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Screening for Anti-Nutrients, Antioxidants, Minerals, and Cannabinoid Content in Hemp (*Cannabis sativa* L.) Seed Cultivars

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Abstract

Hemp is an excellent source of nutrients and phytochemicals that provide various health benefits. However, the presence of anti-nutrient factors (ANFs) limits the effectiveness of these nutrients in conferring health benefits. Additionally, hemp cultivars differ in the concentrations of phytocannabinoids and other polyphenols, making it crucial to investigate the best cultivar that can yield the maximum benefit. To determine this, total phenolic content (TPC), total flavonoid content (TFC), saponins, tannins, and antioxidant activities using ferric reducing antioxidant potential (FRAP) and 2, 2-diphenyl-2-picrylhydrazyl (DPPH) were measured on defatted and non-defatted hemp cultivars, namely Hemp Gen Int. Grandi (HGIG), International Hemp Henola (IHH), and Adams and Family farm (CFX-1). The levels of phytocannabinoids in hemp cultivars were determined using UHPLC-MS/MS. Among the cultivars, HGIG had the highest TPC (mg GAE $100g^{-1}$ dry weight (DW)) of 67.43, while IHH had the highest TFC (mg CE $100g^{-1}$ dry matter (DM)) of 34.55. CFX-1 had the lowest TPC and TFC of 41.47 and 4.49, respectively. Furthermore, HGIG also has the highest antioxidant capacity, with a FRAP value (µmol Fe (II) SO_4 $100g^{-1}$ DW) of 360.74, while CFX-1 had the lowest FRAP value of 105.5. The levels of phytic acid, saponin, and tannins varied significantly among the three hemp cultivars that were studied. Cannabidiolic acid (CBDA) was the most abundant cannabinoid in all three cultivars, with the highest concentration observed in HGIG. The study demonstrated that hemp is an excellent source of flavonoids and phenolic compounds. Additionally, the results indicated differences in the antioxidant properties and ANFs. These findings provide valuable insights for further testing and investigation.

Keywords: *Anti-Nutrients; Antioxidants; Extraction; Nutrients; Processing*

Abbreviations

ANFs: Anti-Nutrient Factors; TPC: Total Phenolics Content; TFC: Total Flavonoids Content; FRAP: Ferric Reducing Antioxidant Potential; DPPH: 2, 2-Diphenyl-2-Picrylhydrazyl; HGIG: Hemp Gen Int. Grandi; IHH: International Hemp Henola; CFX-1: Adams and Family Farm; THC: Tetrahydro *Cannabis*; AOCS: American Oil Chemists Society; AOAC: Association of Official Agricultural Chemists; ICP-OES: Inductively Coupled Plasma Atomic Emission Spectroscopy; PUFAs: Polyunsaturated Fatty Acids; CBG: Cannabigerol; CBD: Cannabidiol; CBC: Cannabichromene; CBDA: Cannabidiolic Acid; CBN: Cannabinol; CBDVA: Cannabidivaric Acid; CBDV: Cannabidivarin; CBGA: Cannabigerolic Acid; THCV: Tetrahydrocannabivarin; THCVA: Tetrahydrocannabivarin Acid; CBNA: Cannabinoid Acid; CBL: Cannabicyclol; D8-THC: Delta-8.Tetrahydrocannabinol; D9-THCA: Delta-9-Tetrahydrocannabinolic Acid; D9-THC: Delta-9-Tetrahydrocannabinol; ND: Non-Detected; ALA: Alpha-Linolenic Acid; DW: Dry Weight; IC_{50}: Inhibitory Concentration 50

Introduction

Industrial hemp (*Cannabis sativa* L.) is an ancient crop grown and cultivated over 10,000 years ago [1,2]. Hemp seeds are the byproduct obtained after the commercial utilization of fiber [3]. Interest in hemp seed has grown over the past 20 years because of its pharmaceutical and nutritional value [1]. The THC content of Industrial hemp is low -< 0.3% [4] and has been explored for varied uses. Whole hemp seeds contain 25% to 35% oil, 20% to 25% protein, 20% to 30% carbohydrates, 10% to 15% insoluble fibers, vitamins and minerals such as potassium, phosphorus, sulfur, magnesium, calcium, iron, and Zinc [5,6]. According to Hullar., *et al*. [7], hemp seed contains 320 to 380 g Kg^{-1} oil, of which 80% is polyunsaturated fatty acids. Hemp oil is rich in omega-6 and omega-3 fatty acids and has a ratio of around 2.5, although this value differs in literature [5].

Hemp seeds contain bioactive compounds, including phytochemicals and phytocannabinoids [8]. The phenolic compounds in hemp seeds are lignanamides, hydroxycinnamic acids, hydroxybenzoic acids, and flavonoids [1]. These phytochemicals contribute to the antioxidant potential of hemp seeds. Antioxidants protect cells against oxidative stress [9], scavenge free radicals, chelate pro-oxidant metals, and inhibit lipid peroxidation [10]. As such, studies have shown that hemp seeds possess numerous health benefits. They have anti-inflammatory and anti-cancer effects based on *in vitro* and *in vivo* studies [1,11].

The phytocannabinoid in hemp is classified into eight sub-groups, which include cannabichromene, Cannabigerol, Cannabidiol, tetrahydrocannabinol, cannabielsoin, isotetrahydrocannabinol, cannabicyclol, cannabicitran [12]. These phytocannabinoids are essential because of their health-promoting benefits, such as anti-depressants, analgesics, and anti-inflammatory properties [13]. However, phytocannabinoids are present in high concentrations in female flowers and occur less in the seeds [14].

Despite containing health-promoting compounds, hemp seeds are prominent sources of anti-nutrients (ANFs) such as phytic acid, saponin, and tannins. Their presence in foods limits the absorption of proteins, vitamins, and minerals and can affect the bioavailability of essential nutrients, leading to their deficiency and impacting overall health. Thus, ANFs levels must be reduced or removed to prevent their negative effect on the body [15,16]. This study was carried out to assess the proximate composition of polyphenol content and antioxidant activities, phytocannabinoid contents, and ANFs of selected industrial hemp seed cultivars.

Materials and Methods

Sample collection

Industrial Hemp seeds were obtained from the Alabama A&M Winifred Thompson Experimental Station (Hazel Green, Alabama). The seeds were packaged in envelopes and transported to the laboratory, where they were ground into fine powder and stored at -20°C until use.

Chemicals

All the chemicals and solvents used for analysis were of pure analytical grade and were obtained from Fisher Scientific (Suwanee, Georgia, Ohio, United States) and Sigma-Aldrich (St. Louis, MO, United States). The phytocannabinoids standards were purchased from Sigma Aldrich (St. Louis, MO, United States).

Determination of proximate composition in industrial hemp seeds

The moisture and fat content of the industrial hemp seeds were determined according to AOCS Official Procedure Am 5-04 [17]. The protein content was determined according to the AOAC m968.06 and 992.15 [18]. The ash content was determined according to the AOAC m923.03 [19]. The identification and quantification of minerals were determined using ICP-OES according to the AOAC International m984.27, 985.01, and 2011.14 [20].

Extraction of phytochemicals from industrial hemp seeds

The polyphenol contents of industrial hemp seeds (defatted and non-defatted) were extracted with 80% ethanol [21] in a 1:10 (w/v) ratio using an ultrasonic bath (Model 8510R-MT, Branson Ultrasonics Corporation, Danbury, Connecticut) for 1 hour. After, the extracts were filtered (Whatman No. 4), and the solvent was removed via rotary evaporation (Model RE301, Yamato Scientific Co., Ltd, Santa Clara, California). The concentrated extracts were stored at -20°C until analysis.

Determination of TPC, TFC, and antioxidant activities in industrial hemp seed

The TPC and TFC of the hemp extracts were determined using the Folin-Ciocalteu method described by Gajula., *et al* [22]. The TPC and TFC were expressed as mg of Gallic acid equivalents (GAE) per gram sample (mg GAE/100 g DW) and mg Catechin equivalent (CE) per gram sample (mg CE/100 g sample), respectively. The ferric-reducing antioxidant potential (FRAP) and 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities were determined according to Chen., *et al.*'s [23] method. The FRAP activity was expressed as an mM Fe^{2+}/g sample; for DPPH radical activities, the decrease in absorbance at 517 nm was monitored for 90 minutes. The IC_{50} was calculated using the calibration curve obtained from the antioxidant activity of the hemp extract at various concentrations.

The DPPH scavenging effect was calculated as follows:

DPPH Scavenging ability (%) Inhibition = () X 100

where A_{blank} is the absorbance of the blank and $A_{testsample}$= absorbance of the sample extract.

Determination of anti-nutrients in industrial hemp seed cultivars

The saponin content was determined according to the method by Chen., *et al* [24]. The Results were expressed using a calibration curve of diosgenin, and results were expressed as diosgenin equivalents per g of sample. Phytic acid was extracted from Hemp seed flour, as described by Raboy [25]. The results were expressed as g of phytic acid equivalents (PAE/100g), and phytic acid sodium salt hydrate was used as standard. The tannin content was determined according to the vanillin method of Herald., *et al*. [26] and expressed as Catechin equivalents (CE g^{-1}) DW /of hemp seeds.

Determination of the cannabinoids in industrial hemp seed

The phytocannabinoid content of the industrial hemp seed cultivars was determined according to the approved AOAC International Official Method of Analysis 2018.11 [27]. The method was evaluated against requirements of standard method performance requirement (SMPR®) quantitation of cannabinoids in plant materials of hemp (Low THC varieties *Cannabis* sp.).

Statistical analysis

The data were analyzed using the SAS 9.0 statistical programs; hence, the Analysis of Variance (ANOVA) was determined, and Tukey's group mean comparison test was used for all significant treatments. All experiments were carried out in triplicates, and statistical tests were performed at a 5% significance level.

Results and Discussion

Proximate composition in industrial hemp seeds

Table 1 shows the proximate composition of industrial hemp seed cultivars. The moisture content (%) of the hemp seed cultivars ranged from 5.32 to 6.82, and there was no significant difference among the cultivars ($p < 0.05$). The results are consistent with other studies on

industrial hemp seeds [1,28]. Hemp seeds with low moisture content have a longer, safe storage time with slight deterioration [29]. The protein content was 23.9, 22.75, and 22.25% for CFX-1, HGIG, and IHH, respectively. Although there were no significant differences ($p \geq 0.05$) among the cultivars, the highest protein content was observed in CFX-1 (23.9%). These values agree with other studies by Farinon., *et al*. [30] and Teleszko., *et al*. [31]. Hemp seed protein is valued for its amino acid composition, high bioavailability, and digestibility. It is a rich source of all nine essential amino acids, making it suitable for children between 2 and 5 years, providing sufficient nutrients [6,5,32-35]. The main proteins that are identified in hemp seeds are the storage protein albumin (globular protein) and edestin (a legume) [33,34]. The high protein content of hemp seed has drawn global attention to its nutritional value and potential as a functional ingredient in food formulations [36]. Hemp protein can also be substituted for other proteins in some food products because of its low allergenicity [36]. Fat is the principal constituent of hemp seeds. The oils obtained from hemp seeds are valuable, especially from an industrial point of view. Hemp oil is utilized for numerous health benefits, as well as in nutraceutical preparations and functional foods. It was found that the fat content was similar in the hemp seed cultivars ($p < 0.05$). The fat content of the cultivars analyzed in this study is 31.13, 30.57, and 27.53% for CFX-1, HGIG, and IHH, respectively. These results agree with previous studies [37-40]. Teleszko., *et al*. [31] also reported a mean fat content of 32.52% in two hemp varieties- Bialobrzeskie and Henola. The fat content in hemp seeds is essential because of their fatty acid composition [8]. Studies have shown that up to 70% and 80% of the fatty acid composition in hemp seeds is PUFAs. The predominant PUFA is omega 3 ALA, which protects against cardiovascular diseases, diabetes, obesity, and certain anti-inflammatory disorders [41]. Although the fatty acid profile was not determined, the crude fat content of the hemp seed cultivars in this study is in the same range known to be present in hemp oil, which is claimed to be ideal for human nutrition [42]. The ash content represents the total mineral content present in a sample. In our study, we observed no significant difference among the cultivars. The values presented here align with other studies [43,44].

Cultivars	Moisture content (%)	Protein (%)	Crude Fat (%)	Ash (%)
CFX-1	6.16[a]	23.9[a]	31.13[a]	4.4a
HGIG	6.21[a]	22.75[a]	30.57[a]	4.11a
IHH	5.81[a]	22.25[a]	27.53[a]	4.47a

Table 1: *Proximate composition in industrial hemp seeds.*

Results are expressed as means ± SEM. Means with the same superscripts within the column are not significantly ($p \geq 0.05$) different using Tukey's means separation test.

Figure 1 shows the pictures of hemp seed cultivars used in this study. The pictures of the seeds, non-defatted and defatted, are shown.

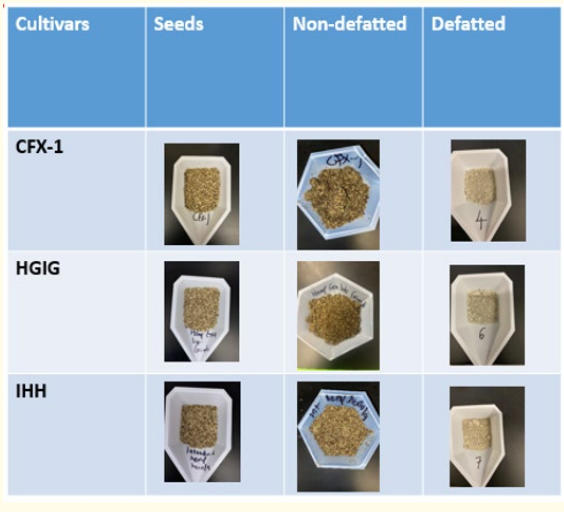

Figure 1: *The hemp seed cultivars used in the study. CFX-1; HGIG; IHH.*

The polyphenol content and antioxidant activities of industrial hemp seed

TPC of hemp seed extracts

Table 2 presents the polyphenol content (TPC, TFC) and antioxidant activities (FRAP, DPPH) of non-defatted and defatted cultivars of industrial hemp seeds. The results indicate that the TPC varied among cultivars, with HGIG showing the highest TPC for defatted seeds (67.43 mg GAE/100 g DW), while IHH had the highest TPC for non-defatted hemp seeds (57.38 mg GAE/100 g DW). Conversely, CFX-1 had the lowest TPC for both defatted (48.58 mg GAE/100 g DW) and non-defatted (41.47 mg GAE/100 g DW) seeds. A study by Benkirane., *et al*. [45] investigated the TPC of defatted hempseeds, reporting TPC ranging from 6.86 to 50.19 mg GAE per g of sample. According to Irakli., *et al*. [38], the TPC of hemp seeds from defatted cultivars ranged from 381.8 to 779.8 mg GAE 100^{-1}. Furthermore, André., *et al*. [46] reported that the TPC of hemp cultivars ranged from 22.05 mg GAE/g to 4.72 mg GAE/g DW. Although we did not consider environmental factors in our research, the TPC contents of hemp seed cultivars varied, which could have been due to environmental factors like temperature, rainfall, humidity, and soil quality during cultivation. In contrast to our findings, Rashid., *et al*. [47] reported TPC in the range of 77.64 µg GAE 100 mg^{-1} DW to 103.62 µg GAE 100 mg^{-1} DW in industrial hemp cultivars. Overall, TPC was higher in defatted hemp seeds than in non-defatted hemp seeds. Although our study did not determine the TPC of hemp oil, other studies have shown that hemp oil is a significant source of TPC. For instance, Yu., *et al*. [48] reported a TPC of 44.0 mg 100 g^{-1} GAE on cold-pressed hemp seed oil extract, while Teh and Birch [49] stated a TPC of 188.23 mg 100^{-1} GAE in hemp seed oil. Defatted hemp seeds have been found to contain compounds with high radical scavenging activity, as reported by Chen., *et al*. [50]. Pojic., *et al*. [51] identified that the cotyledon-containing fractions of the hemp seed meal obtained after oil extraction are rich in polyphenols such as p-hydroxybenzoic acid and catechin. In a recent study, Ozdemir., *et al*. [52] reported that defatted hemp seeds have high TPC and exhibit high antioxidant activity. The study found that defatting helps release phenolic compounds in the hemp seeds, making them a good source of antioxidant compounds. These compounds are beneficial in extending the shelf life of food products without negatively affecting their sensory and nutritional qualities [53].

TFC of hemp seed extracts

The TFC of the hemp seed cultivars was significantly different ($p < 0.05$) for each cultivar. The IHH had the highest TFC (34.55 mg CE/100 g DW) for defatted hemp seeds and non-defatted hemp seeds (33.07 mg CE/100 g DW). The lowest TFC was observed in the CFX-1 cultivar for the defatted (22.28 mg CE/100 g DW) and the non-defatted (4.49 mg CE 100 g DW) hemp seed, and this could be a result of the distribution of flavonoids, which varies during plant development of *C. sativa* [54]. Rashid., *et al*. [47] reported a TFC of hemp ranging from 34.52-µg Quercetin 100 mg-1 DW to 47.12 µg Quercetin 100 mg-1 DW, which aligns with the range detected in this study. Aloo., *et al*. [55] also reported a TFC of 107.50 mg/100 g CE. As stated by Irakli., *et al*. [38], the differences in the levels or content of TFC in hemp seeds may be due to agronomic and genotype factors. Although many flavonoids confer protection to plants, their production is responsive to environmental factors; for example, the accumulation of cannflavin A is determined not only by genetics but also by responses to temperature, humidity, rainfall, and solar radiation in the environment [56].

FRAP activity of hemp seed cultivars

Table 2 displays the FRAP activity results and value for defatted and non-defatted hemp seed cultivars. Our study found a significant difference ($p > 0.05$) between the defatted and non-defatted hemp seed cultivars. The FRAP value of non-defatted hemp seed varied from 105.5 mmolFE/g for CFX-1 cultivar to 222.43 for IHH cultivar, whereas for defatted hemp seed, it ranged from 268.54 mmolFE/g for CFX-1 cultivar to 360.74 for HGIG cultivar. Notably, the HGIG cultivar exhibited the highest FRAP value among defatted and non-defatted hemp seed cultivars, and the lowest value was observed in CFX-1. Irakli., *et al*. [38] reported a FRAP value ranging from 338.4 to 806.8 mg TE 100 $^{g-1}$ of seven hemp cultivars, which were also in the range of the FRAP determined in this study. Benkirane., *et al*. [45] determined the FRAP value of defatted hemp seeds using different solvent combinations, and their FRAP value ranged from 12.21 - 61.01. A study by Aloo., *et al*. [55] reported that hemp seed extract had a FRAP content of 44.42%.

The FRAP value estimates the antioxidant activity in food. The hemp cultivars have shown high FRAP values, meaning they contain rich sources of natural antioxidants. This suggests that hemp might have the potential to protect against various diseases.

DPPH free radical scavenging activity of hemp seed cultivars

The results of the DPPH scavenging activity of three hemp seed cultivars are presented in table 2. Although there were no significant differences between the cultivars, some notable variations were observed. The results revealed that the defatted hemp seed cultivar HGIG had the highest % DPPH inhibition at 78.72%, while CFX-1 had the lowest at 76.19%. Similarly, among the non-defatted hemp seed cultivars, HGIG showed the highest DPPH % inhibition at 79.65%, and CFX-1 had the lowest at 78.05%. In a study by Rashid., *et al*. [47], the highest DPPH (%) inhibition ranged from 44.46 to 49.33%. Frassinetti., *et al*. [57] also evaluated the seeds and sprouts of *Cannabis* and reported DPPH % inhibition ranging from 40 to 52%, respectively.

The findings demonstrate the variability in DPPH scavenging activity among the tested hemp seed cultivars.

Table 2 shows the IC_{50} of the hemp seed cultivars. The IC_{50} indicates the lowest concentration of extracts that can scavenge 50% of the radical. Our study found significant differences ($p < 0.05$) in the IC_{50} for the DPPH among hemp seed cultivars. In the defatted hemp seed cultivars, the HGIG indicated the lowest IC_{50} at 0.030 mg/mL, while the hemp seed cultivar CFX-1 had the highest IC_{50} at 0.050 mg/mL. In contrast, the non-defatted hemp seed cultivar CFX-1 had the lowest IC_{50} at 0.024 mg/mg, while the IHH had the highest IC_{50} at 0.114 mg/mg. According to Chen., *et al*. [50], the IC_{50} for different hemp seed varieties and extracts ranged from 0.09 to 4.55 mg/mL. The high polyphenol content and antioxidant activity of the hemp seed cultivars in this study highlight the potential to be used in food applications due to the presence of antioxidants that help maintain the hemp's oxidative stability.

	Non-defatted					Defatted				
Cultivars	**TPC (mg GAE/100 g)**	**TFC (mg CE/100 g)**	**FRAP (mmol FE/g)**	**DPPH (mg/mL)**	**IC_{50} (mg/mg)**	**TPC (mg GAE/100 g)**	**TFC (mg CE/100 g)**	**FRAP (mmol FE/g)**	**DPPH (%)**	**IC_{50} (mg/mL)**
CFX-1	41.47 ± 0.7^{c}	4.49 ± 0.6^{c}	105.5 ± 1.2^{c}	78.05 ± 3.2^{a}	0.024 ± 0.006^{b}	48.59 ± 0.4^{c}	22.28 ± 0.8^{b}	268.54 ± 11.0^{c}	76.19 ± 3.5^{a}	0.050 ± 0^{a}
HGIG	51.87 ± 0.2^{b}	19.42 ± 2.8^{b}	121.54 ± 11.0^{b}	79.65 ± 1.4^{a}	0.073 ± 0.006^{c}	67.43 ± 2.7^{a}	23.86 ± 1.8^{b}	360.74 ± 6.4^{a}	78.72 ± 1.7^{a}	0.030 ± 0^{b}
IHH	57.38 ± 1.3^{a}	33.07 ± 1.0^{a}	222.43 ± 13.6^{a}	79.31 ± 2.3^{a}	0.114 ± 0.002^{a}	64.71 ± 2.9^{b}	34.55 ± 3.5^{a}	293.26 ± 10^{b}	78.56 ± 2.1^{a}	0.036 ± 0.003^{b}

Table 2: *The polyphenol content (TPC, TFC) and antioxidant activities (FRAP, DPPH, IC_{50}) of non-defatted and defatted industrial hemp seed cultivars.*

Results are expressed as means ± SEM. Means with different superscripts are significantly ($p \leq 0.05$) different, and means with the superscripts are not significantly different using Tukey's means separation test.

The anti-nutrients of hemp seed cultivars

Table 3 presents the ANFs of defatted and non-defatted hemp seed cultivars. The ANFs are a significant issue in hemp seeds as they prevent essential nutrients from being absorbed by the body. According to the study, saponins were the ANFs, followed by phytic acid and tannins. The saponin content of hemp seed were 191.61, 186.72, and 198.94 mg DE/100 g DW for CFX-1, HGIG, and IHH cultivars, respectively, for the defatted hemp seeds cultivars and 155.75, 185.09, and 185.9 mg DE/100 g DW respectively for the non-defatted

hemp seed cultivars. The defatted hemp seed cultivars had more saponin content than the non-defatted hemp seeds. Russo and Reggiani [58] evaluated the ANFs compounds in hemp seed meal of Italian and French varieties and reported a saponin content of around 69.0 mg Kg^{-1} dry matter.

	Non-defatted			Defatted		
Cultivars	**Phytic acid (mg/g)**	**Tannins (mg CE/100 g)**	**Saponins (mg DE/100 g)**	**Phytic acid (mg/g)**	**Tannins (mg CE/100 g)**	**Saponins (mg DE/100 g)**
CFX-1	57.42 ± 1.30^{b}	14.93 ± 0.6^{b}	155.75 ± 2.8^{b}	12.83 ± 0.36^{b}	33.74 ± 2.6^{a}	191.61 ± 2.4^{a}
HGIG	49.71 ± 1.57^{c}	17.29 ± 0.5^{a}	185.09 ± 0.8^{a}	19.50 ± 0.00^{a}	26.64 ± 1.8^{c}	186.72 ± 8.1^{a}
IHH	62.42 ± 1.44^{a}	17.18 ± 2.1^{a}	185.9 ± 3.2^{a}	8.25 ± 0.62^{c}	24.86 ± 1.2c	198.94 ± 4.8^{a}

Table 3: *The anti-nutrients (phytic acid, tannins, and saponins) of non-defatted and defatted hemp seed cultivars.*

Results are expressed as means ± SEM. Means with different superscripts are significantly ($p \leq 0.05$) different using Tukey's means separation test.

Tannins are also phenolic compounds but are considered anti-nutrients because they form insoluble complexes with proteins and minerals, affecting the absorption of these nutrients [35] and reducing the nutritional value of food. Our study determined the tannin levels in hemp seeds, and our findings revealed that the tannin content of defatted hemp seed cultivars was highest at 33.74 mg CE/100 g DW for CFX-1 and lowest at 24.86 mg CE/100 g DW for IHH. On the other hand, the non-defatted hemp seed cultivars had the highest tannin content of 17.29 mg CE/100 g DW for HGIG and the lowest tannin content of 14.93 mg CE/100 g DW for CFX-1. Mattila., *et al*. [32] found that the hull of hempseed had more tannins than the whole hemp seeds. The defatted sample had more tannins than the non-defatted hemp seed cultivars. This indicates that the defatting process did not inhibit the presence of tannins; instead, it increased it.

Phytic acid is the primary storage of phosphorus in plants. The phytic acid content of the three hemp seed cultivars showed that the defatted hemp seed cultivars had significantly lower levels of phytic acid than the non-defatted ones. Specifically, the phytic acid levels for the defatted CFX-1, HGIG, and IHH cultivars were 12.83, 19.50, and 8.25 mg/g, respectively. In contrast, the non-defatted ones had much higher levels of 57.42, 49.71, and 62.42 mg/g, respectively.

Previous studies have also reported varying levels of phytic acid in different hemp cultivars. For instance, Russo and Reggiani [58] found that the phytic acid content in hemp cultivars ranged from 61.2 to 74.1 g/kg dry matter. They also reported that French monoecious varieties had lower phytic acid content than the Italian dioecious ones. However, Galasso., *et al*. [40] analyzed twenty hemp seed varieties and found that French varieties had the highest phytic acid content. These differences could be due to variations in genotype and growing conditions, as Russo and Reggiani [35] noted.

Interestingly, Farinon., *et al*. [4] found that the raw hemp sample had higher levels of phytic acid than the defatted sample, suggesting that the defatting process can effectively reduce phytic acid levels in hemp seed cultivars.

ICP profile of hemp seed cultivars

Table 4 presents the mineral content of three hemp seed cultivars. The primary macro elements in hemp seeds were phosphorus, potassium, magnesium, calcium, and sodium. Iron, manganese, Zinc, and Copper are some of the significant microminerals found in these seeds [8]. These minerals are essential for various physiological functions in the body, as recognized by the European Food Safety Authority in 2017 [59]. According to the ICP analysis, the highest mineral detected in the IHH cultivar was phosphorus, with a concentration of 8.73 mg/g. Among the different hemp cultivars, Copper was found to be the least abundant mineral, with 0.013 mg/g in HGIG.

Minerals	CFX-1	HGIG	IHH
Phosphorus	8.37 ± 0.06[a]	7.95 ± 0.36[a]	8.73 ± 0.4[a]
Potassium	6.63 ± 0.04[b]	6.65 ± 0.15[b]	6.83 ± 0.2[b]
Magnesium	3.86[c]	3.85 ± 0.07[c]	3.99 ± 0.05[c]
Calcium	1.00 ± 0.001[d]	0.97 ± 0.003[d]	0.91 ± 0.005[d]
Iron	0.13 ± 0.0007[e]	0.12 ± 0.002[e]	0.12 ± 0.0007[e]
Zinc	0.05[f]	0.05 ± 0.001[e]	0.07 ± 0.001[e]
Manganese	0.12 ± 0.001[f]	0.10 ± 0.0007[e]	0.14[e]
Copper	0.02[f]	0.01 ± 0.0002[e]	0.02 ± 0.0007[e]

Table 4: *The mineral content (mg/g) in hemp seed cultivars using ICP-OES.*

Results are expressed as means ± SEM. Means with the same superscripts are not significantly different (p ≥ 0.05) using Tukey's means separation test.

In contrast, Zinc was highest in the IHH cultivar at 0.07 mg/g and lowest in CFX-1 at 0.05 mg/g. The highest Manganese content was observed in IHH at 0.14 mg/g, while the lowest was in the HGIG at 0.10 mg/g. Alonso-Esteban., *et al*. [60] conducted a study on minerals in whole and hulled hemp seeds, which revealed that phosphorus was the most abundant mineral in both types of seeds, with 871.2 mg/100g for whole seeds and 1099.5 mg/100g for hulled seeds. The study also found that the potassium content in all hemp seeds ranged from 311.5 to 713.6 mg/100g, and the average magnesium content in whole hemp seeds was 383.4 mg/100g.

Similarly, Callaway [5] reported phosphorus (1160 mg/100g) as the most abundant mineral. This study found that phosphorus is the most abundant mineral in hemp seed cultivars. Potassium, magnesium, and calcium are also present in high quantities, while Copper is the least abundant mineral. The amounts of these minerals may vary depending on environmental conditions, the type of minerals, fertilizers, and the variety used [8].

The results indicate that these cultivars contain essential nutrients required by the body. Zinc, an essential micronutrient that plays a significant role in cellular growth and tissue differentiation, was also present in the cultivars [61]. Regular dietary Zinc intake is necessary since the body does not store it for long. Magnesium is another essential mineral that contributes to heart function and overall health. A magnesium deficiency can result in cardiac dysfunction [4]. Copper is another trace element that the body needs for various cellular pathways. Phosphorus, a macronutrient, is also essential for bone formation. Approximately 70% of phosphorus is in the form of phytic acid [62], which can hinder the bioavailability of nutrients.

There have been insufficient studies on the mineral content of hemp seed cultivars. Therefore, this study highlights that various cultivars contain a range of minerals that are beneficial to health and can be used for their properties.

The cannabinoid profile of hemp seed cultivars

In the study, CFX-1, HGIG, and IHH hemp seed cultivars were analyzed for their cannabinoid profiles, which are shown in figure 2-4. Additionally, table 5 displays the cannabinoid contents of these cultivars. Cannabinoids are bioactive components found in hemp and have numerous health benefits. CBG and CBD have antineoplastic properties, CBC is an anti-depressant, CBD has analgesic, anti-inflammatory, and anxiolytic properties, and D8-THC is effective against glaucoma [63]. CBDA is the most abundant cannabinoid in all three hemp seed cultivars. The HGIG cultivar exhibits the highest concentrations of both CBDA and CBD at 39.25 mg/kg and 10.94 mg/kg, respectively. CBD is one of the main components of hemp products, and the most common CBD product available in the United States is hemp oil, which can be purchased over the counter [64,65].

Cannabinoids	CFX-1 (mg/kg)	HGIG (mg/kg)	IHH (mg/kg)
CBDA	23.39 ± 3.35[a]	39.25 ± 0.91[a]	10.72 ± 1.59[a]
CBN	3.23 ± 0.40[c]	4.04 ± 0.29[c]	3.99 ± 0.09[b]
CBD	5.05 ± 0.30[b]	10.95 ± 0.03[b]	2.35 ± 0.44[b]
CBDVA	0.73 ± 0.12[d]	1.77 ± 0.08[e]	0.35 ± 0.13[c]
THCVA	0.11[d]	0.22 ± 0.01[f]	0.09 ± 0.01[c]
CBNA	0.63 ± 0.02[d]	2.71 ± 0.19[d]	0.07 ± 0.01[c]
CBG	0.11 ± 0.02[d]	0.35[f]	0.04[c]
CBDV	ND	0.11 ± 0.02[f]	ND
CBC	ND	ND	ND
CBL	ND	ND	ND
D8-THC	ND	ND	ND
D9-THC	ND	1.07 ± 0.17[e]	ND
D9-THCA	0.16 ± 0.03[d]	1.68 ± 0.13[e]	ND
THCV	ND	ND	ND
CBGA	ND	0.65 ± 0.05	ND

Table 5: *The cannabinoid content (mg/kg) of selected hemp seed cultivars using ICP-OES.*

Results are expressed as means ± SEM. Means with the same superscripts are not significantly different (p ≥ 0.05) using Tukey's means separation test.

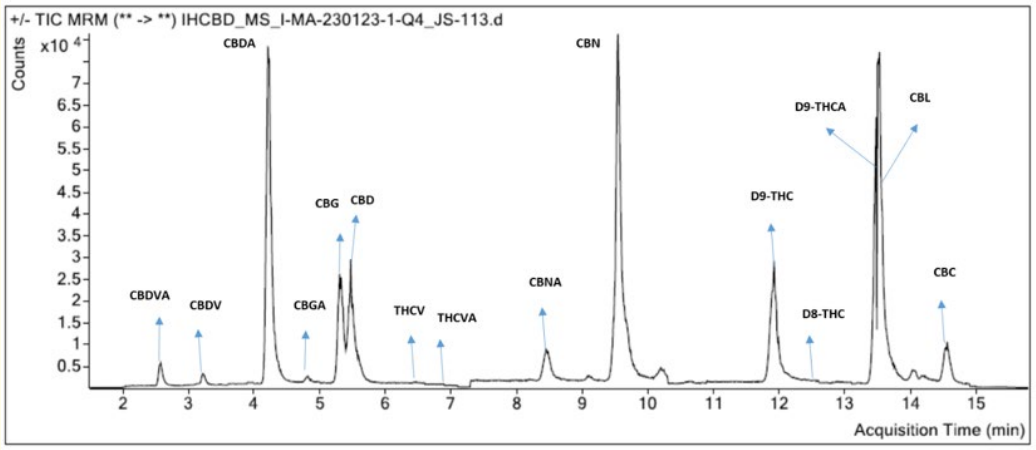

Figure 2: *UHPLC-MS/MS chromatogram of cannabinoids profile of CFX-1.*

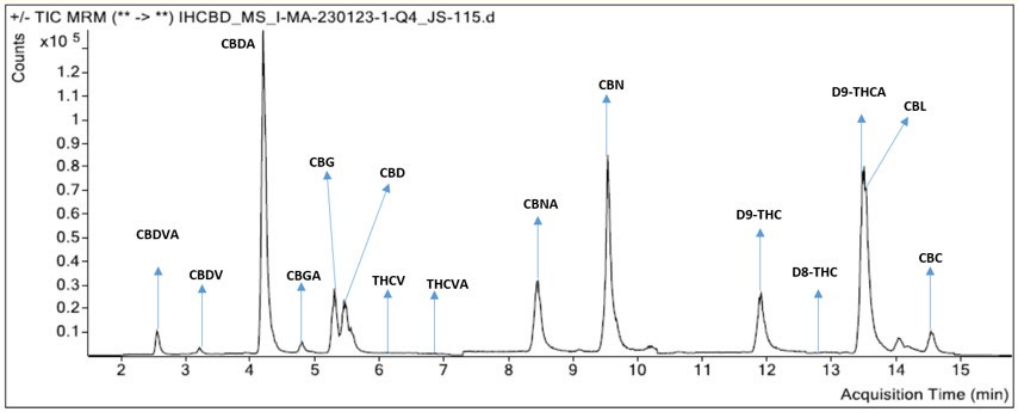

Figure 3: *UHPLC-MS/MS chromatogram of cannabinoids profile of HGIG.*

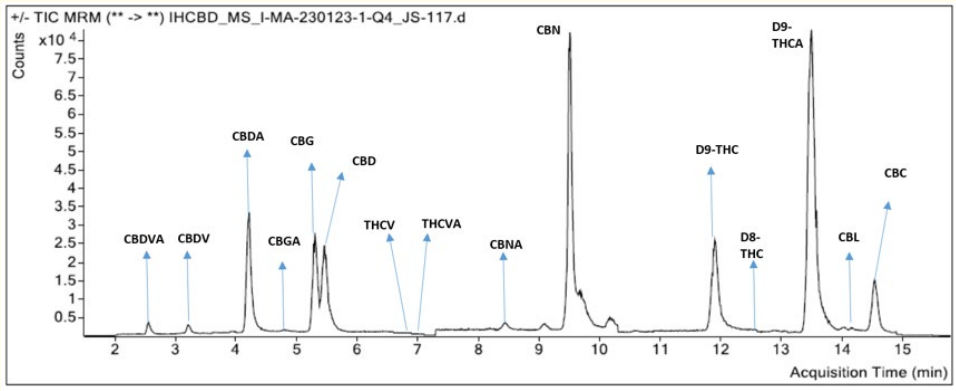

Figure 4: *UHPLC-MS/MS chromatogram of cannabinoids profile of IHH.*

The FDA in the United States has approved CBD as a prescription medication for seizures due to its neuroprotective, anxiety-relieving, and anti-cancer properties [65,66]. Therefore, growers and breeders of hemp aim to produce plants with high CBD and low THC levels, as these are desirable characteristics [67]. Among other cannabinoids evaluated, CBN has been found to have the highest concentration of 4.04 mg/kg in the HGIG cultivar. The test results show that other cannabinoids are present, albeit in small amounts. CBDVA was detected but at low levels. CBDV was not found in CFX-1 and IHH, but 0.11 mg/kg of CBDV was found in the HGIG cultivar. Furthermore, no CBGA was detected in CFX-1 and IHH, but 0.65 mg/kg of CBGA was found in the HGIG cultivar. Chen., *et al*. [68] found that Matterhorn, another hemp variety, had CBGA levels ranging from 62.65 to 80.82 mg/g. In the selected cultivars, the CBG concentration varies. The highest concentration of CBG was found in HGIG at 0.35 mg/kg, while the lowest concentrations were found in CFX-1 and IHH at 0.12 and 0.04 mg/kg, respectively. However, a study by Chen., *et al*. [68] reported a much higher CBG level of 1.34 to 2.93 mg/g. These differences in CBG levels among the cultivars may be due to genetic diversity, environmental conditions, and growers' practices [68]. This information can be helpful for hemp growers when selecting cultivars and aid them in making informed decisions.

Table 6 displays the correlation table of antioxidants and anti-nutrients in non-defatted hemp seeds. The study found a positive correlation between antioxidants and anti-nutrients. The antioxidants increased as the anti-nutrients increased. However, phytic acid

had a lower correlation with the antioxidants, except for FRAP, which had a positive correlation with the phytic acid. The study suggests that the positive correlation between the antioxidants and anti-nutrients may be due to the hemp seeds being in their natural form without being defatted; thus, the antioxidants and anti-nutrients were masked. Table 7, on the other hand, shows the correlation tables of antioxidants and anti-nutrients in defatted hemp seeds. Overall, a negative correlation was observed between the antioxidants and the anti-nutrients. Except for FRAP, the correlation between phytic acid and antioxidants was low. FRAP showed a positive correlation with phytic acid. The same trend was observed in non-defatted hemp seeds. The negative correlation between antioxidants and anti-nutrients indicates that the amount of anti-nutrients decreases as the antioxidants increase. This means that antioxidants play a crucial role in reducing anti-nutrients. The defatting process can release more antioxidants and fewer anti-nutrients from hemp seed cultivars, thus reducing their harmful effects. Therefore, defatting is essential for releasing antioxidants and reducing anti-nutrients in hemp seeds.

	Non-defatted TPC	Non-defatted TFC	Non-defatted FRAP	Non-Defatted DPPH
Non-defatted Saponins	0.935022	0.872739	0.620961	0.343342
Non-defatted Tannins	0.61501	0.631578	0.30317	0.244429
Non-defatted Phytic acid	0.207542	0.350642	0.68629	-0.00462

Table 6: *Correlation table of antioxidants and anti-nutrients in non-defatted hemp seeds.*

	Defatted TPC	Defatted TFC	Defatted FRAP	Defatted DPPH
Defatted Saponins	0.505852	0.39374	0.289299	-0.21803
Defatted Tannins	-0.86354	-0.71117	-0.47883	-0.28971
Defatted Phytic acid	0.222118	-0.6848	0.762409	0.050485

Table 7: *Correlation table of antioxidants and anti-nutrients in defatted hemp seeds.*

Conclusion

Our study showed significant differences and variabilities among the three hemp seed cultivars evaluated. While each cultivar contained antioxidants and anti-nutrients, the levels varied due to factors such as geography and soil. Thus, screening different hemp seed cultivars provided new insights and opportunities for further research. After analyzing three hemp cultivars, we found that each contained varying levels of phytochemicals, anti-nutrients, cannabinoids, and antioxidants. Although all cultivars had different TPC, TFC, FRAP, and DPPH levels, the IHH cultivars showed the most promise due to their unique properties. Our study suggests that hemp seeds have a valuable nutritional and antioxidant profile that could be utilized in the food industry. However, the anti-nutrient saponin was higher than the other anti-nutrient and could impair the beneficial properties of hemp seeds. Our results can help hemp growers decide which cultivars to grow and encourage people to purchase and use hemp seeds for their many benefits.

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Conflict of Interest

The authors declare no conflict of interest.

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