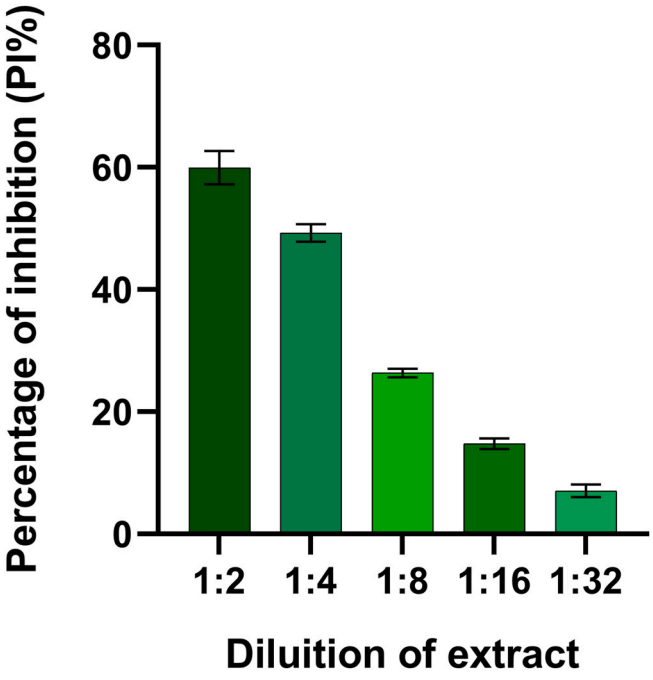

Fig. 3. Percentage inhibition of radical scavenging activity (PI%) at five different dilutions (1:2; 1:4; 1:8; 1:16; 1:32) of hulled hemp seed extract after 30 min of incubation. The data are shown as the means ± standard deviations (SDs) (n = 3).

4. Discussion

4.1. Chemical-nutritional composition

4.1.1. Nutritional composition

The data obtained from the bromatological analysis confirmed that *Cannabis sativa* L. seeds are a highly nutritional source of protein [47]. In

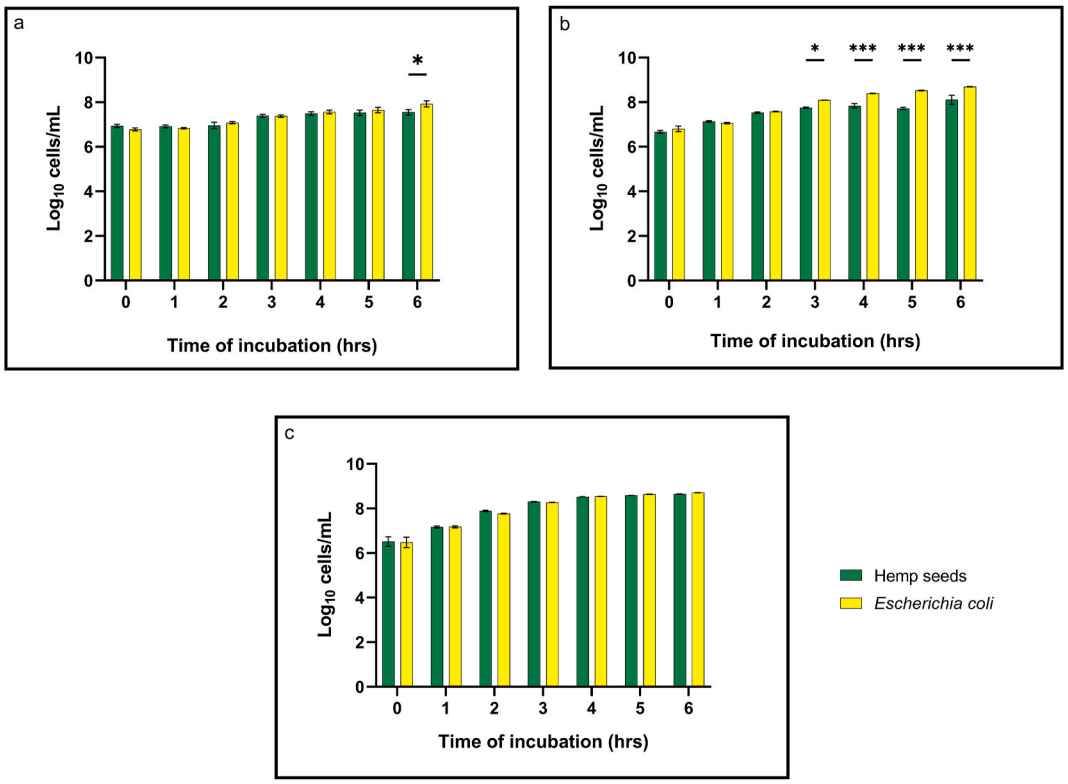

Fig. 4. Hulled hemp seed extract growth inhibition against *E. coli* F18^{+}. (a) Growth inhibition assay at a dilution of 1:4. (b) Growth inhibition assay at a dilution of 1:8. (c) Growth inhibition assay at a dilution of 1:16. The data are shown as the means and standard errors. Asterisks (n = 3) with different superscripts are significantly different; ***p < 0.001, *p < 0.05.

Table 2
Minimal inhibitory concentration (MIC) of hulled hemp seeds against *Escherichia coli* F18^{+}.

Minimal Inhibitory Concentrations (MICs)							
Extract concentration (mg/mL)	20	15	10	5	1	0.5	0.1
Inhibition rate (%)	76.98	86.79	87.01	86.44	75.71	49.08	33.12

the matrix considered in this study, the decortication process enabled us to reduce the fibre content (mostly from the husks and hulls), thus obtaining raw materials with a higher protein and lipid content [48].

This aspect has become increasingly important. The high protein content required for animal production diets is a challenge for Europe in terms of finding alternative protein sources that can replace soybean meal, which is the most commonly used plant protein due to its technical and nutritional properties, but which is increasingly less available on the market [49]. Due to their high protein content, hulled hemp seeds therefore represent an interesting alternative to traditional protein sources.

However, the decortication process also leads to the production of ingredients with a higher lipid content, which need to be considered in the diet formulation. In the case of hulled hemp seeds, the lipids present are mainly polyunsaturated, more than 70% of which are essential fatty acids (n-3 and n-6) which are necessary for regulating the organism's metabolic activity. This highlights the need for appropriate preservation strategies to prevent peroxidative processes [50].

Hemp seeds are therefore a good source of polyunsaturated and essential fatty acids, and the high value of n-3 could contribute to the favourable shift in the omega-6/omega-3 ratios [51]. For this reason, hemp oil, which is made by pressing whole seeds and is available on the market, is regarded as an excellent source of essential fatty acids. In addition, the low amount of moisture matter ensures good product conservation. In fact, the controlled humidity of the seeds used as a food resource is considered the most important process for maintaining the quality of non-perishable preserved foods, such as legumes and different varieties of cereals. In fact, inadequate food storage leads to a loss of quality and nutrients and, consequently, to the wastage as it can no longer be consumed.

4.1.2. Evaluation of the metabolomic profiles of the main molecules with bioactive properties

Most of the molecules found through the HPLC-QTOF-MS/MS analysis are phenols and flavonoids that are known for their biological properties, such as antioxidant, antimicrobial, and anti-inflammatory activities [52]. The molecules belonging to the phenol chemical class, found by metabolomic analysis have antioxidant, antimicrobial, and anti-inflammatory effects. The antioxidant capacity of phenols, characterized by the presence of OH groups, decreases the rate of organic matter oxidation by transferring a hydrogen atom from their OH groups to chain-carrying ROO• radicals. This transfer likely involves the synchronized movement of hydrogen as a proton and one electron between the two oxygen atoms, known as the proton-coupled electron transfer mechanism (O–H···O•) [53].

Phenols and their derivatives are also known for their antimicrobial activity. The effects of phenolic compounds on bacterial cells can damage the bacterial membrane, inhibit virulence factors such as enzymes and toxins, and suppress the formation of bacterial biofilms [54].

Among the other molecules revealed through HPLC-QTOF-MS/MS,

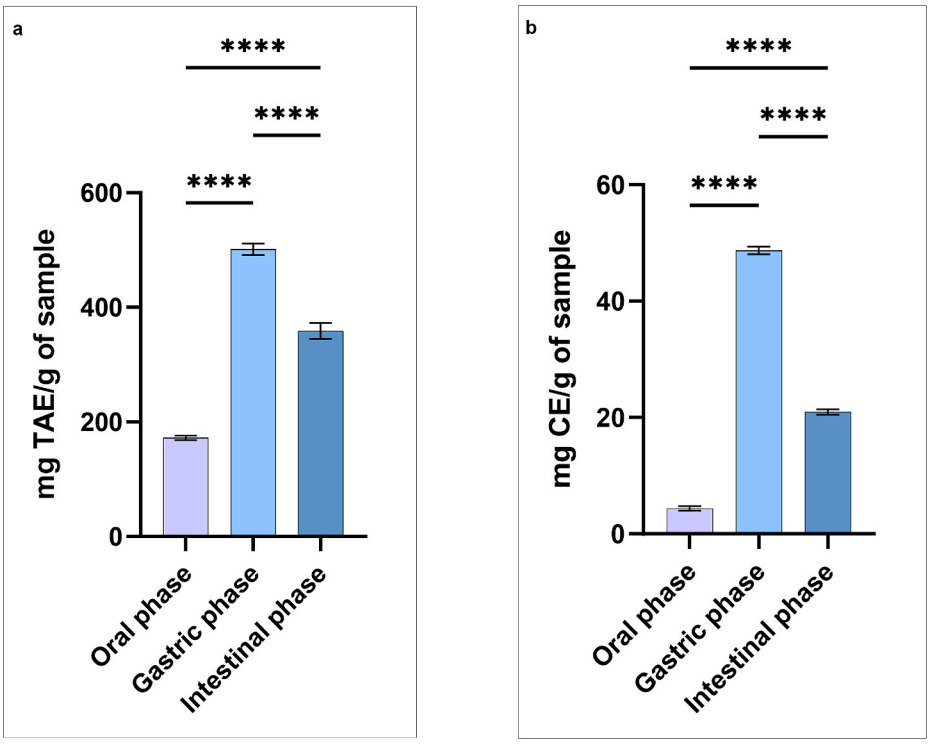

Fig. 5. Bioaccessibility of the principal bioactive compounds in hulled hemp seeds. (a) Total phenolic content (TPC) after *in vitro* digestion. (b) Total flavonoid content (TFC) after *in vitro* digestion. TAE: tannic acid equivalent; CE: catechin equivalent. The data are shown as the means ± standard deviations (SDs) (n = 3). Asterisks (n = 3) with different superscripts are significantly different; ****$p < 0.0001$.

linoleic acid regulates inflammatory responses [55]. EFAs give rise to biologically active hormones called eicosanoids, which have short half-lives. Eicosanoids derived from n-3 EFAs have anti-inflammatory properties, whereas those originating from n-6 EFAs are proinflammatory. Geniposide is a natural component of the iridoid glycoside class with several bioactive properties [56], such as antioxidant [57], anti-inflammatory [58], and antifungal [59] properties.

Lastly, our analysis revealed the presence of citric acid, which is an organic acid that is not typically considered as a bioactive compound. However, in addition to improving the shelf life of food products, it is essential for various biological processes that indirectly affect the body [60].

4.2. Evaluation of TPC and TFC

Our results of the analysis of the polyphenol content of hulled hemp seeds were in line with those found in the literature [61]. Although TPC was found to be strongly correlated with several factors, such as hemp variety, growing conditions, and processing method [62–64], it is well known that hemp seeds are rich in bioactive components, including polyphenols [65]. One of the primary polyphenols found in hemp seeds is Cannflavin A, a flavone that has been shown to have potential anti-inflammatory properties [66]. Additionally, hemp seeds contain other polyphenolic compounds, such as proanthocyanidins, flavonoids, lignans, hydroxycinnamic acids, and hydroxybenzoic acid [17,65]. These compounds have antioxidant, anti-inflammatory, prebiotic, and antimicrobial properties and may help protect the body against oxidative stress and damage caused by free radicals [67–69]. In addition, flavonoids and other metabolites are believed to interact with cannabinoids and alter their characteristics, resulting in a diverse array of pharmacological effects based on distinct cannabis chemotypes [70].

4.3. Evaluation of the functional properties of Cannabis sativa L. Seeds

4.3.1. Assessment of antioxidant activity

The results from the TAC, are in line with the data in the literature [71,72], and are also supported by the metabolomic analyses which showed the presence of several molecules with antioxidant activities. In fact, in addition to the presence of polyphenolic compounds, the metabolomic profile showed the presence of molecules, such as linoleic acid and geniposidic acid. Although they are not polyphenols, these molecules still have several bioactive properties [56]. As with all vegetable matrices, hemp seeds produce a wide variety of secondary metabolites that serve as defence and signaling compounds [73]. The active principles of secondary metabolites and plants can form a complex that combines the effects of different compounds to ensure a wide array of biological and pharmacological properties [74].

Following the determination of total antioxidant activity, our study also evaluated the ability of hemp seed extract to neutralize the free radicals generated by the ABTS radical system.

The results obtained firstly showed that the incubation time needed to achieve total scavenging activity was 30 min, which is in agreement with the literature [75]. Although the used incubation time for the ABTS assay is approximately 6 min [76], the incubation time can vary depending on the research methodology, the objectives of the study, and the nature of the samples being tested [77]. When the ABTS assay is used for fat matrix samples, such as hemp seeds, an extended incubation time is often employed compared to that used for aqueous samples. This is because the oxidation reactions in fats tend to be slower than those in aqueous systems [78]. Moreover, Dawidowicz and Olszowy [79] reported that the type of solvent used significantly affects the estimated antioxidant activity of the phenolic compounds in the ABTS assay. In particular, their antioxidant activity increases with the aliphatic chain elongation in alcohol. This difference is mainly due changes in the solvation energy of $ABTS^{\bullet+}$ by the solvent used rather than from changes in the dissociation of the phenolic compounds.

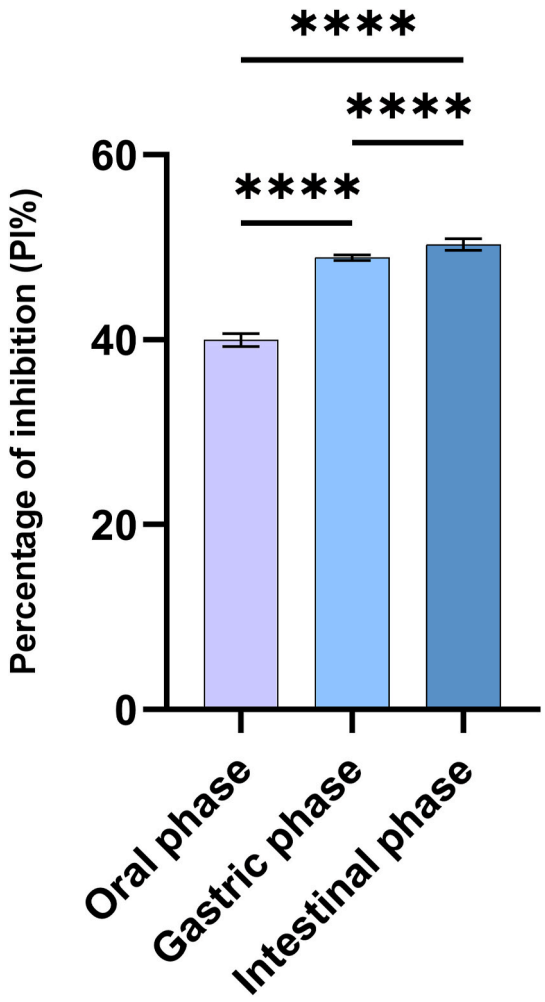

Fig. 6. Percentage inhibition of radical scavenging activity (PI%) after *in vitro* digestion of hulled hemp seed extract after 30 min of incubation. The data are shown as the means ± standard deviations (SDs) (n = 3). Asterisks (n = 3) with different superscripts are significantly different; ****p < 0.0001.

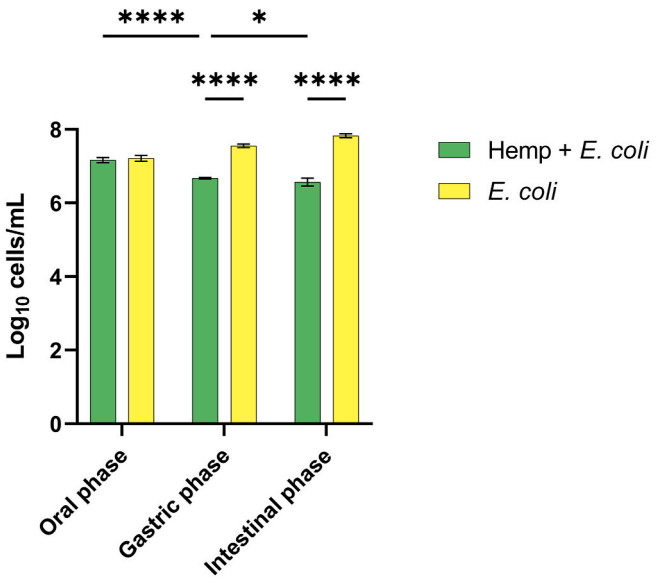

Fig. 7. Growth inhibition of hulled hemp seeds against *E. coli* F18+ during the digestive process. The data are shown as the means ± standard deviations (SDs) (n = 3). Asterisks (n = 3) with different superscripts are significantly different; ****p < 0.0001, *p = 0.023.

At the peak of scavenging activity, after 30 min of incubation, the antioxidant activity values expressed in terms of percentage inhibition showed that the decorticated hemp seeds had a good antioxidant power. Our data values appear lower than those of Kalinowska and colleagues [80], who reported that the antioxidant activity of hemp seeds was 93.30 ± 1.09%. This difference can be explained by considering the hulling process of the hemp seeds we analysed, which can affect the antioxidant capacity. The hull or outer shell of seeds typically contains a significant portion of the phenolic compounds and antioxidants. Therefore, removing the hulls can potentially reduce the antioxidant capacity of hemp seeds [61,81]. In any case, as shown from our results and other studies [61,82], hulled hemp seeds can retain a considerable antioxidant capacity even after hull removal.

4.3.2. Assessment of antimicrobial activity

The antimicrobial activity of the hemp seed extract was evaluated, since several studies have shown that some secondary metabolites (such asphytoalexins, phenolic compounds, and terpenes) produced by plants can perform a variety of functions, including defence against pathogenic microorganisms, thus demonstrating an antimicrobial activity [83–85]. In the present study, we evaluated the antimicrobial activity of hulled hemp seeds against *Escherichia coli*, specifically against the *E. coli* O138 strain.

Our study showed that hemp seeds can only partially inhibit the growth of *E. coli*, however their antimicrobial ability was observed only at high concentrations. The results obtained are in line with those found in the literature. In fact, the inhibition of the growth of *E. coli* is likely due to the high content of polyphenols present in the hemp seed extract [40].

However in terms of antimicrobial efficacy, the results in the literature are mixed. Comparable results to ours were found by Pasquali and colleagues [86], who reported that industrial hemp plants (*Cannabis sativa* L. variety Futura 75) exhibited *in vitro* antimicrobial effects against foodborne pathogens such as *L. monocytogenes* and *Staphylococcus* spp. but not against *S. Typhimurium* or *E. coli*. On the other hand, some authors report greater antimicrobial power against gram-negative bacteria [87].

This discrepancy could be explained by the solvent used for the extraction. Khan et al. [88] reported that oil, aqueous, and ethanol extracts were found to be only slightly active against *E. coli*. In contrast, high activity was attributed to methanol and petroleum hemp extracts. In addition, the antioxidant activity of hemp seeds is frequently associated with the content of cannabidiol (CBD) [87,89] since the hemp seeds under consideration complied with the legally prescribed levels of CBD, which could explain the overall antioxidant activity found.

4.4. Evaluation of TPC and TFC following in vitro digestion

Our evaluation of the bioactive molecules, polyphenols, and flavonoids following *in vitro* digestion was in line with the literature [90], revealing that at the end of the oral phase, there was partial degradation, with a consequent reduction in phenolic compounds [91,92]. This degradation was followed by an increase in TPCs during the gastric phase. This can be attributed to the fact that in the gastric tract, the pH is low (pH 2), which promotes phenol release. Finally, following the intestinal phase, the TPC decreases again due to the instability of polyphenols at a neutral pH (pH 7) [90]. The same trend was also observed for flavonoids. The observed trend in flavonoid content following the different stages of digestion could be related to the chemical transformations that occur during the digestive process. The increase in flavonoid content observed at the end of the gastric phase could be the result of the action of enzymes on the residual matrix. In contrast, the lower values observed during the oral and intestinal stages could be due

to degradation or isomerization in the presence of oxygen and/or transition metal ions under near-neutral conditions [93]. In the oral phase, although saliva seems to play a role in enhancing the availability of polyphenols, the initial interaction of oral enzymes with these compounds is limited, especially for carbohydrate-rich foods [94,95]. In the stomach, flavonoids degrade, and in the small intestine, various processes occur, such as deglycosylation and hydroxylation [96]. The compounds then undergo further transformations in the large intestine by microbial action [97]. Accurately determining the performance of these phenolic compounds during the digestive process is complex and closely related to the methodology employed [98]. However, our results agree with those found in the literature [99–101].

4.5. Evaluation of the bioaccessibility indices of phenolic and flavonoid compounds

Calculation of the bioavailability of bioactive compounds revealed that phenolic compounds have a high bioavailability since the bioavailability of phenolic compounds may increase following salivation, which allows for better solubilization of the phenolic compounds themselves [95]. In addition, the pH is lower (pH 2) during the gastric phase, which could promote the release of phenols, thereby increasing their availability at the intestinal level [90]. In contrast, molecules belonging to the flavonoid class are less bioavailable. Balakrishnan and Schneider [102] reported that flavonoids are bioaccessible during digestion and are largely released during the gastrointestinal phase. However, the low bioavailability of flavonoids disclosed in our study may be related to interactions at various stages of digestion, which are strongly affected by the molecular structure of flavonoids [103].

4.6. Evaluation of functional activity after in vitro digestion

The results of our antioxidant activity analysis following the digestive process are consistent with the scientific evidence available. According to Pinacho et al. [104], phenolic compounds undergo a reaction in response to the alkaline pH of the intestinal phase. The passage from an acidic medium to an alkaline medium is related to an increase in phenolic and flavonoid compounds, which in turn are associated with increased antioxidant activity [105]. This phenomenon arises due to the deprotonation of hydroxyl groups within aromatic rings [106,107]. Another aspect that influences antioxidant capacity is the interaction of phenolic compounds with other compounds released during digestion, such as minerals or dietary fibre, which affects solubility and phenol availability [108].

As shown by the analysis conducted in this study, hemp seeds can also exert antimicrobial activity, possibly because of their high content of polyphenols, which, as reported by Patnaik and colleagues [109], are known for their antibacterial activity, especially against *S. aureus* and *E. coli*. The results obtained after the *in vitro* digestion highlighted that hemp seeds retain their growth-inhibiting ability even during this process. Although kinetics studies should be conducted to assess the exact behavior during digestion, with these preliminary data, we can therefore state that the secondary metabolites in hemp seeds that confer antimicrobial activity were not affected by changes in the environmental conditions occurring during digestion.

5. Conclusion

This study investigated the functional properties of hulled hemp seeds by employing a metabolomics approach supplemented with an *in vitro* evaluation of several significant functional characteristics. Our study revealed the presence of bioactive molecules, such as polyphenols and flavonoids, within hulled hemp seeds. These results help to explain the results later found during functional assays carried out *in vitro*, which showed that hemp seeds possess strong antioxidant and antimicrobial capabilities. Our results also highlighted that the functional properties of hemp seeds were maintained during the digestion process, further reinforcing the beneficial effects that introducing this by-product within the diet. The high nutritional value of hemp seeds combined with their functional value suggest that hemp seeds can be considered as viable ingredients for the animal feed industry. We believe that the encouraging results obtained in this study therefore lay a solid foundation for future in vivo studies.

Data and model availability statement

The data presented in this study are not deposited in an official repository. The data are available within the article and from the corresponding author upon reasonable request.

CRediT authorship contribution statement

S. Frazzini: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M.C. Torresani:** Methodology, Investigation. **G. Roda:** Investigation. **M. Dell'Anno:** Writing – review & editing, Validation. **G. Ruffo:** Writing – review & editing, Supervision. **L. Rossi:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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.

Acknowledgements

The authors would like to thank HEMPET S.r.l. who provided the hulled seeds of *Cannabis sativa* L.

References

[1] S.A. Hassan, M. Abbas, S. Zia, A.A. Maan, M.K.I. Khan, A. Hassoun, A. Shehzad, R. Gattin, R.M. Aadil, An appealing review of industrial and nutraceutical applications of pistachio waste, Crit. Rev. Food Sci. Nutr. (2022) 1–19, https://doi.org/10.1080/10408398.2022.2130158.
[2] M.A. Shalaby, R.A. Ghandour, S.R. Emam, Coadministration of ginger roots extract and vitamin E improves male fertility of streptozotocin-induced diabetic rats, Int. J. Vet. Sci. 11 (2022) 183–188, https://doi.org/10.47278/journal.ijvs/2021.083.
[3] A. Mubashir, A. Ghani, A. Mubashar, Common medicinal plants effective in peptic ulcer treatment: a nutritional review, Int. J. Agric. Biosci. 11 (2022) 70–74, https://doi.org/10.47278/journal.ijab/2022.010.
[4] A. Misganew, Nutritional and anti-nutritional characteristics of okra (abelmoschus esculents (L.) moench) accessions grown in pawe district, northwestern Ethiopia, Int. J. Agric. Biosci. 12 (2023) 18–21, https://doi.org/10.47278/journal.ijab/2022.040.
[5] G. della Rocca, A. Di Salvo, Hemp in veterinary medicine: from feed to drug, Front. Vet. Sci. 7 (2020), https://doi.org/10.3389/fvets.2020.00387.
[6] G. Piluzza, G. Delogu, A. Cabras, S. Marceddu, S. Bullitta, Differentiation between fiber and drug types of hemp (Cannabis sativa L.) from a collection of wild and domesticated accessions, Genet. Resour. Crop Evol. 60 (2013) 2331–2342, https://doi.org/10.1007/s10722-013-0001-5.
[7] I. Adesina, A. Bhowmik, H. Sharma, A. Shahbazi, A review on the current state of knowledge of growing conditions, agronomic soil health practices and utilities of hemp in the United States, Agriculture 10 (2020) 129, https://doi.org/10.3390/agriculture10040129.

[8] M. Hejna, E. Onelli, A. Moscatelli, M. Bellotto, C. Cristiani, N. Stroppa, L. Rossi, Heavy-metal phytoremediation from livestock wastewater and exploitation of exhausted biomass, Int. J. Environ. Res. Publ. Health 18 (2021) 2239, https://doi.org/10.3390/ijerph18052239.

[9] C. Moscariello, S. Matassa, G. Esposito, S. Papirio, From residue to resource: the multifaceted environmental and bioeconomy potential of industrial hemp (Cannabis sativa L.), Resour. Conserv. Recycl. 175 (2021) 105864, https://doi.org/10.1016/j.resconrec.2021.105864.

[10] G. Kaur, R. Kander, The sustainability of industrial hemp: a literature review of its economic, environmental, and social sustainability, Sustainability 15 (2023) 6457, https://doi.org/10.3390/su15086457.

[11] M. Geissdoerfer, P. Savaget, N.M.P. Bocken, E.J. Hultink, The Circular Economy – a new sustainability paradigm? J. Clean. Prod. 143 (2017) 757–768, https://doi.org/10.1016/j.jclepro.2016.12.048.

[12] V.M.C. Moliterni, M. Pojić, B. Tiwari, Industrial hemp by-product valorization, in: Industrial Hemp, Elsevier, 2022, pp. 301–340, https://doi.org/10.1016/B978-0-323-90910-5.00007-5.

[13] Report ID: GVR-2-68038-389-8, Industrial Hemp Market Size, Share & Trends Analysis Report By Product (Seeds, Fiber, Shivs), By Application (Animal Care, Textiles, Food & Beverages, Construction Materials, Personal Care), By Region, And Segment Forecasts, 2023 – 2030; Report ID: GVR-2-68038-389-8, (n.d.). https://www.grandviewresearch.com/industry-analysis/industrial-hemp-market#:~:text=What%20is%20the%20industrial%20hemp,USD%2016.75%20billion%20by%202030 (accessed October 30, 2023).

[14] R.A. Burton, M. Andres, M. Cole, J.M. Cowley, M.A. Augustin, Industrial hemp seed: from the field to value-added food ingredients, J Cannabis Res 4 (2022) 45, https://doi.org/10.1186/s42238-022-00156-7.

[15] G. Dhadwal, M.G. Kirchhof, The risks and benefits of cannabis in the dermatology clinic, J. Cutan. Med. Surg. 22 (2018) 194–199, https://doi.org/10.1177/1203475417738971.

[16] S. Kumar, R. Singh, V. Kumar, A. Rani, R. Jain, Cannabis sativa: a plant suitable for phytoremediation and bioenergy production, in: Phytoremediation Potential of Bioenergy Plants, Springer Singapore, Singapore, 2017, pp. 269–285, https://doi.org/10.1007/978-981-10-3084-0_10.

[17] B. Farinon, R. Molinari, L. Costantini, N. Merendino, The seed of industrial hemp (cannabis sativa L.): nutritional quality and potential functionality for human health and nutrition, Nutrients 12 (2020) 1935, https://doi.org/10.3390/nu12071935.

[18] F. Semwogerere, C.L.F. Katiyatiya, O.C. Chikwanha, M.C. Marufu, C. Mapiye, Bioavailability and bioefficacy of hemp by-products in ruminant meat production and preservation: a review, Front. Vet. Sci. 7 (2020), https://doi.org/10.3389/fvets.2020.572906.

[19] D. Lanzoni, E. Skřivanová, R. Rebucci, A. Crotti, A. Baldi, L. Marchetti, C. Giromini, Total phenolic content and antioxidant activity of in vitro digested hemp-based products, Foods 12 (2023) 601, https://doi.org/10.3390/foods12030601.

[20] S. Frassinetti, E. Moccia, L. Caltavuturo, M. Gabriele, V. Longo, L. Bellani, G. Giorgi, L. Giorgetti, Nutraceutical potential of hemp (Cannabis sativa L.) seeds and sprouts, Food Chem. 262 (2018) 56–66, https://doi.org/10.1016/j.foodchem.2018.04.078.

[21] E. Kasapidou, E. Sossidou, P. Mitlianga, Fruit and vegetable Co-products as functional feed ingredients in farm animal nutrition for improved product quality, Agriculture 5 (2015) 1020–1034, https://doi.org/10.3390/agriculture5041020.

[22] European Commission, Regulation (EU), 2019/6 of the European parliament and of the council of 11 december 2018 on veterinary medicinal products and repealing directive 2001/82/EC, Off. J. Eur. Union L4, (2019) 43–167.

[23] W. Windisch, K. Schedle, C. Plitzner, A. Kroismayr, Use of phytogenic products as feed additives for swine and poultry1, J. Anim. Sci. 86 (2008) E140–E148, https://doi.org/10.2527/jas.2007-0459.

[24] M. Hejna, L. Kovanda, L. Rossi, Y. Liu, Mint oils: in vitro ability to perform anti-inflammatory, antioxidant, and antimicrobial activities and to enhance intestinal barrier integrity, Antioxidants 10 (2021) 1004, https://doi.org/10.3390/antiox10071004.

[25] W.B. Adisasmito, S. Almuhairi, C.B. Behravesh, P. Bilivogui, S.A. Bukachi, N. Casas, N. Cediel Becerra, D.F. Charron, A. Chaudhary, J.R. Ciacci Zanella, A. A. Cunningham, O. Dar, N. Debnath, B. Dungu, E. Farag, G.F. Gao, D.T. S. Hayman, M. Khaitsa, M.P.G. Koopmans, C. Machalaba, J.S. Mackenzie, W. Markotter, T.C. Mettenleiter, S. Morand, V. Smolenskiy, L. Zhou, One Health: a new definition for a sustainable and healthy future, PLoS Pathog. 18 (2022) e1010537, https://doi.org/10.1371/journal.ppat.1010537.

[26] EFSA, Scientific Opinion on the safety of hemp (Cannabis genus) for use as animal feed, EFSA J. 9 (2011), https://doi.org/10.2903/j.efsa.2011.2011.

[27] F.G. Silversides, M.R. LefranÇois, The effect of feeding hemp seed meal to laying hens, Br. Poultry Sci. 46 (2005) 231–235, https://doi.org/10.1080/00071660500066183.

[28] N. Gakhar, E. Goldberg, M. Jing, R. Gibson, J.D. House, Effect of feeding hemp seed and hemp seed oil on laying hen performance and egg yolk fatty acid content: evidence of their safety and efficacy for laying hen diets, Poultry Sci. 91 (2012) 701–711, https://doi.org/10.3382/ps.2011-01825.

[29] E.M. Goldberg, N. Gakhar, D. Ryland, M. Aliani, R.A. Gibson, J.D. House, Fatty acid profile and sensory characteristics of table eggs from laying hens fed hempseed and hempseed oil, J. Food Sci. 77 (2012), https://doi.org/10.1111/j.1750-3841.2012.02626.x.

[30] T. Taubner, M. Skřivan, M. Englmaierová, L. Malá, Effects of hemp seed and flaxseed on enzyme activity in the broiler chicken digestive tract, Animal 17 (2023) 100765, https://doi.org/10.1016/j.animal.2023.100765.

[31] A. Hessle, M. Eriksson, E. Nadeau, T. Turner, B. Johansson, Cold-pressed hempseed cake as a protein feed for growing cattle, Acta Agric Scand A Anim Sci 58 (2008) 136–145, https://doi.org/10.1080/09064700802452192.

[32] L. Karlsson, M. Finell, K. Martinsson, Effects of increasing amounts of hempseed cake in the diet of dairy cows on the production and composition of milk, Animal 4 (2010) 1854–1860, https://doi.org/10.1017/S1751731110001254.

[33] S. Frazzini, P. Cremonesi, E. Scaglia, B. Castiglioni, F. Biscarini, V. Besana, L. Rossi, Effect of hulled Cannabis sativa L. seeds in a home-made diet for adult dogs, Ital. J. Anim. Sci. 22 (2023) 784–797, https://doi.org/10.1080/1828051X.2023.2241489.

[34] AOAC, AOAC. Official Methods of Analysis, sixteenth ed., vol. 2019, Association of Official Analytical Chemists, Washington, DC, USA, 2019.

[35] J. Xiang, F.B. Apea-Bah, V.U. Ndolo, M.C. Katundu, T. Beta, Profile of phenolic compounds and antioxidant activity of finger millet varieties, Food Chem. 275 (2019) 361–368, https://doi.org/10.1016/j.foodchem.2018.09.120.

[36] E. Attard, A rapid microtitre plate Folin-Ciocalteu method for the assessment of polyphenols, Open Life Sci. 8 (2013) 48–53, https://doi.org/10.2478/s11535-012-0107-3.

[37] T.J. Herald, P. Gadgil, M. Tilley, High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour, J. Sci. Food Agric. 92 (2012) 2326–2331, https://doi.org/10.1002/jsfa.5633.

[38] C.F. Hsu, H. Peng, C. Basle, J. Travas-Sejdic, P.A. Kilmartin, ABTS $^{\bullet+}$ scavenging activity of polypyrrole, polyaniline and poly(3,4-ethylenedioxythiophene), Polym. Int. 60 (2011) 69–77, https://doi.org/10.1002/pi.2912.

[39] S. Frazzini, A. Zuorro, S. Panseri, R. Pavlovic, C.A. Sgoifo Rossi, L. Rossi, Repurposing hazelnut waste products for a sustainable economy: a metabolomic analysis of cuticles and shells to highlight their antioxidant potential and inhibitory activity against verocytotoxic Escherichia coli, Sustainability 15 (2023) 3268, https://doi.org/10.3390/su15043268.

[40] S. Reggi, C. Giromini, M. Dell'Anno, A. Baldi, R. Rebucci, L. Rossi, In vitro digestion of chestnut and quebracho tannin extracts: antimicrobial effect, antioxidant capacity and cytomodulatory activity in swine intestinal IPEC-J2 cells, Animals 10 (2020) 195, https://doi.org/10.3390/ani10020195.

[41] S. Frazzini, E. Scaglia, M. Dell'Anno, S. Reggi, S. Panseri, C. Giromini, D. Lanzoni, C.A. Sgoifo Rossi, L. Rossi, Antioxidant and antimicrobial activity of algal and cyanobacterial extracts: an in vitro study, Antioxidants 11 (2022) 992, https://doi.org/10.3390/antiox11050992.

[42] C.H. Collins, P.M. Lyne, J. Grange, Collins and Lyne's Microbiological Methods, eighth ed., Hodder Education Publishers, 2004.

[43] M. Minekus, M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carrière, R. Boutrou, M. Corredig, D. Dupont, C. Dufour, L. Egger, M. Golding, S. Karakaya, B. Kirkhus, S. Le Feunteun, U. Lesmes, A. Macierzanka, A. Mackie, S. Marze, D. J. McClements, O. Ménard, I. Recio, C.N. Santos, R.P. Singh, G.E. Vegarud, M.S. J. Wickham, W. Weitschies, A. Brodkorb, A standardised static *in vitro* digestion method suitable for food – an international consensus, Food Funct. 5 (2014) 1113–1124, https://doi.org/10.1039/C3FO60702J.

[44] J.K. Santana Andrade, R.G. Chagas Barros, U.C. Pereira, J.P. Nogueira, N. C. Gualberto, C. Santos de Oliveira, S. Shanmugam, N. Narain, Bioaccessibility of bioactive compounds after in vitro gastrointestinal digestion and probiotics fermentation of Brazilian fruits residues with antioxidant and antidiabetic potential, LWT 153 (2022) 112469, https://doi.org/10.1016/j.lwt.2021.112469.

[45] K.S. Pettersen, T. Skjerdal, Y. Wasteson, T. Lindbäck, G. Vegarud, I. Comi, M. Aspholm, Survival of Listeria monocytogenes during in vitro gastrointestinal digestion after exposure to 5 and 0.5 % sodium chloride, Food Microbiol. 77 (2019) 78–84, https://doi.org/10.1016/j.fm.2018.08.010.

[46] J.A. Myers, B.S. Curtis, W.R. Curtis, Improving accuracy of cell and chromophore concentration measurements using optical density, BMC Biophys. 6 (2013) 4, https://doi.org/10.1186/2046-1682-6-4.

[47] O.V. Yermishev, A.K. Rudkevych, O.S. Luka, Correlation of protein content and other nutritions in human diet, Journal of Education, Health and Sport 10 (2020) 226–237, https://doi.org/10.12775/JEHS.2020.10.11.022.

[48] N.E. Buitimea-Cantúa, P.I. Torres-Chávez, A.I. Ledesma-Osuna, B. Ramírez-Wong, R.M. Robles-Sánchez, S.O. Serna-Saldívar, Effect of defatting and decortication on distribution of fatty acids, phenolic and antioxidant compounds in sorghum (<*scp*>*S*</*scp*> *orghum bicolor*) bran fractions, Int. J. Food Sci. Technol. 48 (2013) 2166–2175, https://doi.org/10.1111/ijfs.12201.

[49] C.A. Bittencourt, R.T. Alves Júnior, E.E.P. Silva, J.R.S. Meneguette, B.R.F. Schuh, V.L. Daley, S.R. Fernandes, R.D. Signoretti, J.A. Freitas, Replacement of soybean meal with alternative protein sources in the concentrate supplement for lactating Holstein × Gyr cows in an intensive tropical pasture-based system: effects on performance, milk composition, and diurnal ingestive behavior, Livest. Sci. 259 (2022) 104898, https://doi.org/10.1016/j.livsci.2022.104898.

[50] F. Oppedisano, R. Macrì, M. Gliozzi, V. Musolino, C. Carresi, J. Maiuolo, F. Bosco, S. Nucera, M. Caterina Zito, L. Guarnieri, F. Scarano, C. Nicita, A.R. Coppoletta, S. Ruga, M. Scicchitano, R. Mollace, E. Palma, V. Mollace, The anti-inflammatory and antioxidant properties of n-3 PUFAs: their role in cardiovascular protection, Biomedicines 8 (2020) 306, https://doi.org/10.3390/biomedicines8090306.

[51] A.P. Simopoulos, The importance of the ratio of omega-6/omega-3 essential fatty acids, Biomed. Pharmacother. 56 (2002) 365–379, https://doi.org/10.1016/S0753-3322(02)00253-6.

[52] R.E. Mutha, A.U. Tatiya, S.J. Surana, Flavonoids as natural phenolic compounds and their role in therapeutics: an overview, Futur J Pharm Sci 7 (2021) 25, https://doi.org/10.1186/s43094-020-00161-8.

[53] M.C. Foti, Antioxidant properties of phenols, J. Pharm. Pharmacol. 59 (2010) 1673–1685, https://doi.org/10.1211/jpp.59.12.0010.

[54] M. Miklasińska-Majdanik, M. Kępa, R. Wojtyczka, D. Idzik, T. Wąsik, Phenolic compounds diminish antibiotic resistance of Staphylococcus aureus clinical strains, Int. J. Environ. Res. Publ. Health 15 (2018) 2321, https://doi.org/10.3390/ijerph15102321.
[55] K.-T. Teng, C.-Y. Chang, L.F. Chang, K. Nesaretnam, Modulation of obesity-induced inflammation by dietary fats: mechanisms and clinical evidence, Nutr. J. 13 (2014) 12, https://doi.org/10.1186/1475-2891-13-12.
[56] N. Li, L. Li, H. Wu, H. Zhou, Antioxidative property and molecular mechanisms underlying geniposide-mediated therapeutic effects in diabetes mellitus and cardiovascular disease, Oxid. Med. Cell. Longev. 2019 (2019) 1–20, https://doi.org/10.1155/2019/7480512.
[57] J. Wang, Y. Zhang, R. Liu, X. Li, Y. Cui, L. Qu, Geniposide protects against acute alcohol-induced liver injury in mice via up-regulating the expression of the main antioxidant enzymes, Can. J. Physiol. Pharmacol. 93 (2015) 261–267, https://doi.org/10.1139/cjpp-2014-0536.
[58] H.-J. Koo, K.-H. Lim, H.-J. Jung, E.-H. Park, Anti-inflammatory evaluation of gardenia extract, geniposide and genipin, J. Ethnopharmacol. 103 (2006) 496–500, https://doi.org/10.1016/j.jep.2005.08.011.
[59] R.A.A. Lelono, S. Tachibana, K. Itoh, Isolation of antifungal compounds from gardenia jasminoides, Pakistan J. Biol. Sci. 12 (2009) 949–956, https://doi.org/10.3923/pjbs.2009.949.956.
[60] L. Česonienė, J. Labokas, I. Jasutienė, A. Šarkinas, V. Kaškonienė, P. Kaškonas, R. Kazernavičiūtė, A. Pažereckaitė, R. Daubaras, Bioactive compounds, antioxidant, and antibacterial properties of Lonicera caerulea berries: evaluation of 11 cultivars, Plants 10 (2021) 624, https://doi.org/10.3390/plants10040624.
[61] L. Montero, D. Ballesteros-Vivas, A.F. Gonzalez-Barrios, A. del P. Sánchez-Camargo, Hemp seeds: nutritional value, associated bioactivities and the potential food applications in the Colombian context, Front. Nutr. 9 (2023), https://doi.org/10.3389/fnut.2022.1039180.
[62] Z. Drinić, S. Vidović, J. Vladić, A. Koren, B. Kiprovski, V. Sikora, Effect of extraction solvent on total polyphenols content and antioxidant activity of Cannabis sativa L, Lekovite Sirovine (2018) 17–21, https://doi.org/10.5937/leksir1838017D.
[63] M. Irakli, E. Tsaliki, A. Kalivas, F. Kleisiaris, E. Sarrou, C.M. Cook, Effect of genotype and growing year on the nutritional, phytochemical, and antioxidant properties of industrial hemp (cannabis sativa L.) seeds, Antioxidants 8 (2019) 491, https://doi.org/10.3390/antiox8100491.
[64] D. Motiejauskaitė, S. Ullah, A. Kundrotaitė, R. Žvirdauskienė, A. Bakšinskaitė, K. Barčauskaitė, Isolation of biologically active compounds from cannabis sativa L. Inflorescences by using different extraction solvents and evaluation of antimicrobial activity, Antioxidants 12 (2023) 998, https://doi.org/10.3390/antiox12050998.
[65] W. Leonard, P. Zhang, D. Ying, Z. Fang, Hempseed in food industry: nutritional value, health benefits, and industrial applications, Compr. Rev. Food Sci. Food Saf. 19 (2020) 282–308, https://doi.org/10.1111/1541-4337.12517.
[66] J.L. Bautista, S. Yu, L. Tian, Flavonoids in *Cannabis sativa* : biosynthesis, bioactivities, and biotechnology, ACS Omega 6 (2021) 5119–5123, https://doi.org/10.1021/acsomega.1c00318.
[67] N. Majidiyan, M. Hadidi, D. Azadikhah, A. Moreno, Protein complex nanoparticles reinforced with industrial hemp essential oil: characterization and application for shelf-life extension of Rainbow trout fillets, Food Chem. X 13 (2022) 100202, https://doi.org/10.1016/j.fochx.2021.100202.
[68] J.I. Alonso-Esteban, J. Pinela, A. Ćirić, R.C. Calhelha, M. Soković, I.C.F. R. Ferreira, L. Barros, E. Torija-Isasa, M. de C. Sánchez-Mata, Chemical composition and biological activities of whole and dehulled hemp (Cannabis sativa L.) seeds, Food Chem. 374 (2022) 131754, https://doi.org/10.1016/j.foodchem.2021.131754.
[69] M. Grzesik, G. Bartosz, A. Dziedzic, D. Narog, J. Namiesnik, I. Sadowska-Bartosz, Antioxidant properties of ferrous flavanol mixtures, Food Chem. 268 (2018) 567–576, https://doi.org/10.1016/j.foodchem.2018.06.076.
[70] G. Nahler, Cannabidiol and contributions of major hemp phytocompounds to the “entourage effect”; possible mechanisms, alternative, Complementary & Integrative Medicine 5 (2019) 1–16, https://doi.org/10.24966/ACIM-7562/100066.
[71] K. Ning, C. Hou, X. Wei, Y. Zhou, S. Zhang, Y. Chen, H. Yu, L. Dong, S. Chen, Metabolomics analysis revealed the characteristic metabolites of hemp seeds varieties and metabolites responsible for antioxidant properties, Front. Plant Sci. 13 (2022), https://doi.org/10.3389/fpls.2022.904163.
[72] L.R. Bhatt, P. Dawadi, G. Syangtan, M.A. Siddiqui, B. Lama, K. Nepal, D.R. Joshi, Nutritional value and antioxidant properties of Cannabis seeds from Makwanpur district of central Nepal, Sci. World 15 (2022) 103–112, https://doi.org/10.3126/sw.v15i15.45657.
[73] M. Wink, Modes of action of herbal medicines and plant secondary metabolites, Medicines 2 (2015) 251–286, https://doi.org/10.3390/medicines2030251.
[74] Regina de Caridad Rodríguez Muñoz, Ana Elena Rodríguez Cadalso, Viviana Silvia Rodríguez Jiménez, Geuny Vázquez Bandomo, Yosimy Seijas Delgado, Reydilexis Forestal Gonzáles, Those responsible for the pharmacological actions of plants are the active secondary metabolites, J. Pharm. Pharmacol. 9 (2021), https://doi.org/10.17265/2328-2150/2021.03.003.
[75] C. Benkirane, A. Ben Moumen, M.-L. Fauconnier, K. Belhaj, M. Abid, H.S. Caid, A. Elamrani, F. Mansouri, Bioactive compounds from hemp (*Cannabis sativa* L.) seeds: optimization of phenolic antioxidant extraction using simplex lattice mixture design and HPLC-DAD/ESI-MS ^{2} analysis, RSC Adv. 12 (2022) 25764–25777, https://doi.org/10.1039/D2RA04081F.
[76] I.R. Ilyasov, V.L. Beloborodov, I.A. Selivanova, R.P. Terekhov, ABTS/PP decolorization assay of antioxidant capacity reaction pathways, Int. J. Mol. Sci. 21 (2020) 1131, https://doi.org/10.3390/ijms21031131.
[77] M. Olszowy-Tomczyk, How to express the antioxidant properties of substances properly? Chem. Pap. 75 (2021) 6157–6167, https://doi.org/10.1007/s11696-021-01799-1.
[78] E.D.N.S. Abeyrathne, K. Nam, D.U. Ahn, Analytical methods for lipid oxidation and antioxidant capacity in food systems, Antioxidants 10 (2021) 1587, https://doi.org/10.3390/antiox10101587.
[79] A.L. Dawidowicz, M. Olszowy, The importance of solvent type in estimating antioxidant properties of phenolic compounds by ABTS assay, Eur. Food Res. Technol. 236 (2013) 1099–1105, https://doi.org/10.1007/s00217-013-1982-1.
[80] M. Kalinowska, A. Płońska, M. Trusiak, E. Gołębiewska, A. Gorlewska-Pietluszenko, Comparing the extraction methods, chemical composition, phenolic contents and antioxidant activity of edible oils from Cannabis sativa and Silybum marianu seeds, Sci. Rep. 12 (2022) 20609, https://doi.org/10.1038/s41598-022-25030-7.
[81] Nazneen G. Kundgol, K.K. Math, B. Kasturiba, M.Y. Kamatar, M. Usha, Impact of decortication on chemical composition, antioxidant content and antioxidant activity of little millet landraces, Int. J. Eng. Res. Technol. 2 (2013).
[82] E. Pontonio, M. Verni, C. Dingeo, E. Diaz-de-Cerio, D. Pinto, C.G. Rizzello, Impact of enzymatic and microbial bioprocessing on antioxidant properties of hemp (cannabis sativa L.), Antioxidants 9 (2020) 1258, https://doi.org/10.3390/antiox9121258.
[83] L. Othman, A. Sleiman, R.M. Abdel-Massih, Antimicrobial activity of polyphenols and alkaloids in middle eastern plants, Front. Microbiol. 10 (2019), https://doi.org/10.3389/fmicb.2019.00911.
[84] K. Allemailem, Antimicrobial potential of naturally occurring bioactive secondary metabolites, J. Pharm. BioAllied Sci. 13 (2021) 155, https://doi.org/10.4103/jpbs.JPBS_753_20.
[85] P. Cavazos, D. Gonzalez, J. Lanorio, R. Ynalvez, Secondary metabolites, antibacterial and antioxidant properties of the leaf extracts of Acacia rigidula benth. and Acacia berlandieri benth, SN Appl. Sci. 3 (2021) 522, https://doi.org/10.1007/s42452-021-04513-8.
[86] F. Pasquali, M. Schinzari, A. Lucchi, M. Mandrioli, T.G. Toschi, A. De Cesare, G. Manfreda, Preliminary data on the antimicrobial effect of Cannabis sativa L. variety Futura 75 against food-borne pathogens in vitro as well as against naturally occurring microbial populations on minced meat during storage, Ital J Food Saf 9 (2020), https://doi.org/10.4081/ijfs.2020.8581.
[87] J.A. Karas, L.J.M. Wong, O.K.A. Paulin, A.C. Mazeh, M.H. Hussein, J. Li, T. Velkov, The antimicrobial activity of cannabinoids, Antibiotics 9 (2020) 406, https://doi.org/10.3390/antibiotics9070406.
[88] B.A. Khan, P. Warner, H. Wang, Antibacterial properties of hemp and other natural fibre plants: a review, BioRes 9 (2014) 3642–3659.
[89] L. Schofs, M.D. Sparo, S.F. Sánchez Bruni, The antimicrobial effect behind *Cannabis sativa*, Pharmacol Res Perspect 9 (2021), https://doi.org/10.1002/prp2.761.
[90] M. Friedman, H.S. Jürgens, Effect of pH on the stability of plant phenolic compounds, J. Agric. Food Chem. 48 (2000) 2101–2110, https://doi.org/10.1021/jf990489j.
[91] Y. Ma, Y. Yang, J. Gao, J. Feng, Y. Shang, Z. Wei, Phenolics and antioxidant activity of bamboo leaves soup as affected by in vitro digestion, Food Chem. Toxicol. 135 (2020) 110941, https://doi.org/10.1016/j.fct.2019.110941.
[92] V. Goulas, A. Hadjisolomou, Dynamic changes in targeted phenolic compounds and antioxidant potency of carob fruit (Ceratonia siliqua L.) products during in vitro digestion, LWT 101 (2019) 269–275, https://doi.org/10.1016/j.lwt.2018.11.003.
[93] M. Alminger, A.-M. Aura, T. Bohn, C. Dufour, S.N. El, A. Gomes, S. Karakaya, M. C. Martínez-Cuesta, G.J. McDougall, T. Requena, C.N. Santos, In vitro models for studying secondary plant metabolite digestion and bioaccessibility, Compr. Rev. Food Sci. Food Saf. 13 (2014) 413–436, https://doi.org/10.1111/1541-4337.12081.
[94] S.J. Hur, B.O. Lim, E.A. Decker, D.J. McClements, In vitro human digestion models for food applications, Food Chem. 125 (2011) 1–12, https://doi.org/10.1016/j.foodchem.2010.08.036.
[95] I. Ginsburg, E. Koren, M. Shalish, J. Kanner, R. Kohen, Saliva increases the availability of lipophilic polyphenols as antioxidants and enhances their retention in the oral cavity, Arch. Oral Biol. 57 (2012) 1327–1334, https://doi.org/10.1016/j.archoralbio.2012.04.019.
[96] J.P.E. Spencer, Metabolism of tea flavonoids in the gastrointestinal tract, J. Nutr. 133 (2003) 3255S–3261S, https://doi.org/10.1093/jn/133.10.3255S.
[97] N. Mateo Anson, R. van den Berg, R. Havenaar, A. Bast, G.R.M.M. Haenen, Bioavailability of ferulic acid is determined by its bioaccessibility, J. Cereal. Sci. 49 (2009) 296–300, https://doi.org/10.1016/j.jcs.2008.12.001.
[98] K. Wojtunik-Kulesza, A. Oniszczuk, T. Oniszczuk, M. Combrzyński, D. Nowakowska, A. Matwijczuk, Influence of in vitro digestion on composition, bioaccessibility and antioxidant activity of food polyphenols—a non-systematic review, Nutrients 12 (2020) 1401, https://doi.org/10.3390/nu12051401.
[99] Q. Zhang, B. Xing, M. Sun, B. Zhou, G. Ren, P. Qin, Changes in bio-accessibility, polyphenol profile and antioxidants of quinoa and djulis sprouts during in vitro simulated gastrointestinal digestion, Food Sci. Nutr. 8 (2020) 4232–4241, https://doi.org/10.1002/fsn3.1718.
[100] D. de Paulo Farias, F.F. de Araújo, I.A. Neri-Numa, F.L. Dias-Audibert, J. Delafiori, R.R. Catharino, G.M. Pastore, Effect of in vitro digestion on the bioaccessibility and bioactivity of phenolic compounds in fractions of Eugenia pyriformis fruit,

Food Res. Int. 150 (2021) 110767, https://doi.org/10.1016/j.foodres.2021.110767.

[101] M. Li, Q. Bai, J. Zhou, T.S.P. de Souza, H.A.R. Suleria, In vitro gastrointestinal bioaccessibility, bioactivities and colonic fermentation of phenolic compounds in different vigna beans, Foods 11 (2022) 3884, https://doi.org/10.3390/foods11233884.

[102] G. Balakrishnan, R.G. Schneider, Quinoa flavonoids and their bioaccessibility during in vitro gastrointestinal digestion, J. Cereal. Sci. 95 (2020) 103070, https://doi.org/10.1016/j.jcs.2020.103070.

[103] G.B. Gonzales, G. Smagghe, C. Grootaert, M. Zotti, K. Raes, J. Van Camp, Flavonoid interactions during digestion, absorption, distribution and metabolism: a sequential structure–activity/property relationship-based approach in the study of bioavailability and bioactivity, Drug Metab. Rev. 47 (2015) 175–190, https://doi.org/10.3109/03602532.2014.1003649.

[104] R. Pinacho, R.Y. Cavero, I. Astiasarán, D. Ansorena, M.I. Calvo, Phenolic compounds of blackthorn (Prunus spinosa L.) and influence of in vitro digestion on their antioxidant capacity, J. Funct.Foods 19 (2015) 49–62, https://doi.org/10.1016/j.jff.2015.09.015.

[105] X.C. Sollano-Mendieta, O.G. Meza-Márquez, G. Osorio-Revilla, D.I. Téllez-Medina, Effect of in vitro digestion on the antioxidant compounds and antioxidant capacity of 12 plum (spondias purpurea L.) ecotypes, Foods 10 (2021) 1995, https://doi.org/10.3390/foods10091995.

[106] R.L.T. Dutra, A.M. Dantas, D. de A. Marques, J.D.F. Batista, B.R.L. de A. Meireles, Â.M.T. de Magalhães Cordeiro, M. Magnani, G. da S.C. Borges, Bioaccessibility and antioxidant activity of phenolic compounds in frozen pulps of Brazilian exotic fruits exposed to simulated gastrointestinal conditions, Food Res. Int. 100 (2017) 650–657, https://doi.org/10.1016/j.foodres.2017.07.047.

[107] A.M. Dantas, I.M. Mafaldo, P.M. de L. Oliveira, M. dos S. Lima, M. Magnani, G. da S.C. Borges, Bioaccessibility of phenolic compounds in native and exotic frozen pulps explored in Brazil using a digestion model coupled with a simulated intestinal barrier, Food Chem. 274 (2019) 202–214, https://doi.org/10.1016/j.foodchem.2018.08.099.

[108] Ł. Sęczyk, U. Gawlik-Dziki, M. Świeca, Influence of phenolic-food matrix interactions on in vitro bioaccessibility of selected phenolic compounds and nutrients digestibility in fortified white bean paste, Antioxidants 10 (2021) 1825, https://doi.org/10.3390/antiox10111825.

[109] T. Patnaik, R.K. Dey, P. Gouda, Isolation of triterpenoid glycoside from bark of *Terminalia arjuna* using chromatographic technique and investigation of pharmacological behavior upon muscle tissues, E-Journal of Chemistry 4 (2007) 474–479, https://doi.org/10.1155/2007/538932.