

ANALYSIS OF PROXIMATE COMPOSITION, FATTY ACIDS PROFILE, AND MINERAL ELEMENTS OF HEMP (*CANNABIS SATIVA*)

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Abstract

For decades, hemp has been used as a medicinal plant. The present study aimed to evaluate the nutritional importance of hemp products by analyzing their proximate composition in hemp seed and leaf, fatty acids profile in hemp seed oil and minerals in hempseed, hemp oil, and leaf as per standard methods. In hempseed, dry matter, crude protein, ether extract and energy are resulted significantly ($p < 0.05$) higher in comparison to hemp leaf. In hemp leaf, ash are resulted significantly ($p < 0.05$) higher in comparison to hemp seed. Moisture content are resulted significantly similar in hemp seed and hemp leaf. In the fatty acids profile, among saturated fatty acids (SFA), palmitic acid (4.35 ± 0.78), and stearic acid (3.67 ± 0.14) resulted higher in concentrations as compared to other SFA. In monounsaturated fatty acids (MUFA), oleic acid, (12.61 ± 0.73) resulted higher in concentrations as compared to other MUFAs. In polyunsaturated fatty acids (PUFA), linoleic acid (39.72 ± 0.62), and linolenic acid (18.31 ± 0.02) resulted higher in concentrations as compared to other PUFA. Total percentage of SFA, MUFA and PUFA are 8.89%, 16.30%, and 63.70% respectively. In hemp seed, Magnesium, Potassium, Manganese, Copper, and Zinc are resulted significantly ($p < 0.05$) higher in comparison to hemp oil and hemp leaf. In hemp oil Chromium are resulted significantly higher in comparison to hemp seed and hemp leaf. In hemp leaf Calcium and Iron are resulted significantly higher in comparison to hemp seed and hemp leaf. We recommend hemp products as a feed ingredient in the diets of fishes, and poultries due to its remarkable nutritive nature.

Key words: Hemp products; Proximate composition; Fatty acids profile; Mineral elements

Introduction

Cannabis sativa L. (commonly known as marijuana or Indian hemp) is widely distributed across tropical and temperate regions worldwide (Small, 2017; Chandra *et al.*, 2017). As one of best humanity's oldest cultivated crops, *Cannabis* has a documented history of use back 6000 years. The plant is native to western and central Asia and is now commercially cultivated in Europe and parts of China, Canada, the United States, and Japan. In Pakistan, its cultivation is primarily limited to the former Federally Administered Tribal Area (Ex-FATA), where it typically matures within 120 days, reaching heights of 10–15 feet (Borhade, 2013). Unlike many crops, *Cannabis sativa* does not require herbicides and pesticides for growth, but it grows best in fertile soil with moderate water availability.

Cannabis is an annual herbaceous plant, agriculturally cultivated for its oil and fiber (Small, 2015). Female plants are useful due to their pharmacological properties. Hemp phytochemistry is quite complex and cannabinoids are its most typical compounds (Flores-Sanchez & Verpoorte,

2008). Phytochemical analysis of the leaves revealed the presence of alkaloids, flavonoids, steroids, cardiac glycosides, resins, and terpenes (Audu *et al.*, 2014). Cannabinoids are a group of compounds present in the form of resin extracted from flowers of glandular trichomes of female plants, which are commonly located in the bracts of female flowers (Small & Naraine, 2016).

In the late 1930s in North America hempseed and all *Cannabis* varieties were prohibited because of the presence of the phytochemical drug δ -9-tetrahydrocannabinol (THC) (Huestis, 2005). Within the last 10 years, hempseed has been legally used as food for humans in both Canada and the United States. Since 2003, *C. sativa* L., varieties with a low concentration of the psychoactive substance delta-9-tetrahydrocannabinol has been permitted within the European Union (Council of the European Communities, 1993) (Ballotta *et al.*, 2008). Non-drug varieties of *C. sativa* L., collectively namely as “hemp” have been an interesting source of food, fiber, and medicine for thousands of years (Montserrat-De *et al.*, 2014). In China, roasted hempseed is still sold as a snack by street vendors.

According to novel research, applications for hemp seed oil contribute to the pharmaceutical, cosmetic, food, and other non-food industries (Montserrat-De *et al.*, 2014). Its excessive use can cause melancholy, cough, impotence, dyspepsia, dropsy, insanity, and restlessness (Zade *et al.*, 2013). In Pakistan there is a limited study on hemp, and its applications, however Afridi *et al.*, (2019), reported nutritional importance of hemp, and therapeutic role of hemp against copper induced toxicity in freshwater fishes (Afridi *et al.*, 2024). In world some other researcher reported antibacterial effect of hemp by (Ali *et al.*, 2012), pharmacological activities by (Whiting *et al.*, 2015), antifungal, and anti-leishmanial activity by (Radwan *et al.*, 2009).

Hemp plants possess a remarkable chemical composition. Hemp seed typically contains over 300 g oil/kg, about 250 g protein/kg, and considerable amounts of dietary fiber, vitamins, and minerals (Callaway, 2004). It contains 20–25% protein, 20–30% carbohydrates, 25–35% oil, and 10–15% insoluble fiber. Hempseed has elevated levels of vitamins A, C, and E, β -carotene (Orhan *et al.*, 2000), Thiamine (B1), Riboflavin (B2), and a rich set of minerals such as Phosphorous (P), Potassium (K), Magnesium (Mg), Calcium (Ca), Iron (Fe), Sodium (Na), Manganese (Mn), Zinc (Zn) and Copper (Cu) (Borhade, 2013; Isahq *et al.*, 2015). The two main proteins in hempseed are edestin and albumin. Both high-quality storage proteins are easily digested and contain nutritionally significant amounts of all essential amino acids. In addition, hempseed has exceptionally elevated levels of the amino acid arginine but scarce information is available about their proximate, fatty acid and mineral elements. To fill this gap, the objectives of this study were to determine the nutritional importance of hemp products by assessing the proximate analysis, fatty acid profile, and mineral element content of whole hemp plant.

Material and Methods

Specimen collection: Hemp seeds and leaves were collected from Tirah Maidan, district Khyber, ground well, and made a powder, kept in a zip lock shopper for further analysis.

Proximate Composition: The moisture, dry matter, ash content, crude protein, crude fats (ether extract), and gross energy contents were determined of hemp seed and hemp leaf as per the methodology of Anon., (2000) from Veterinary Research Institute, Peshawar. The moisture content was determined by weighing the initial wet weight of a fresh sample and then drying it in the oven at 45°C for 72 h until obtained a constant weight. Crude protein was determined by nitrogen determination using the Kjeldahl micro method (Sutharshiny & Sivashanthini 2011), and conversion of nitrogen to protein by factor 6.25. The lipid content of the powdered hemp seed was determined with the help of the Soxhlet apparatus using the non-polar organic solvent hexane. For ash contents, a pre-washed crucible was placed in a muffle furnace at 100°C for an hour, then cooled and weighed. Now 2 g sample was placed in the crucible and again placed in the muffle furnace at 550°C for 5 h. Later, it was again placed in a desiccator, cooled, and weighed quickly to prevent moisture absorption. The proximate composition were determined on basis of dry methods. Gross energy was measured through a bomb calorimeter (cal/g).

Oil extraction: First, powdered hemp seeds were kept in n-hexane from 8 to 10 days, and extracted oil was condensed by evaporation of extra n-hexane through a Rotary vacuum evaporator (PLC/FTC/Chem. MB12 Eyela Japan) and shifted to a vial. The vial was left overnight to evaporate the extra hexane (Anon., 1972).

Fatty acids methyl esters analysis: FAMES (fatty acids methyl esters) were carried out according to the standard procedure mentioned in the association of official analytical chemists (Anon., 1999). FAMES were prepared and filtered through 0.45 μ m membrane filter paper. The composition analysis of fatty acid methyl esters (FAMES) was done with help of a gas chromatography-mass spectrometer (GC-MS-QP Plus 2010 Shimadzu Japan). GC-MS conditions with 100 m CP Sil 88 capillary column (i.d. 0.25 μ m, film thickness, 0.20 μ m, Chrompack, Middleburg, Netherlands) and a flame ionization detector. The column temperature was 80°C for 1 min at the time of sample injection, then ramped at 2°C min⁻¹ to 215 C and maintained for 30 min. Inlet temperatures were 220°C and detector temperatures were 230°C. The split ratio was 100:1. The flow rate for H₂ carrier gas was 1 mL/min. Most of the fatty acid peaks were identified and quantified using either a quantitative mixture or pure methyl ester standards using a software program chem station and data processor Chem.

Sample preparation and Mineral analysis: For mineral element analysis, briefly, 1 g of each sample (hemp seed, leaf, and oil) was transferred into an oven-dried 50 mL volumetric flask, and added 5 mL of 69 % of nitric acid (HNO₃) and 1 mL 60% of perchloric acid (HClO₄). Yousafzai & Shakoori, (2006) method was adopted for the digestion of samples. The flasks were tightly packed and kept overnight at room temperature. The next day, again 5 mL of HNO₃ and 1 mL of HClO₃ were added to each flask and placed on a hot plate at 200 to 250°C for the digestion of the sample.

The appearance of brown-red fumes and conversion into white fumes was an indication of the completion of the digestion process. The digestion process continued until a clear and transparent solution was obtained. At the end of complete digestion, samples were cooled and the volume of the digest was raised to 50 mL by the addition of double distilled water (Nussey *et al.*, 2000). Each digest was filtered through 10 mm Whatman filter paper in a 50-mL round bottom and this prepared sample was stored at room temperature till analysis. Macro and microelements like Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K), Iron (Fe), Chromium (Cr) Manganese (Mn), Copper (Cu), and Zinc (Zn) were analyzed with help of Atomic Absorption Spectrophotometer (AAS) (Thermo made in USA) at Quaid-e- Azam University, Islamabad.

For gaining a standard curve on spectrophotometer, analyzed standard solution in different ranges (ppm) before analyzing the selected element. The concentration of each element was calculated in prepared samples with the help of the standard curve.

Statistical Analysis

Data were presented as mean \pm S.E. Data of Fatty acids profile are represented in percentage (%) as mean. For significant differences of proximate composition, and Mineral elements the comparison among the means was evaluated by One-way ANOVA followed by Tukey HSD post hoc test using R-statistical package.

Results

Proximate composition: The proximate composition of hemp leaf and hemp seed are shown in (Table 1). Proximate composition are determined in two selected parts of hemp (*Cannabis sativa*) Viz hemp seed and hemp leaf. For significant variation One-way ANOVA followed by Tukey HSD post hoc test are used among them. In hemp seed the dry matter, crude protein, ether extract and energy are resulted significantly ($p < 0.05$) higher in comparison to hemp leaf. In hemp leaf only ash content are resulted significantly ($p < 0.05$) higher in comparison to hemp seed. Moisture content are resulted significantly similar in both hemp seed and hemp leaf. Both parts showing good composition especially of crude protein and ether extract.

Table 1. Showing the proximate composition of hemp seed and hemp leaf.

Parameter	Hemp Seed	Hemp Leaf
Moisture	5.36 \pm 0.10a	4.73 \pm 0.32a
Dry matter (D. M)	94.64 \pm 0.10a	92.70 \pm 0.53b
Ash	9.05 \pm 0.51b	12.30 \pm 0.93a
Crude protein (C.P)	20.79 \pm 0.34a	15.67 \pm 1.01b
Ether extract	27.94 \pm 0.17a	15.79 \pm 0.56b
Energy	3410.30 \pm 1.16a	3163.30 \pm 40.96b

Data are represented by Means \pm SE; n= number of the sample (n=6) One-way ANOVA followed by Tukey HSD post hoc test. Different letters within the row showing significantly different ($p < 0.05$)

Fatty acids profile: Fatty acids profile in hemp seed oil in percentage (%) are shown in (Table 2). The contents of saturated fatty acids (SFA) like Palmitic acid (C12:0), Stearic acid (C18:0) were showed comparatively higher in concentration as compared to other SFA like Myristic acid (C14:0), Pentadecanoic acid (C15:0), Margaric acid (C17:0), Behenic acid (C22:0), and Tetracosanoic acid (C24:0). Among monounsaturated fatty acids (MUFA), the contents of Oleic acid, (C18:1c) were resulted higher as compared to others MUFA like Heptadecenoic acid (C17:1), Eicosanoic acid (C20:1) and Erucic acid (C22:1). Among polyunsaturated fatty acids (PUFA) the contents of Linoleic acid (C18:2c), Linolenic acid (Omega 3) (C18:3n3), were resulted higher as compared to others PUFA like Octadecadienoic acid (C18:2t), Eicosadienoic acid (C20:2), Docosahexaenoic acid (DHA) (C22:6n3). Three essential fatty acids like Linolenic acid (C18:3n3), (omega-3 fatty acids), Linoleic acid (C18:2c) (Omega-6 fatty acids) and Oleic acids (C18:1c) (Omega-9 fatty acids) resulted in higher concentration as compares to others. Overall, the total % age contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were 8.89, 16.30, and 73.70, respectively. Hemp seed oil has shown best percentage of polyunsaturated fatty acids especially of essential fatty acids such as Oleic acids, Linoleic acid, and Linolenic acid.

Minerals element: The resulting minerals element of hemp seed, hemp seed oil, and hemp leaf are shown in (Table 3). In three selected parts of hemp, four macro elements (Ca, Mg, Na, and K) and five microelements (Fe, Cr, Mn, Cu, and Zn) were analyzed and One-way ANOVA tracked by Tukey HSD post hoc test are used for significant variation among them. In hemp seed mineral such as Magnesium, Potassium, Manganese, Copper, Zinc are resulted significantly ($p < 0.05$) higher in comparison to hempseed oil and hemp leaf. In hempseed oil only Chromium are resulted significantly higher in comparison to hemp seed and hemp leaf. In hemp leaf Calcium and Iron are resulted significantly ($p < 0.05$) higher in comparison to hemp seed and hemp leaf. Sodium are significantly similar in hemp seed and hemp leaf, but higher than hemp oil. The resulting concentration order of Microelements in hemp seed are Fe > Cr > Cu > Mn > Zn; in hemp seed oil are Fe > Cr > Mn > Cu > Zn; and in hemp leaf are Fe > Cr > Cu > Mn > Zn. The resulting concentration order of the Macro element in hemp seed are K > Mg > Ca > Na; in hemp oil are K > Na > Mg > Ca and in hemp leaf are Ca > Mg > K and > Na respectively. All of three parts showed best results of mineral followed by seed > leaf > oil. The mineral element of hemp seed were better in concentration in comparison to hemp seed oil and hemp leaf.

Discussion

Proximate analysis: *Cannabis sativa* is an important source of nutrition and considered a medicinal plant for thousands of years. Hemp seeds contain large amounts of protein, carbohydrates, oil, and insoluble fiber at 20–25%, 20–30%, 25–35%, and 10–15%, respectively (Small, 2017; Chandra *et al.*, 2017). Hemp products represent good chemical composition in this study as well as reported by Orhan *et al.*, (2000) as a food ingredient and a super food by Afridi *et al.*, (2019). Besides protein and fibers, novel research represents, that hemp as a good source of essential amino acids, PUFA, vitamins, minerals, and antioxidants (Cerino *et al.*, 2021; Mihoc *et al.*, 2022).

In hempseed, dry matter, crude protein, ether extract and energy are resulted significantly ($p < 0.05$) higher in comparison to hemp leaf. In hemp leaf, ash are resulted significantly ($p < 0.05$) higher in comparison to hemp seed. Moisture content are resulted significantly similar in hemp seed and hemp leaf. Diet formulation and preparation is a crucial part of animal nutrition and plays a significant role in the growth and development of organisms. In the results of this study, proximate composition of this study indicate a good proportion of crude protein and ether extract of both hemp seed and hemp leaf. However, hemp seed showed higher contents of crude protein and ether extract in comparison to hemp leaf. In artificial feed preparation two factors such as crude protein and ether extract are of prime importance. From field studies, it comes under observation that hemp is traditionally used as a feed ingredient in the diet of various animal feeds in Pakistan. Hemp and their products are cheap and easily available in Pakistan. Nutritionist always prefers such type of feed, which enhances growth factor and determines the quality of animal meat, which consume them. Literature reported the nutritional application of hemp products, such as hempseed

cake (HC) are the by-product obtained after extracting the oil, as a source of protein for the feed of ruminants like goats, cows, and buffaloes was recommended by Bailoni *et al.*, (2021) and Karlsson (2010). Karlsson *et al.*, (2010) reported that increasing the proportion of HC in the diet of cows significantly increased milk production and composition, while Tang *et al.*, (2006) reported by comparing the hemp protein isolate (HPI) and soy protein isolate (SPI) that HPI can be used as a valuable source of nutrition for infants and children. In contrast to other animals, limited information exists on the use of hemp seed products in aquaculture. Afridi *et al.*, (2019) reported that

hemp product in fish meal significantly increased muscle total fatty acid contents, improved ω -3/ ω -6 ratios, and decreased SFA / USFA ratio in a % age inclusion-dependent manner. A few investigators replaced the fish meal with a hempseed meal in feed formulation and suggested its potential in fish culture (Webster *et al.*, 2000). However, some scientists reported the beneficial effects of hempseed products (hempseed and hempseed oil) on the nutritional value of eggs i.e., increased ω -3 PUFA and decreased ω -6/ ω -3 PUFA ratio (Gakhar *et al.*, 2012; Neijat *et al.*, 2016) and serum fatty acids composition of animals (Klir *et al.*, 2019).

Table 2. Showing the fatty acids profile in hemp seed oil.

Fatty acids	No of carbon atoms	Types of fatty acids	Fatty acids (% age)
Myristic acid	C14:0	SFA	0.21 ± 0.03
Pentadecanoic acid	C15:0	SFA	0.18 ± 0.03
Palmitic acid	C16:0	SFA	4.35 ± 0.78
Margaric acid	C17:0	SFA	0.21 ± 0.00
Heptadecenoic acid	C17:1	MUFA	1.59 ± 0.68
Oleic acids (Essential)	C18:1c	MUFA	12.61 ± 0.73
Stearic acid	C18:0	SFA	3.67 ± 0.14
Lenoliec acid (Essential)	C18:2c	PUFA	39.72 ± 0.62
Octadecadienoic acid	C18:2t	PUFA	2.70 ± 0.45
Linolenic acid (Omega 3) (Essential)	C18:3n3	PUFA	18.31 ± 0.02
Eicosanoid acid	C20:1	MUFA	1.99 ± 0.12
Eicosadienoic acid	C20:2	PUFA	0.22 ± 0.18
Behenic acid	C22:0	SFA	0.04 ± 0.00
Errucic acid	C22:1	MUFA	0.11 ± 0.00
Docosahexaenoic acid (DHA)	C22:6n3	PUFA	2.58 ± 0.15
Tricosanoic acid	C23:0	PUFA	0.17 ± 0.12
Tetracosanoic acid	C24:0	SFA	0.23 ± 0.01
SFAs		=	8.89
UFAs		MUFAs =	16.30
Total percentage (%) contents of fatty acids		PUFAs =	63.70

Data are represented in % age as mean ± SE; n = 6

Table 3. Showing the mineral elements in hemp seed, hemp seed oil, and hemp leaf.

Minerals types	Hemp seed	Hemp oil	Hemp leaf
Ca	42.22 ± 5.85 ^b	11.84 ± 3.27 ^c	55.02 ± 5.85 ^a
Mg	46.63 ± 3.02 ^a	14.53 ± 0.99 ^c	39.02 ± 4.04 ^b
Na	31.20 ± 2.80 ^a	16.26 ± 1.23 ^b	28.24 ± 3.00 ^a
K	94.17 ± 5.67 ^a	17.00 ± 1.81 ^c	28.24 ± 3.00 ^b
Fe	49.55 ± 12.08 ^b	10.82 ± 1.11 ^c	74.08 ± 10.89 ^a
Cr	7.75 ± 0.37 ^b	10.82 ± 1.11 ^a	6.06 ± 0.44 ^b
Mn	5.82 ± 1.47 ^a	4.51 ± 1.13 ^b	3.58 ± 0.87 ^b
Cu	5.92 ± 0.91 ^a	3.22 ± 0.50 ^b	3.88 ± 0.52 ^{ab}
Zn	2.53 ± 0.41 ^a	0.44 ± 0.09 ^b	1.89 ± 0.55 ^{ab}

Data are represented by Means ± SE; n = number of a sample (n=5). One-way ANOVA followed by Tukey HSD post hoc test. Different letters within the row showing significantly different ($p < 0.05$)

Fatty acids: In this study, three essential fatty acids like Linolenic acid (C18:3n3), (omega-3 fatty acids), Linoliec acid (C18:2c) (Omega-6 fatty acids) and Oleic acids (C18:1c) (Omega-9 fatty acids) are resulted higher in concentration as also reported by Kriese *et al.*, (2004). Results of this study showing the percentage (%) of the total contents of unsaturated fatty acids (UFA) are greater in comparison to saturated fatty acids (SFA). Reported literature agrees with this study, that hempseed contains significant amounts of linoleic acid “omega-6” (18:2n6,

LA) which represents 50% of total fatty acids, and “omega-3” alpha-linolenic acid (18:3n3, ALA) occurs at about 20% and 12–17% of oleic acid (Montserrat-De *et al.*, 2014; Alonso-Esteban *et al.*, 2022; Vonapartis *et al.*, 2015). However, the Linoliec acid (C18:2c) (Omega-6 fatty acids) and α -linolenic acid (ALA), an omega-3 fatty acid, are essential for the human body (cannot be synthesized by the human body). Adili *et al.*, (2018) reported that the deficiencies of essential fatty acids and an imbalance of omegas 6/3 lead to cardiovascular disease. Most animals including humans, are unable to synthesize essential fatty acids, like alpha-linolenic acids (ALA, ω -3 fatty acids) and linoleic acids (LA, ω -6 fatty acid), thus indicating the need to supply through supplemented food (Das, 2006; Wallis & Watts, 2002; Kiralan *et al.*, 2010). Both these FAs are essential as several other fatty acids are synthesized in a cascade reaction downstream from these fatty acids. Among highly physiologically downstream products, arachidonic acid (20:4n-6), derives from linoleic acid (LA) (linoleic acid → gamma-linolenic acid (GLA) → arachidonic acid (AA)) while α -linolenic acid is the precursor of docosahexaenoic acid (DHA) (22:6n-3) and eicosapentaenoic acid (EPA) (20:5n-3) (Salim *et al.*, 2015). Hemp seed possesses a good level of essential fatty acids but its market value is equal to zero in the majority of world countries. Some seed have high market values and their

remains are used as the common sources of protein in livestock nutrition like soybean meal and canola meal, but not contains all essential fatty acids. Some seeds contain a high level of α -linolenic acid (nuts, flaxseeds, soybean, canola oil, and walnuts), while some seed contains an abundant amount of linoleic acid but contains very little α -linolenic acid (corn oil, sunflower oil, and safflower oil). Comparatively hemp seeds contain both α -linolenic acid and linoleic acid in abundant amounts. According to literature hemp seed oil is over 80% in polyunsaturated fatty acids (PUFAs) with an abundant amount of linoleic, α -linolenic acid, and tocopherols (Vitamin E) which act as good antioxidants (Kriese *et al.*, 2004). The ratio of hemp oil of omega 6 and 3 (n6/n3) is 2.5 which is almost equal to fish oil (Stastnik *et al.*, 2022).

Mineral elements: Macronutrients provide energy and are used in the growth of living organisms while micronutrients control metabolism, health, and many physiological processes. In the present study, the resulting concentration order of macro-elements in hemp seed are $K > Mg > Ca, > Na$; in hempseed oil are $K > Na > Mg > Ca$, and in hemp leaf are $Ca > Mg > K$ and $> Na$, respectively. Studies about mineral elements in hemp seeds are scarce. However, Mihoc *et al.*, (2012), affirmed the remarkable contents of minerals such as Potassium, Phosphorus, Calcium, Magnesium, Manganese, Copper, Iron, and Zinc in agreement with this study. Alonso-Esteban *et al.*, (2022) reported similar results with this study of minerals in whole hemp seed. In macro element Potassium in hemp seed and hemp seed oil, and Calcium in hemp leaf resulted in higher concentrations in comparison to other elements. Mihoc *et al.*, (2012), also reported a high level of Potassium in hemp seed and a low level of Sodium with agreement to this study.

Microelements are essential dietary elements for mammals and are involved in many physiological processes (Anon., 2017). Their deficiencies are linked to mineral deficiencies in animals. A remarkable concentration of microelements was detected in three parts of hemp products i.e hemp seed, hemp seed oil, and leaf. Results showing significant variation in mineral in three parts of hemp. In hemp seed, mineral such as Magnesium, Potassium, Manganese, Copper, and Zinc are resulted significantly ($p < 0.05$) higher in concentration in comparison to hemp oil and hemp leaf. Chromium is resulted significantly ($p < 0.05$) higher in hempseed oil in comparison to hemp seed and hem leaf, while in hemp leaf Calcium and Iron are resulted significantly ($p < 0.05$) higher in concentration in comparison to hemp seed and hemp leaf. The resulting element's concentration varies from part to part. Mihoc *et al.*, (2012), reported element concentrations of hempseed differ from one variety to another variety of monoecious and dioecious hemp plant. Alonso-Esteban *et al.*, (2022), reported that mineral elements vary in whole hemp seeds and commercial hulled hemp seeds of eight different varieties of hemp. Few studies on the mineral elements of hemp in different parts are available, so it is difficult to compare the present study with previous studies.

The concentration order of microelements in hemp seed are $Fe > Cr > Cu > Mn > Zn$; in hemp seed oil are $Fe, > Cr > Mn > Cu > Zn$; and in hemp leaf are $Fe, > Cr, > Cu, > Mn > Zn$. In mineral elements, Calcium, Magnesium, Sodium, Potassium, Iron, Copper, and Zinc resulted in the

order of seed $>$ leaf $>$ oil; while Chromium resulted in the order of oil $>$ seed $>$ leaf; and Manganese resulted in the order of seed $>$ oil $>$ leaf. Iron has resulted in the order of leaf $>$ seed $>$ oil. The mineral element of hemp seed resulted better in concentration in comparison to hemp seed oil and hemp leaf. We recommend hemp products as a feed ingredient in the diets of fishes, and poultries due to its remarkable nutritive nature.

Conclusion

This study investigated the best nutritional importance of hemp. In hempseed, dry matter, crude protein, ether extract and energy are resulted significantly ($p < 0.05$) higher in comparison to hemp leaf. The oil analysis revealed a significantly higher proportion of unsaturated fatty acids compared to saturated fatty acids. Notably, three essential fatty acids (linoleic acid (C18:3n3, omega-3), linoleic acid (C18:2c, omega-6), and oleic acid (C18:1c, omega-9) are present at substantially higher concentrations than other essential fatty acids. In hemp seed, mineral such as Magnesium, Potassium, Manganese, Copper, and Zinc are resulted significantly ($p < 0.05$) higher in comparison to hemp oil and hemp leaf. Chromium is resulted significantly ($p < 0.05$) higher in hemp oil in comparison to hemp seed and hem leaf, while in hemp leaf Calcium and Iron are resulted significantly ($p < 0.05$) higher in comparison to hemp seed and hemp leaf. Overall, hemp seeds demonstrated superior mineral concentrations compared to hemp seed oil and leaves. In summary, due to their exceptional nutritional profile, hemp-based products show promising potential as high-quality feed ingredients for fish, poultry, cows, and cattle.

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References

- Adili, R., M. Hawley and M. Holinstat. 2018. Regulation of platelet function and thrombosis by omega-3 and omega-6 polyunsaturated fatty acids. *PG. Other Lipid Mediat*, 139: 10-18.
- Afridi, A.J., A. Zuberi, A.M. Yousafzai and M. Kamran. 2024. Therapeutic role of hemp (*Cannabis sativa*) against copper-induced toxicity in *Labeo rohita* and *Cirrhinus mrigala*. *Biol. Trace Elem. Res.*, 202(1): 307-318.
- Afridi, A.J., A. Zuberi, A.M. Yousafzai, M. Kamran and S. Ullah. 2019. Hemp (Marijuana) reverted Copper-induced toxic effects on the essential fatty acid profile of *Labeo rohita* and *Cirrhinus mrigala*. *Mol. Boil. Reports.*, 46: 391-401.
- Ali, E.M., A.Z. Almagboul, S.M. Khogali and U.M. Gergeir. 2012. Antimicrobial activity of *Cannabis sativa* L. *CHI. Med.*, 3(1): 61-64.
- Alonso-Esteban, J.I., M.E. Torija-Isasa and M. de Cortes Sánchez-Mata. 2022. Mineral elements and related antinutrients, in whole and hulled hemp (*Cannabis sativa* L.) seeds. *J. Food Compos. Anal.*, 109: 104516.

- Anonymous. 1972. Oil and fat. Chap 41, p.13 17th edition, first action 1972.
- Anonymous. 1999. Oil and fat. Chap, 41 p, 26 17th editions.
- Anonymous. 2000. Official Methods of Analysis, 16th ed. Washington, DC: Association of Official Analytical Chemists.
- Anonymous. 2017. European Food Safety Authority (EFSA). Dietary reference values for nutrients summary report, Vol. 14(12): p. e15121E).
- Audu, B.S., P.C. Ofojekwu, A. Ujah and M.N.O. Ajima. 2014. Phytochemical, proximate composition, amino acid profile and characterization of Marijuana (*Cannabis sativa* L.). *J. Phytopharmacol.*, 3(1): 35-43.
- Bailoni, L., E. Bacchin, A. Trocino and S. Arango. 2021. Hemp (*Cannabis sativa* L.) seed and co-products inclusion in diets for dairy ruminants: *A Rev. Anim.*, 11(3): 856.
- Ballotta, D., H. Bergeron and B. Hughes. 2008. Cannabis control in Europe. *EMCDDA MONOGRAPHS.*, 99.
- Borhade, S.S. 2013. Chemical composition and characterization of hemp (*Cannabis sativa*) seed oil and essential fatty acids by HPLC method. *Arch Appl Sci Res.*, 5(1): 5-8.
- Callaway, J.C. 2004. Hemp seed as a nutritional resource: an overview. *Euphytica.*, 140(1): 65-72.
- Cerino, P., C. Buonerba, G. Cannazza, J. D'Auria, E. Ottoni, A. Fulgione and A. Gallo. 2021. A review of hemp as food and nutritional supplement. *Cannabis & Cannabinoid Res.*, 6(1): 19-27.
- Chandra, S., H. Lata, M.A. ElSohly, L.A. Walker and D. Potter. 2017. Cannabis cultivation: Methodological issues for obtaining the medical-grade product. *Epilepsy Beh.*, 70: 302-312.
- Das, U.N. 2006. Essential fatty acids: biochemistry, physiology, and pathology. *Biotech. J.*, 1(4): 420-439.
- Flores-Sanchez, I.J. and R. Verpoorte. 2008. Secondary metabolism in cannabis. *Phytochem. Rev.*, 7: 615-639.
- Gakhar, N, E. Goldberg, M. Jing, R. Gibson and J. House. 2012. 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. *Poult. Sci.*, 91(3): 701-711.
- Huestis, M.A. 2005. Pharmacokinetics and metabolism of the plant cannabinoids, Δ 9-tetrahydrocannabinol, cannabidiol, and cannabinol. *Cannabinoids*, 657-690.
- Isahq, M.S., M.S. Afridi, J. Ali, M.M. Hussain, S. Ahmad and F. Kanwal. 2015. Proximate composition, phytochemical screening, GC-MS studies of biologically active cannabinoids and antimicrobial activities of Cannabis indica. *Asian. Pac. J. Trop. Dis.*, 5(11): 897-902.
- Karlsson, L. 2010. Hemp seed cake as a protein feed for ruminants. Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences (Vol. 2010, No. 86).
- Karlsson, L., M. Finell and K. Martinsson. 2010. Effects of increasing amounts of hempseed cake in the diet of dairy cows on the production and composition of milk. *Anim.*, 4(11): 1854-1860.
- Kiralan, M., V. Gül and S.M. Kara. 2010. Fatty acid composition of hempseed oils from different locations in Turkey. *Span. J. Agric. Res.*, 8(2): 385-390.
- Klir, Z., J. Novoselec and Z. Antunovic. 2019. An overview on the use of hemp (*Cannabis sativa* L.) in animal nutrition. *Poljoprivreda*, 25(2): 52-61.
- Kriese, U., E. Schumann, W.E. Weber, M. Beyer and L. Brühl. 2004. Oil content, tocopherol composition and fatty acid patterns of the seeds of 51 *Cannabis sativa* L. genotypes. *Euphytica*, 137(3): 339-351.
- Mihoc, M., G. Pop, E. Alexa and I. Radulov. 2012. Nutritive quality of Romanian hemp varieties (*Cannabis sativa* L.) with special focus on oil and metal contents of seeds. *Chem. Cent. J.*, 6(1): 1-12.
- Montserrat-de la Paz, S., F. Marín-Aguilar, M.D. García-Gimenez and M.A. Fernández-Arche. 2014. Hemp (*Cannabis sativa* L.) seed oil: Analytical and phytochemical characterization of the unsaponifiable fraction. *J. Agric. Food Chem.*, 62(5): 1105-1110.
- Neijat, M., M. Suh, J. Neufeld and J. House. 2016. Hemp seed products fed to hens effectively increased n-3 polyunsaturated fatty acids in total lipids, triacylglycerol, and phospholipid of egg yolk. *Lipids*, 51(5): 601-614.
- Nussey, G., J.H.J. van Vuren and H.H. du Preez. 2000. Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeo umbratus* (Cyprinidae), from Witbank Dam, Mpumalanga. *Water Sa.*, 26(2): 269-284.
- Orhan, I., S. Kumenoglu and B. Sener. 2000. GC-MS analysis of the seed oil of *Cannabis sativa* L. Cultivated in Turkey, 17: 79-81.
- Radwan, M.M., M.A. El-Sohly, D. Slade, S.A. Ahmed, I.A. Khan and S.A. Ross. 2009. Biologically active cannabinoids from high potency *Cannabis sativa*. *J. Nat. Prod.*, 72(5): 906-911.
- Salim de Castro, G.R. Deminice, L.M.C. Simões-Ambrosio, P.C. Calder, A.A. Jordão and H. Vannucchi. 2015. Dietary docosahexaenoic acid and eicosapentaenoic acid influence liver triacylglycerol and insulin resistance in rats fed a high-fructose diet. *Mar. Drugs.*, 13(4): 1864-1881.
- Small, E. 2015. Evolution and classification of *Cannabis sativa* (marijuana, hemp) in relation to human utilization. *Bot. Rev.*, 81: 189-294.
- Small, E. 2017. Classification of *Cannabis sativa* L. in relation to agricultural, biotechnological, medical, and recreational utilization. In *Cannabis sativa L.-Bot. Biotechnol.*, (pp. 1-62).
- Small, E. and S.G. Naraine. 2016. Expansion of female sex organs in response to prolonged virginity in *Cannabis sativa* (marijuana). *Genet. Resour. Crop Evol.*, 63(2): 339-348.
- Stastnik, O., E. Mrkvicova and L. Pavlata. 2022. Industrial hemp in animal feed applications. In *Industrial hemp*,). *Acad. Press*. pp. 341-365.
- Sutharshiny, S. and K. Sivashanthini. 2011. Proximate composition of three species of Scomberoides fish from Sri Lankan waters. *Asian J. Clin. Nutr.*, 3(3): 103-111.
- Tang, C.H., Z. Ten, X.S. Wang and X.Q. Yang. 2006. Physicochemical and functional properties of hemp (*Cannabis sativa* L.) protein isolate. *J. Agric. Food Chem.*, 54(23): 8945-8950.
- Vonapartis, E., M.P. Aubin, P. Seguin, A.F. Mustafa and J.B. Charron. 2015. Seed composition of ten industrial hemp cultivars approved for production in Canada. *J. Food Compos. Anal.*, 39: 8-12.
- Wallis, J.G. and J.L. Watts. 2002. Polyunsaturated fatty acid synthesis: what will they think of next?. *Trends Biochem. Sci.*, 27(9): 467-473.
- Webster C.D, K.R. Thompson, A.M. Morgan, E.J. Grisby and A.L. Gannam. 2000. Use of hemp seed meal, poultry by-product meal, and canola meal in practical diets without fish meal for sunshine bass (*Morone chrysops* \times *M. saxatilis*). *Aquac.*, 188(3-4): 299-309.
- Whiting P.F., R.F. Wolff, S. Deshpande, M. Di Nisio, S. Duffy, A.V. Hernandez, J.C. Keurentjes, S. Lang, K. Misso, S. Ryder and S. Schmidtkofer. 2015. Cannabinoids for medical use: A systematic review and meta-analysis. *JAMA.*, 313(24): 2456-2473.
- Yousafzai, A.M. and A.R. Shakoori. 2006. Bioaccumulation of chromium, nickel, lead, copper, and zinc in the skin of *Tor putitora* as an indicator of the presence of the heavy metal load in River Kabul Pakistan. *Pak. J. Zool.*, 38: 341-346.
- Zade, V., M. Wikhe, D. Dabhadkar, S. Dawada and U. Patil. 2013. Antifertility efficacy of *Cannabis sativa* leaves on female Albino rats. *IJSIT.*, 2(2): 107-117.