Heliyon 10 (2024) e35186

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

CBD oil by-product (*Hemp flakes*): Evaluation for nutritional composition, heavy metals and functionality as a food ingredient

Elvis A. Baidoo [a], Ernst Cebert [b], Regine Mankolo [c], Martha Verghese [a], Joshua L. Herring [a,*]

[a] *Department of Food and Animal Sciences, Alabama Agricultural and Mechanical University, Normal, AL, USA*
[b] *Winfred Thomas Agricultural Research Station, Hazel Green, AL, USA*
[c] *Department of Biological and Environmental Sciences, Alabama Agricultural and Mechanical University, Normal, AL, USA*

ARTICLE INFO

Keywords:
Industrial hemp by-products
Cannabinoids
Antioxidants
Heavy metals
Functional ingredients

ABSTRACT

Background: The recent interest among consumers in industrial hemp due to health and wellness benefits has led to several products from industrial hemp, including cannabidiol (CBD) oil. CBD oil extraction from hemp buds and flowers generates by-product biomass (*hemp flakes*), often posing disposal challenges and with little or no applications. We hypothesized that hemp flakes possess residual compounds with nutritional and health value that could be used to improve utilization.

Methods: Locally sourced hemp flakes were compared to three commercial hemp protein products. The nutritional composition (proximate analysis), heavy metals (Al, Cu, As, Pb, Co, Cd), and functional composition (phenolic and antioxidant properties–total phenolic compounds (TPC), total flavonoid compounds (TFC), ferric reducing antioxidant potential (FRAP), 1,1–diphenyl-1-picrylhydrazyl (DPPH), Trolox equivalent antioxidant capacity (TEAC)), (CBD, cannabiodiolic acid–CBDA, cannabichromene–CBC, cannabigerol–CBG, and cannabinol–CBN) contents were determined and compared.

Findings: Hemp flakes had a similar nutritional composition to commercial hemp protein products, with heavy metal levels within FDA allowed limits. The by-product had significantly higher CBDA levels than commercial products. Overall, hemp flakes had comparable nutrient composition and antioxidant capabilities. Based on the protein composition of hemp flakes (31.62 %) versus the highest commercial product (43 %), hemp flakes are an acceptable functional food ingredient.

1. Introduction

Industrial hemp (*Cannabis sativa* L.) is a versatile plant now cultivated globally for industrial, medicinal, and food purposes [1]. Unlike its counterpart, marijuana, industrial hemp contains less than 0.3 % of the psychoactive compound tetrahydrocannabinol (THC). Cannabidiol (CBD), a non-psychoactive compound, has shown therapeutic potential for treating conditions like body pain, anxiety, and insomnia [2]. Other reported benefits of industrial hemp include anti-inflammatory, antipsychotic, and anticonvulsant

* Corresponding author.
E-mail addresses: elvis.baidoo@bulldogs.aamu.edu (E.A. Baidoo), martha.verghese@aamu.edu (M. Verghese), josh.herring@aamu.edu (J.L. Herring).

https://doi.org/10.1016/j.heliyon.2024.e35186
Received 27 February 2024; Received in revised form 4 June 2024; Accepted 24 July 2024
Available online 25 July 2024
2405-8440/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

properties [3,4]. Its mechanisms of action are linked to its ability to mimic endocannabinoids that regulate the nervous system. These potentials have fueled consumer demand for CBD-infused products, prompting manufacturers to incorporate them into various available food and beverage items [5].

Hemp was not legally recognized in the United States until the 2018 Farm Bill [3], and it still has use limitations by state. Around the same time, regulatory bodies in the US and UK approved CBD-based medications for treating epilepsy and multiple sclerosis symptoms.

CBD is not allowed in foods on a federal basis [6,7]; however, some states have legalized recreational (including food applications) and medicinal use, while others approved it only for therapeutic use. CBD is extracted from industrial hemp through cold pressing or chemical extraction. The by-product of CBD extraction are hemp flakes. Hemp flakes contain residual CBD, antioxidant compounds, and nutritional value. The cannabionoids and antioxidiant compounds can account for functional properties. A functional ingredient is one that provides benefits to the consumer beyond nutrition and is capable of mitigating a specific disease or illness condition [8]. Further, consumers have turned their attention to plant sources for antioxidants instead of synthetic ones, which are feared to possess carcinogenic properties.

Several reports indicate that *Cannabis* species is a metal hyperaccumulator as it grows and has been used for soil bioremediation to remove contaminants [9,10]. Therefore, the safety of any hemp-derived product must be considered for heavy metal contamination. This implies that food products potentially made using hemp as an ingredient must be checked for safety before consumption.

Studies on the utilization of hemp-based products have suggested industrial and agricultural products such as the extraction of valuable chemical components, including fatty acids, cannabinoids, and terpenoids, as well as micro and nanofibers [11]. Furthermore, ongoing work is on its feasibility for feeding livestock and soil composting [12]. Little or no information exists presently on applying hemp by-products in food systems. Thus, this research aimed to assess the nutritional, safety (heavy metals), and functional (antioxidant properties and cannabinoid content) values of hemp flakes by comparing it with existing commercial hemp protein products. This work fits into the circular bio-economy [13], a proposed sustainability method for managing agro-resources, promising to be a better approach than our current harvest-use-and-dump approach. These research results could inform the food industry to incorporate hemp flakes as a safe, functional ingredient.

2. Materials and methods

2.1. Materials

A by-product obtained from a local facility expressing CBD oil from the *Jinma* variety's hemp buds and seeds was used. The dried material, hereafter referred to as 'hemp flakes' (H), was used in addition to three commercial hemp products (A, B, and C) purchased online (Fig. 1). The compared products were hemp protein powders selected from different geographical regions across the United States, Canada, and Italy with their specific names coded to prevent disclosing commercial products.

2.2. Sample preparation

All samples were milled with a laboratory Waring blender (Dynamics Corporation of America, McConnelsburg, PA) and screened through a 2 mm mesh for proximate analytical determinations. All solvents used for extraction were obtained from Sigma-Aldrich (St. Louis, MO). Solvent ET–100 % ethanol (70 % v/v), solvent MET–100 % methanol (80 % v/v), solvent AQ–100 % distilled water, solvent EM–50 % methanol, 50 % ethanol) were used for extraction. For antioxidant assays with respective solvents, 5.0 ± 0.05 g of sample was added to 100 ml of the solvent and sonicated for 2 h, after which it was centrifuged (Thermo Fisher Scientific Sorvall Legend XTR, Waltham, MA, USA) at 3000×*g* at 4 °C for 20 min. The supernatant was filtered with Whatman #1 filter paper and evaporated to dryness using a rotary evaporator (Buchi Rotavapor R–215, New Castle, DE, USA). The dried extract was reconstituted to 10 ml by adding the respective solvent. All extracts were stored at 4 °C until further analysis.

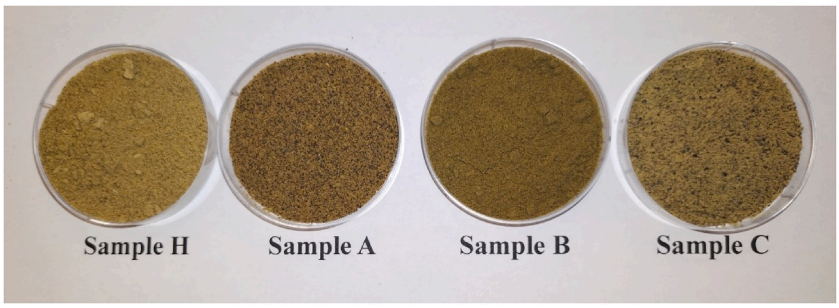

Fig. 1. Milled hemp samples showing H (hemp flakes) and three commercial hemp products, A, B, and C. (specific commercial names of hemp products are not provided so as not to identify commercial products).

2.3. Proximate analysis

Proximate analysis of samples was conducted using the AACC-approved standard methods [14]. All chemicals were obtained from Sigma-Aldrich (St. Louis, MO). The air oven method was applied for moisture content determination (AACC44-15) using Sartorius (Practicum 224-1s, Wood Dale, IL, USA) weighing balance and a convection laboratory oven (Fisher Scientific Model # 6925, New Hampton, NH USA) at 130 °C for 60 min. For ash (AACC 08–01.01a), 3–5 g of sample was added to a pre-ignited, cooled, and weighed ashing porcelain crucibles and ignited at (600 ± 20 °C) overnight using a (Sybron Thermolyne model # 6020–12325, Dubuque, Iowa, USA). Crude protein (NX6.25) was determined by the Kjeldahl nitrogen method following AACC method 46–30.01. A Foss Tecator digestion system 2508 (Eden Prairie, MN, USA) and a Kjeltec auto 1030 analyzer (Waltham, MA, USA) were used. Crude fat was determined by following the AACC method 30–25.01 in a Foss Soxtec system 1043 H T (Eden Prairie, MN, USA). The crude fiber content (AOCS-approved method Ba 6a-05) of hemp samples was determined using an Ankom 2000 fiber analyzer (Ankom Technology, NY, USA). Approximately 0.95–1.00 g of milled hemp sample (passed through a 2 mm sieve) was placed in sealed filter bags and two blank bags for reference. The filter bags were then immersed in petroleum ether for 10 min to extract fat, drained, and placed in the analyzer's vessel with a bag suspender. The bags underwent digestion with 0.225 N H_2SO4 and 0.313 N NaOH solutions, followed by rinsing with distilled water. After drying in an oven (102 ± 2 °C), the bags were ashed in a furnace (600 ± 15 °C) and weighed. The percentage of crude fiber was calculated by comparing the weight before and after ashing using Eq. (1). Carbohydrate content was determined by difference.

$$\% \text{ Crude fiber} = 100 \text{ x } [(W3 - (W1 \text{ x } C1)]/W2 \quad (1)$$

Where W1 is the bag tare weight, W2 is the weight of the sample, W3 is the weight of organic matter, and C1 is the ash corrected factor.

2.4. Antioxidant potential and free radical scavenging properties of hemp samples

2.4.1. Total phenolic content

The Folin-Ciocalteau method was used to determine the total phenolic content in hemp samples as described by Singleton et al., [15]. Unknown samples were prepared by mixing 12.5 μl of the sample with 50 μl of distilled water. 12.5 μl of Folin-Ciocalteau reagent (Fisher Chemicals, Pittsburgh, PA, USA) was added to the mixture for 5 min. Finally, 125 μl of 7 % sodium carbonate anhydrous solution was added and shaken at room temperature for 90 min. Absorbance was read at a wavelength of 750 nm against a blank of distilled water using a microplate reader (Synergy HT, BioTek Instruments Inc. Winooski, Vermont, USA). The standard curve for determining total phenolic compounds was conducted with gallic acid (0–500 mg/l) (Fisher Chemicals, Pittsburgh, PA, USA), and the results were stated as mg GAE/100 g.

2.4.2. Total flavonoids content

The aluminum chloride colorimetric method by Marinova et al. [16], was applied with little modifications for determining the total flavonoids in the samples. A standard curve of catechin (0.02–0.5 mg/l) (Cayman chemicals, Ann Arbor, MI, USA) was used, and the results were expressed as mg CE/100 g). Unknown samples were prepared by adding 125 μl of distilled water to 25 μl of extract in a well plate, followed by adding 7.5 μl of 5 % sodium nitrite and allowed to stand for 5 min 50 μl of 1M sodium hydroxide solution and 25.5 μl of distilled water were mixed briefly. The absorbance of the mixture was read against a blank distilled water at 510 nm with a microplate reader (Synergy HT, BioTek Instruments Inc. Winooski, Vermont, USA).

2.4.3. Ferric reducing antioxidant potential (FRAP)

The ferric-reducing antioxidant potential of the samples was determined following the Benzie et al. [17], method with slight modifications. The FRAP reagent, freshly prepared, was prepared with 10 mmol 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) in 40 mmol hydrochloric acid, 20 mmol ferric chloride ($FeCl_3$–$6H_20$), and 300 mmol acetate buffer (pH 3.6). A standard curve was prepared using ferrous sulfate (0.1–1.0 mmol). Distilled water, 30 μl, and FRAP reagent, 30 μl, were added to 10 μl of the sample, allowed to mix briefly and read at an absorbance of 593 nm using a microplate reader (Synergy HT, BioTek Instruments Inc. Winooski, Vermont, USA).

2.4.4. 1,1–diphenyl-1-picrylhydrazyl (DPPH) assay

A modified assay method described by Brand-Williams et al. [18], was applied to determine the radical scavenging ability of samples. DPPH solution, 0.1 mmol, was prepared in methanol, and 200 μl of the DPPH solution was added to 40 μl of sample in a well plate with the absorbance read at 517 nm using a plate reader (Synergy HT, BioTek Instruments Inc. Winooski, Vermont, USA). Results were expressed as a reduction in the percentage of DPPH using Eq. (2).

$$\% \text{ DPPH} = (Abs.control - Abs.sample)/(Abs.control) * 100 \% \quad (2)$$

where $Abs_{control}$ is the absorbance of the control and Abs_{sample} is the sample absorbance.

2.4.5. Trolox equivalent antioxidant capacity (TEAC)

The Trolox equivalent antioxidant activity for samples was determined using the Miller et al. [19], method. A standard curve was first prepared with Trolox (0.02–0.80 mmol). 2,2-azino-bis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS) was diluted with ethanol to obtain an absorbance of 0.70 ± 0.025 at a wavelength of 734 nm. The ABTS solution (7 mmol) was prepared and left overnight in the

dark for the radical to develop. In the assay, 10 μl of sample and blank was combined with 290 μl of ABTS, followed by the absorbance measurement at 734 nm at 6 min using a microplate reader (Synergy HT, BioTek Instruments Inc. Winooski, Vermont, USA). The results were reported as micromole Trolox equivalent (μmol TE/100 g) of sample dry weight.

2.5. Heavy metal content determination

Samples were tested for selected heavy metals (lead, cobalt, cadmium, arsenic, copper, and aluminum) using an inductively coupled plasma-optical emission spectrometry (ICP-OES) (Model–Optima 2100 DV, PerkinElmer Inc, Waltham, MA) and a modified method described by Tasie et al., [20]. Samples were prepared by dissolving ashed samples [14] with 5 ml of 5 % hydrochloric acid. The solution was heated to dryness and dissolved with 2 ml of concentrated hydrochloric acid. Further heating of the mixture with a covered watch glass was conducted, and the final mixture was filtered and topped up to 100 ml in a volumetric flask. The standard was IV–STOCK–4 solution (1000 ppm each of 23 element ICP calibration/quality control standard dissolved in nitric acid) purchased from Inorganic Ventures (Christiansburg, VA, USA). Equipment was recalibrated after every sample during the analysis as a quality control check. All experiments were completed in duplicate for each heavy metal.

2.6. Determination of cannabinoids by high-pressure liquid chromatography (HPLC)

Cannabinoids analysis of samples was conducted based on a modified method of Aubin et al., [21]. The analytical HPLC apparatus comprised a Varian Prostar LC system (USA) equipped with Prostar autosampler attached to a 100 μl loop and a Prostar 310 UV-VIS detector. Samples and standards were analyzed by reverse phase HPLC on a C18 column with 250 × 4.6 mm inner diameter and column particle size of 5 μm (Waters part #WAT054275, Ireland). The column heater was set to 25 °C to maintain a consistent temperature. DEA-exempt cannabidiol (CBD), cannabiodiolic acid (CBDA), cannabigerol (CBG), cannabichromene (CBC), and cannabinol (CBN) standards were obtained from Sigma-Aldrich Aldrich (St. Louis, MO, USA).

For cannabinoids analysis, an isocratic mobile phase consisting of A: HPLC-grade water acidified with 0.1 % trifluoroacetic acid and B:100 % HPLC-grade acetonitrile was employed at 20 %:80 % at a flow rate of 0.8 ml per min for separation and run for 30 min. Data was collected on a computer equipped with Varian Star Chromatography software – LC workstation version 6.41. UV spectra were recorded at 228 nm, allowing a 5-min re-equilibrium period within injections. Cannabinoid peak identification was conducted based on the standard peaks and was further employed for calculating the respective concentrations of all selected cannabinoids in the samples. Confirmatory tests were completed by spiking the samples with individual standards. The determination of concentrations of analytes was conducted in triplicate.

2.7. Statistical analysis

Data was collected in triplicate, except ICP, which was completed in duplicate and reported as least square mean ± standard error of the means. Analysis of variance (ANOVA) was conducted at $p \leq 0.05$, and the means were separated using Tukey's method. The statistical software used were SAS 9.4 (SAS Institute Inc, Cary, NC, 2020) for all ANOVA and means separation and Minitab 17.3.0 (Minitab Inc., 2016, PA, USA) for correlation analyses.

3. Results and discussion

3.1. Proximate analysis

Proximate composition of products depends on the species, geographical location of growth, growing season, and handling and processing conditions. The results of the proximate analysis of samples used in this work are shown in Table 1. The moisture content of the samples was within a narrow range of 6.0 and 7.0 %, and all samples were deemed dry and stable. Ash content ranged between 4.72 and 7.21 %, with hemp flakes (H) recording a significantly low amount of ash at 4.72 % ($p \leq 0.05$). Crude fat content was highest ($p \leq 0.05$) in H and could be attributed to the processing technique of adding a neutral oil to aid CBD oil extraction. Crude protein and carbohydrate content values for H were comparable to commercial hemp protein samples. Except for carbohydrate content, which was determined by difference, data on the chemical composition of moisture, ash, protein, and fat for all samples were similar to data reported by Pojic et al. [22], who worked on a similar by-product, a mechanically cold-pressed hemp meal. Hemp flake's crude fiber

Table 1
Proximate analysis of hemp flakes (H) and three commercial hemp products, A, B, and C[a].

Sample	Moisture content (%)	Ash (%)	Crude fat (%)	Crude protein (%)	Crude fiber (%)	Carbohydrates %
H	7.73 ± 0.16^{a}	4.72 ± 0.01^{c}	15.38 ± 0.14^{a}	31.62 ± 0.08^{b}	33.91 ± 1.25^{c}	6.64 ± 0.35^{c}
B	6.16 ± 0.84^{a}	7.21 ± 0.30^{a}	10.88 ± 1.11^{b}	43.03 ± 0.18^{a}	31.84 ± 1.49^{d}	0.88 ± 0.66^{d}
A	7.74 ± 0.48^{a}	5.47 ± 0.03^{b}	10.09 ± 0.38^{b}	22.00 ± 0.71^{d}	42.24 ± 0.72^{a}	12.46 ± 1.51^{a}
C	6.51 ± 0.48^{a}	6.67 ± 0.23^{a}	8.99 ± 0.02^{b}	29.87 ± 0.42^{c}	38.33 ± 0.64^{b}	9.63 ± 0.61^{b}

[a] Sample abbreviations, H - hemp flakes, A, B, and C (names not provided so as not to identify commercial products). Data (n = 3) are LS means ± SEM. Means in a column with common superscripts in small letters are not significantly different at $p \leq 0.05$.

content (33.91 %) was within the range of the commercial hemp protein samples, which recorded crude fiber between 31.8 % and 42.4 %.

3.2. Antioxidant and radical scavenging properties of hemp samples

3.2.1. Total phenolic content

Total phenolic content measures the holistic content of all bioactive phytochemicals present in a product. Table 2 shows the total polyphenol content of hemp flakes and three commercial hemp protein products. The results ranged from a minimum of 1.40 mg GAE/100 g DW for hemp flake (H) from aqueous extraction to a maximum of 11.25 mg GAE/100 g DW in 1:1 ethanol/methanol extract for sample A. These results are similar to reported data when using similar extraction methods reported by Zago et al., [23]. The results for hemp flakes were similar for the ethanol (ET), methanol (MET), and 1:1 ethanol/methanol extracts compared to the commercial hemp protein products. The total polyphenol content in hemp flakes extracted from ethanol and 1:1 ethanol/methanol extracts were not significantly different ($p \leq 0.05$) from those of sample B. The results suggest that the hemp flakes contained comparable or competitive total polyphenol content among the compared commercial protein samples. These results agree with [24] while investigating the effect of malting on hemp varieties' nutritional and antioxidant content. Azad et al. [25], also reported similar levels of hemp total polyphenol contents (7.30–15.40 mg GAE/100 g) while studying the impact of hot melt extrusion on hemp leaves for hemp decarboxylation of cannabidiolic acid into cannabidiol. The effect of solvent systems in the extraction of total polyphenols (Table 2) indicated ethanol extracts as the highest ($p \leq 0.05$) in all samples except C and A, where 1:1 ethanol/methanol extracts produced significantly higher total polyphenol contents. These findings suggest that contrary to expectations, 1:1 ethanol/methanol solvent supersede ethanol or methanol extracts only with some samples.

3.2.2. Total flavonoid content determination

The total flavonoid content of the hemp samples is presented in Table 2. Different solvents and samples yielded different flavonoid contents. The highest ($p \leq 0.05$) flavonoid content (0.93 mg CE/100 g DW) was observed in sample A after extraction with 1:1 ethanol: methanol solvent. Aqueous extracts produced the lowest flavonoid content in all samples ($p \leq 0.05$). The ethanol extract of flavonoids (0.30 mg CE/100 g DW) showed comparable (not significantly different $p \leq 0.05$) amounts of flavonoids with C (0.30 mg CE/100 g DW) and higher when compared with B. The results indicate comparable properties to the flavonoid content of the commercial hemp protein products.

3.2.3. DPPH free radical scavenging properties

In this experiment, antioxidant capacity was determined by the ability of the sample to scavenge the DPPH radical (1,1-diphenyl-2-picrylhydrazyl). DPPH radical is visually recognized by the decolorization in the cell plate during the experiment. A DPPH inhibition of more than 50 % is reported in the literature to signify a potent antioxidant [26]. The ethanol extract of sample H produced the highest ($p \leq 0.05$) DPPH inhibition of 58.70 % among the hemp samples (Table 3). Aqueous and 1:1 ethanol/methanol extracts of H recorded DPPH inhibition rates similar to the commercial hemp protein samples. This observation suggests that the CBD by-product is a source of antioxidants and has similar powers to scavenge free radicals compared to commercial hemp protein products when processed similarly. Within H, it was observed that the ethanol extract produced the highest DPPH radical inhibition, followed by methanol and 1:1 ethanol/methanol solvent extraction. The observation here is that when ethanol and methanol are combined in a 1:1 ratio, the strength of the extraction for DPPH inhibition is lowered significantly ($p \leq 0.05$) than using ethanol alone for the extraction.

3.2.4. Trolox equivalent antioxidant activity (TEAC)

This Trolox equivalent assay determines the ability of antioxidants in a product to scavenge the 2,2′–azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation, with the extent of reaction visually captured as the bleaching of the blue-green color of ABTS [19]. The result of TEAC among hemp samples is shown in Table 3. It was generally high for all samples extracted with

Table 2
Total phenolic and total flavonoid content of hemp flakes (H) compared with three commercial hemp products, coded A, B, and C (LS mean ± SE). Samples were extracted in aqueous (AQ), ethanol (ET), methanol (MET), and a 1:1 ratio of ethanol and methanol (EM). Different letter suffixes on the means represent significant differences at $p \leq 0.05$.

	TPC mg GAE/g DW				TFC mg CE/100 g DW			
	Extraction solvent				Extraction solvent			
Hemp	AQ	ET	MET	EM	AQ	ET	MET	EM
H	$1.40 \pm 0.01^{c,D}$	$2.53 \pm 0.05^{a,C}$	$2.23 \pm 0.04^{b,D}$	$2.48 \pm 0.04^{a,C}$	$0.04 \pm 0.00^{d,B}$	$0.30 \pm 0.00^{a,B}$	$0.11 \pm 0.01^{c,C}$	$0.17 \pm 0.01^{b,D}$
B	$2.69 \pm 0.03^{a,B}$	$2.48 \pm 0.06^{b,C}$	$2.49 \pm 0.01^{b,C}$	$2.57 \pm 0.02^{b,C}$	$0.17 \pm 0.01^{c,A}$	$0.19 \pm 0.01^{b,C}$	$0.24 \pm 0.01^{a,AB}$	$0.23 \pm 0.01^{a,C}$
A	$2.12 \pm 0.05^{d,C}$	$7.85 \pm 0.05^{b,A}$	$4.73 \pm 0.05^{c,A}$	$11.25 \pm 0.01^{a,A}$	$0.01 \pm 0.00^{d,D}$	$0.37 \pm 0.00^{b,A}$	$0.13 \pm 0.00^{c,BC}$	$0.93 \pm 0.03^{a,A}$
C	$3.09 \pm 0.05^{b,A}$	$2.98 \pm 0.01^{bc,B}$	$2.88 \pm 0.03^{c,B}$	$3.33 \pm 0.04^{a,B}$	$0.02 \pm 0.00^{b,C}$	$0.30 \pm 0.01^{a,B}$	$0.30 \pm 0.07^{a,A}$	$0.29 \pm 0.00^{a,B}$

*Sample abbreviations, H - hemp flakes, A, B, and C (names not provided so as not to identify commercial products). Data (n = 3) are LS means ± SEM. Solvent abbreviations: AQ – aqueous, ET– ethanol, MET – methanol, EM – 1:1 ratio of ethanol and methanol. *Means in a column (effect of hemp type) with common superscripts ^{A,B,C,D} letters are not significantly different at $p \leq 0.05$. Means in a row with common superscripts ^{a,b,c,d} letters (effect of solvent type) are not significantly different at $p \leq 0.05$.

Table 3
DPPH scavenging properties and antioxidative potential of hemp flakes (H) compared with three commercial hemp products, coded A, B, and C (LS mean ± SE). Samples were extracted in aqueous (AQ), ethanol (ET), methanol (MET), and a 1:1 ratio of ethanol and methanol (EM). Different letter suffixes on the means represent significant differences at $p \leq 0.05$.

	DPPH %				TEAC (mmol TE/100 g)			
	Extraction solvent				Extraction solvent			
Hemp sample	AQ	ET	MET	EM	AQ	ET	MET	EM
H	29.81 ± 0.65^{c,A}	58.70 ± 0.87^{a,A}	48.33 ± 0.23^{b,D}	49.45 + 0.94^{b,C}	55.83 ± 0.68^{c,C}	121.03 ± 0.47^{a,A}	121.28 ± 0.08^{a,A}	107.94 ± 0.08^{b,A}
B	28.28 ± 1.21^{d,A}	52.44 ± 0.15^{c,B}	55.96 ± 0.23^{b,C}	64.10 + 0.56^{a,A}	87.14 ± 0.34^{c,B}	120.86 ± 0.00^{a,AB}	120.86 ± 0.00^{a,AB}	109.36 ± 1.46^{b,A}
A	22.97 ± 0.82^{c,B}	52.10 ± 0.52^{b,B}	57.75 ± 0.45^{a,B}	50.47 + 0.70^{b,C}	103.77 ± 4.50^{ab,A}	121.03 ± 0.15^{a,A}	121.37 ± 0.15^{a,A}	97.42 ± 3.14^{b,A}
C	22.19 ± 0.56^{d,B}	53.64 ± 0.34^{c,B}	62.64 ± 0.23^{a,A}	58.01 + 0.30^{b,B}	98.66 ± 2.89^{b,A}	121.03 ± 0.08^{a,A}	120.18 ± 0.45^{a,B}	102.91 ± 3.40^{b,A}

*Sample abbreviations, H - hemp flakes, A, B, and C (names not provided so as not to identify commercial products). Data (n = 3) are LS means ± SEM. Solvent abbreviations: AQ – aqueous, ET – ethanol, MET – methanol, EM – 1:1 ratio of ethanol and methanol. *Means in a column (effect of hemp type) with common superscripts ^{A,B,C,D} letters are not significantly different at $p \leq 0.05$. Means in a row with common superscripts ^{a,b,c,d} letters (effect of solvent type) are not significantly different at $p \leq 0.05$.

ethanol, methanol, or ethanol and methanol combination, ranging from 97.42 mmol TE/100 g in A extracted with EM to 121.86 mmol TE/100 g in B extracted with ethanol and methanol. Except for the aqueous extract, all hemp flake (H) extracts were among the highest ($p \leq 0.05$) when compared with the commercial hemp samples. This result suggests that hemp flakes possess a parallel antioxidant capacity as commercial hemp products.

3.2.5. Ferric reducing antioxidant power (FRAP)

The determination of FRAP of a biological material employs the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) by an antioxidant. The results of hemp samples in the present study are shown in Fig. 2. Hemp flake's aqueous extract recorded one of the least ($p \leq 0.05$) FRAP, but when compared to the commercial varieties, it was not significantly different ($p \geq 0.05$) from that of sample A aqueous extract. The ethanol extract for hemp flakes (H) recorded 68.00 ± 0.017 mmol Fe(II) and was intermediate among the results of corresponding commercial samples. Extracts from methanol and 1:1 ethanol/methanol recorded the highest FRAP values ($p \leq 0.05$). Significant differences ($p \leq 0.05$) were found in the antioxidant levels due to FRAP from the hemp samples. The decreasing order for FRAP recorded among the samples extracted with ethanol was A > B > H > C. These differences may be due to the varying sources of hemp samples used in this work. The results further indicate the impact of different solvents on the extraction of antioxidants. Ethanol extracts produced the highest FRAP, while aqueous extracts generally recorded the least significantly different ($p \leq 0.05$) FRAP values. Different organic and aqueous solvent systems used singly or in multiple combinations of different ratios have been reported to impact antioxidant extracts significantly [27]. This is because the different solvent systems possess different polarities and can only dissolve antioxidants of similar polarities. Hemp flake's FRAP potential was superior to C and not statistically different from B with ethanol extraction, while aqueous extraction was similar to A. This indicates that hemp flakes have similar FRAP potential to commercial hemp products.

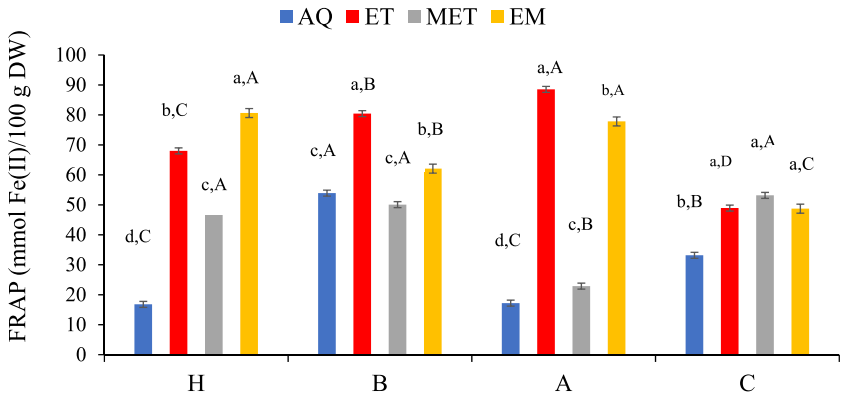

Fig. 2. Ferric reducing antioxidant potential (FRAP) of hemp by-product (H) and three commercial hemp products, A, B, and C (names not provided to identify commercial products). Data (n = 3) are LS means ± SEM. Solvent abbreviations: AQ – aqueous, ET– ethanol, MET – methanol, EM – 1:1 ratio of ethanol and methanol. Means with common superscripts ^{A,B,C,D} letters (effect of hemp type) are significantly different at $p \leq 0.05$. Means with common superscripts ^{a,b,c,d} letters (effect of solvent type) are significantly different at $p \leq 0.05$.

3.3. Heavy metal content of hemp samples

The heavy metal contents in the hemp samples studied are presented in Fig. 3. Arsenic and lead, whose accumulation effects are among the most deleterious with implications for heart disease, high blood pressure, kidney, and bone disease [28], were not detected. Cadmium and cobalt recorded insignificant amounts in all the hemp samples; however, aluminum and copper were present in all samples. In decreasing order of content among samples, aluminum was A > H > C > B ($p \leq 0.05$), while copper was A > C > H > B ($p \leq 0.05$). This might be a characteristic behavior of industrial hemp since commercial samples were sourced from various locations, representing different geographical and growing conditions. The highest ($p \leq 0.05$) heavy metal contamination in this work was in A (0.71± mg/kg DW) for aluminum. Establishing the level of heavy metals in hemp products is crucial, considering the nature of *Cannabis* species being used for phytoremediation of soils. *Cannabis* species are reportedly capable of extracting and bioaccumulating various metals from the soil to their stem, leaves, and branches compared to other species due to the possible presence of stress-tolerant genes [29]. For this reason, food product formulations constituted with hemp must be assessed to assure the safety of the consumer.

The results further indicate that all the levels of heavy metals found in all samples were within the permissible levels according to the Environmental Protection Agency (EPA) and the US Food and Drug Administration (FDA). The EPA has a permissible level of 10 ppm of arsenic in drinking water [30]. Copper and aluminum are not regarded as heavy metals because they have relatively low molecular weights; however, their accumulation in the body can pose risks to humans, such as Wilson's Disease. Copper is an essential mineral known to have critical functions in metabolism, first being able to take part in oxidation-reduction reactions as it moves from its cuprous (Cu^{+}) to cupric forms (Cu^{2+}). It also serves as a cofactor for many enzymes (cuproenzymes), including cytochrome *c* oxidase, whose action generates a proton gradient necessary for ATP synthesis, and lysyl oxidase, which initiates the formation of cross-links that stabilize elastin and collagen for the formation of strong connective tissue [31]. Despite all these essential attributes of copper, there could be an issue when it is overconsumed; even though it is rare in the general population, it is still essential to check the levels of copper in all foods.

Data comparison was conducted between the samples' chemical composition and heavy metal content (Table 4). Negative correlations were found between protein and copper ($r = -0.832$, $p = 0.001$) and protein and aluminum ($r = -0.773$, $p = 0.003$), as well as a positive correlation between fat and cobalt ($r = 0.775$, $p = 0.003$). These relationships, while correlation does not represent causation, and may need further research as we did not find similar reports in literature and more research on the topic would be needed to confirm the notion. If proven by further research, the results could be valuable information for hemp breeders aiming to develop high-protein or high-fat varieties.

Overall, our results have provided evidence to posit that the oil by-product (hemp flakes) obtained from CBD oil extraction could potentially be regarded as a functional ingredient, as described by Ref. [32], "functional foods contain bioactive components with positive health effects reduce a compound with negative effects." Further to the nutritional benefits obtained from the consumption, hemp flakes also possess bioactive compounds that confer systemic health benefits, such as reducing the risk of chronic disease or nullifying the effect of another component with a negative effect.

3.4. Cannabinoid content in hemp samples

Hemp cannabinoids are known to be synthesized in acidic forms while hemp plants grow and, later, during harvest and processing operations, converted to their neutral forms by spontaneous heat or light-mediated reactions [4,33]. The calibration data and regression for five selected cannabinoids (cannabiodiolic acid–CBDA, cannabigerol–CBG, cannabidiol–CBD, cannabinol–CBN, and cannabichromene–CBC) and the results of cannabinoid content in hemp flakes and commercial hemp protein products are presented in Figs. 4 and 5, and Table 5, respectively. Fig. 4 shows the regression coefficients of the standard analytes. The cannabinoids studied eluted in order of retention times as CBDA, CBG, CBD, CBN, and CBC (Fig. 5). Similar order of elution of these cannabinoids has been reported by several authors, including [21,34]. The results show variation in cannabinoids among the samples studied. This could be due to differences in hemp varietal and growing conditions since commercial samples were from various locations. All samples tested,

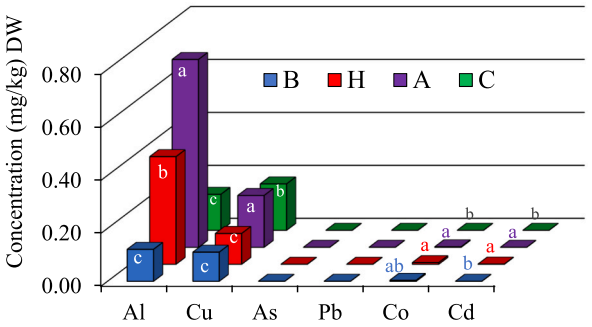

Fig. 3. Heavy metal content of hemp by-product H and three commercial hemp products A, B, and C. Hemp sample abbreviations, H- hemp flakes, A, B, and C (names not provided to identify commercial products). Data is mean ± SE (n = 3). The heavy metals are Cd = cadmium, Co = cobalt, Pb = lead, As = arsenic, Cu = copper, and Al = aluminum. The same letters on bars in a column represent non–significance at $p \leq 0.05$.

Table 4
Correlation matrix between chemical composition of all hemp samples (hemp flakes –H and three commercial hemp products, A, B, and C*) and heavy metals in hemp samples.[a].

	Protein	Ash	MC	CFat	Al	Cu	As	Pb	Co
Ash	0.58								
	0.05								
MC	−0.54	−0.33							
	0.07	0.30							
CFat	0.15	−0.65	−0.14						
	0.64	0.02	0.66						
Al	−0.77	−0.64	0.64	0.17					
	0.003	0.01	0.03	0.59					
Cu	−0.83	−0.08	0.47	−0.60	0.52				
	0.001	0.81	0.13	0.04	0.08				
As	**	**	**	**	**	**			
Pb	**	**	**	**	**	**	**		
Co	0.26	−0.06	−0.11	0.78	0.15	−0.53	**	**	
	0.37	0.87	0.73	0.003	0.64	0.08			
Cd	−0.004	−0.54	−0.06	0.64	0.05	−0.41	**	**	0.09
	0.99	0.07	0.87	0.03	0.88	0.18			0.78

[a] Each data represents correlation coefficient followed by the *p value,* ** As and Pb were not detected. *Sample abbreviations, H – hemp flakes, A, B, and C (names not provided so as not to identify commercial products).

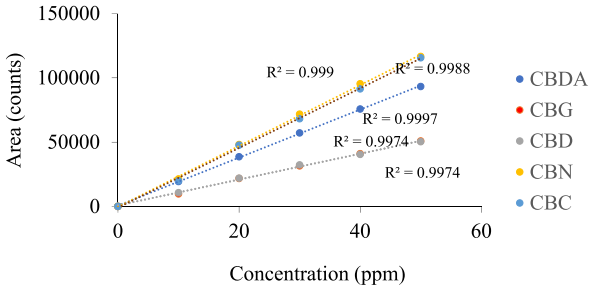

Fig. 4. Calibration curves for five cannabinoid standards (cannabidiol–CBD, cannabiodiolic acid–CBDA, cannabichromene–CBC, cannabigerol–CBG, and cannabinol–CBN) and their regression coefficients.

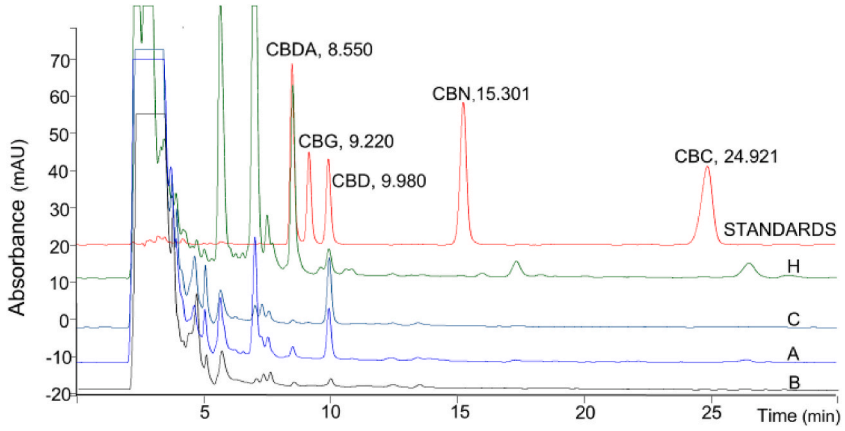

Fig. 5. HPLC chromatograms of five cannabinoids (cannabidiol–CBD, cannabiodiolic acid–CBDA, cannabichromene–CBC, cannabigerol–CBG, and cannabinol–CBN) in hemp flakes (H), and three commercial hemp products, A, B, and C (names not provided to identify commercial products) measured at 280 nm. The standard chromatogram used to detect individual cannabinoids in hemp samples is in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

including hemp flakes, contained CBDA and CBD. However, CBC was not detected in any of the samples, per the peaks and retention times during the HPLC (Fig. 5). CBN was only detected in hemp flakes (H) as was CBG only in sample C. Hemp flakes (H) showed the highest significantly different ($p \leq 0.05$) amount of CBDA (0.144 mg/g DW) (Table 5) among all samples and cannabinoids studied. In

Table 5
Detected and quantified cannabinoids (cannabiodiolic acid–CBDA, cannabidiol–CBD, cannabichromene–CBC, cannabigerol–CBG, and cannabinol–CBN) determined in mg/g DW for hemp flakes and three commercial hemp powders A, Band C[a].

Hemp sample				
Cannabinoid	C	H	A	B
CBDA	$0.02^{b,B}$	$0.14^{a,A}$	$0.04^{b,C}$	$0.003^{b,D}$
CBG	0.01^{c}	ND[a]	ND[a]	ND[a]
CBD	$0.57^{a,A}$	$0.04^{b,C}$	$0.45^{a,B}$	$0.02^{a,D}$
CBN	ND[a]	0.001^{c}	ND[a]	ND[a]
CBC	ND[a]	ND[a]	ND[a]	ND[a]

[a] Sample abbreviations, H– hemp flakes, A, B and C (names not provided so not to identify commercial products). Data (n = 3) are LS means ± SEM. *Means in a column (effect of hemp type) with common superscripts^{a,b,c,d} letters are significantly different at $p \leq 0.05$. Means in a row with common superscripts^{A,B,C,D} letters are significantly different at $p \leq 0.05$.

comparison, sample C showed the highest significantly different ($p \leq 0.05$) amount of CBD (0.574 mg/g).

The highest content of CBDA observed in hemp flakes could be attributed to the source of the hemp plant from where it was made (buds and flowers) versus the commercial hemp products, which are typically processed from hemp seeds. Hemp seeds have been commercially marketed for their nutrition, including essential fatty acids and high proteins, but not as a primary source of cannabinoids [35]. Samples A and C recorded the highest amounts of CBD, while hemp flakes recorded 0.038 mg/g DW. The heating process applied during the processing of hemp powders might have converted some CBDA into CBD. On the contrary, hemp flakes were obtained as a by-product of cold pressing for CBD oil extraction and thus might explain the low amounts of CBD and elevated levels of CBDA in hemp flakes since heat and light help the conversion of CBDA into its neutral form by decarboxylation into CBD [25,36].

Currently, in the United States, and depending on local regulations, the use of hemp ingredients and extracted CBD cannot be applied in foods on a federal basis [37]; however, some state/local regulations do allow them for recreational applications, including foods [38]. Cannabinoids, especially non-psychotic CBD, are reported to present functional benefits, including anti-inflammatory, anxiolytic, and neuroprotective properties, as well as being a muscle relaxant [3,4]. The by-product tested in this work was shown to contain similar kinds of cannabinoids and even higher amounts of specific cannabinoids compared to related commercial hemp food ingredients on the market and could, therefore, be recommended for use as a food ingredient to benefit consumers' health.

4. Conclusions

It is predicted that by 2050, the world's population will reach 9 billion, and a sustained food supply will be a concern; therefore, it is appropriate to examine alternatives, including the exploration of agricultural waste materials. Hemp flakes as a by-product from CBD oil extraction could be utilized due to their nutritional and functional value.

The hemp flake used in this work demonstrated to hold nutritional and health components comparable to related commercial products. The antioxidant levels showed variations attributed to the source of hemp material and solvent extraction method. Hemp flakes exhibited high and similar antioxidant properties as measured by TPC, TFC, FRAP, and TEAC and possessed comparable radical scavenging properties as measured by DPPH. The hemp by-product showed comparative amounts of cannabinoids with the highest content of cannabidiolic acid, which is known to break down to cannabidiol and possess functional benefits. Further, the results of this work have exemplified that hemp flakes generated from CBD oil extraction have a considerable nutritional and functional value that supports its potential to be incorporated in food preparations as an ingredient. It was also established that the hemp flakes contained levels below the permissible levels of heavy metals in foods, according to health and environmental agencies. It is concluded that the by-product from CBD oil extraction could be utilized as an ingredient in food processing, such as a composite with other ingredients to complement nutrition and health functionality for consumers.

Ethics statement

This work did not use animal or human subjects to collect data.

Data availability

Data supporting this work's findings have not been deposited in any repository and would be available upon reasonable request.

CRediT authorship contribution statement

Elvis A. Baidoo: Writing – original draft, Software, Investigation, Data curation, Conceptualization. **Ernst Cebert:** Validation, Resources. **Regine Mankolo:** Validation, Data curation. **Martha Verghese:** Supervision, Project administration, Funding acquisition. **Joshua L. Herring:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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

Acknowledgment

We acknowledge funding from the USDA Evans Allen Program (ALAX - 012-1422 EA) and Alabama Agricultural & Mechanical University for this project. We acknowledge Dr. Lloyd Walker for allowing us to work in the Chemistry Laboratory (HPLC) in the Agricultural Research Center and Mr. Simon Ogutu of the Food and Animal Sciences Department, AAMU, for providing technical laboratory support.

References

[1] P. Cerino, et al., A review of hemp as food and nutritional supplement, Cannabis Cannabinoid Res 6 (1) (2021) 19–27.
[2] I. Urits, et al., Use of cannabidiol (CBD) for the treatment of chronic pain, Best Pract. Res. Clin. Anaesthesiol. (2020).
[3] J. Corroon, R. Kight, Regulatory status of cannabidiol in the United States: a perspective, Cannabis Cannabinoid Res 3 (1) (2018) 152–161.
[4] P.X. Chen, M.A. Rogers, Opportunities and challenges in developing orally administered cannabis edibles, Curr. Opin. Food Sci. 28 (2019) 7–13.
[5] A.E. White, C. Van Tubbergen, B. Raymes, A.E. Contreras, E.J. Scallan Walter, Cannabis-infused edible products in Colorado: food safety and public health implications, Am. J. Publ. Health 110 (6) (2020) 790–795.
[6] A. Mead, Legal and regulatory issues governing cannabis and cannabis-derived products in the United States, Front. Plant Sci. 10 (June) (2019) 1–10.
[7] L.A. Walker, et al., Cannabidiol (CBD) in dietary supplements: perspectives on science, safety, and potential regulatory approaches, J. Diet. Suppl. 17 (5) (2020) 493–502.
[8] A. Saviano, C. Zanza, Y. Longhitano, E.C. Nista, F. Franceschi, V. Ojetti, Effects of functional foods, nutraceuticals, and herbal products on pancreas? Chin. Med. J. (Engl). Publish Ah (5) (2023) 2022–2023.
[9] A. Ferrarini, et al., Bioaugmented phytoremediation of metal-contaminated soils and sediments by hemp and giant reed, Front. Microbiol. 12 (April) (2021) 1–20.
[10] G. Todde, G. Carboni, S. Marras, M. Caria, C. Sirca, Industrial hemp (Cannabis sativa L.) for phytoremediation: energy and environmental life cycle assessment of using contaminated biomass as an energy resource, Sustain. Energy Technol. Assessments 52 (PA) (2022) 102081.
[11] K. Pacaphol, D. Aht-ong, Preparation of hemp nanofibers from agricultural waste by mechanical defibrillation in water, J. Clean. Prod. 142 (2016) 1283–1295.
[12] K. Rajasekhar, et al., Characterization of the nutritional and safety properties of hemp seed cake as animal feed ingredient, Int. J. Livest. Prod. 12 (2) (2021) 53–63.
[13] J. Metic, T.C. McAloone, D.C.A. Pigosso, A look into circular economy research: exploring the bio and techno cycles and the need for dual circularity, Proc. Des. Soc. 1 (AUGUST) (2021) 121–130.
[14] AACC International, Approved methods of analysis, in: Method 44–15.02, Method 08–01.01a, Method 46–30.01, Approved, 12 Ed., 2012.
[15] V. Singleton, R. Orthofer, R. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, in: J.H. Hasperué, L.M. Rodoni, L.M. Guardianelli, A.R. Chaves, G.A. Martínez (Eds.), Polyphenols and Flavonoids, 299, 1999, pp. 152–178.
[16] D. Marinova, F. Ribarova, M. Atanassova, Total phenolics and total flavonoids in Bulgarian fruits and vegetables, Univ. Chem. Technol. Metall. 40 (3) (2005) 255–260.
[17] I.F.F. Benzie, J.J. Strain, Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, Methods Enzymol. 299 (1999) 15–27.
[18] W. Brand-Williams, M. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity. Food science and Technology, Food Sci. Technol. 28 (1995) 25–30.
[19] N.J. Miller, C. Rice-Evans, M. Davies, V. Gopinathan, A. Milner, A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates, Clin. Sci. 4 (84) (1993) 407–412.
[20] O. Tasie, L. Kassama, J. Boateng, I. Tazisong, R. Mankolo, The bioavailability of nutrients , antioxidants , and short-chain fatty acids in vitro fermentation of hydrolyzed canned red kidney beans (phaseolus vulgaris L.) in a simulated gastrointestinal tract system, J. Food Nutr. Sci. 2 (3) (2020) 110–124.
[21] A.J. Aubin, C. Layton, S. Helmueller, Waters Technical Report - 'Separation of 16 Cannabinoids in Cannabis Flower and Extracts Using a Reversed-phase Isocratic HPLC Method,', 2018.
[22] M. Pojić, et al., Characterization of by-products originating from hemp oil processing, J. Agric. Food Chem. 62 (51) (2014) 12346–12442.
[23] E. Zago, R. Nandasiri, U. Thiyam-Holländer, N.A. Michael Eskin, Influence of thermal treatments on the antioxidant activity of hemp cake polar extracts, J. Food Sci. Technol. 59 (8) (2022) 3256–3265.
[24] B. Farinon, R. Molinari, L. Costantini, N. Merendino, Biotechnological Transformation of Hempseed in the Food Industry, 2022.
[25] M.O.K. Azad, B.R. Ryu, M.S. Rana, M.H. Rahman, J.D. Lim, Y.S. Lim, Enhancing the cannabidiol (CBD) compound in formulated hemp (Cannabis sativa L.) leaves through the application of hot-melt extrusion, Processes 9 (5) (2021).
[26] S.H. Nile, Y.S. Keum, A.S. Nile, S.S. Jalde, R.V. Patel, Antioxidant, anti-inflammatory, and enzyme inhibitory activity of natural plant flavonoids and their synthesized derivatives, J. Biochem. Mol. Toxicol. 32 (1) (2018).
[27] J. Thomas, A. Barley, S. Willis, J. Thomas, M. Verghese, J. Boateng, Effect of different solvents on the extraction of phytochemicals in colored potatoes, Food Nutr. Sci. 11 (10) (2020) 942–954.
[28] J. Hirsch, Arsenic and Lead Are in Your Fruit Juice: what You Need to Know Consumer Reports Finds Concerning Levels of Heavy Metals in Almost Half of Tested Juices, Here's How to Protect Yourself and Your Family, 2019. *Available online:*, https://www.consumerreports.org/food-safety/arsenic-and-lead-are-in-your-fruit-juice-what-you-need-to-know/. (Accessed 5 September 2022).
[29] R. Ahmad, et al., Phytoremediation potential of hemp (cannabis sativa L.): identification and characterization of heavy metals responsive genes, Clean 44 (2) (2016) 195–201.
[30] L.S. Neuwirth, E. Cabañas, P. Cadet, W. Zhu, M.E. Markowitz, Cereal and juice, lead and arsenic, our children at risk: a call for the FDA to Re-evaluate the allowable limits of lead and arsenic that children may ingest, Int. J. Environ. Res. Publ. Health 19 (10) (2022).
[31] C. Carpéné, N. Boulet, A. Chaplin, J. Mercader, Past, present and future anti-obesity effects of flavin-containing and/or copper-containing amine oxidase inhibitors, Medicine (Baltim.) 6 (1) (2019) 9.
[32] E. Fuentes-Zaragoza, M.J. Riquelme-Navarrete, E. Sánchez-Zapata, J.A. Pérez-Álvarez, Resistant starch as functional ingredient: a review, Food Res. Int. 43 (4) (2010) 931–942.
[33] A. Hazekamp, The trouble with CBD oil, in: Med Cannabinoids, 2018, pp. 65–72.
[34] E.C. Berthold, et al., Regulatory sampling of industrial hemp plant samples (Cannabis sativa L.) using UPLC-MS/MS method for detection and quantification of twelve cannabinoids, J. Cannabis Res. 2 (1) (2020).
[35] E. Jang, et al., Concentrations of THC, CBD, and CBN in commercial hemp seeds and hempseed oil sold in Korea, Forensic Sci. Int. 306 (2020) 110064.

[36] C. Citti, B. Pacchetti, M.A. Vandelli, F. Forni, G. Cannazza, Analysis of cannabinoids in commercial hemp seed oil and decarboxylation kinetics studies of cannabidiolic acid (CBDA), J. Pharm. Biomed. Anal. 149 (2018) 532–540.
[37] P.J. Zettler, E. Lietzan, A special exception for CBD in foods and supplements? Drug Discov. Today 25 (3) (2020) 467–469.
[38] H. Peng, F. Shahidi, Cannabis and cannabis edibles: a review, J. Agric. Food Chem. 69 (6) (2021) 1751–1774.