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Nutritional qualities of hemp seed (*Cannabis sativa* L.): An underutilized source of protein and fat

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Abstract
The present work was undertaken to study the physicochemical and nutritional properties of hemp seeds. Hemp, a wild plant, is scantly cultivated for fibre and its seeds are an undervalued co-product resulting from the cultivation of industrial hemp. The underutilized seeds of hemp can be used to enhance the nutritional value of staple foods in terms of plant-based protein, good quality fat and fibre. Different physicochemical and nutritional characteristics of whole hemp seed were analyzed. The seed weight, seed volume, hydration and swelling capacity, bulk density and pericarp color were found to be 19.29 g, 18.66 ml, 0.03 g/seed, 0.07 ml, 0.56 g/ml, and light olive brown color respectively. The nutritional composition results revealed that whole hemp seed has good quality of crude protein (20.40%) and crude fat (28.70%). Whole hemp seed has good amount of dietary fibre (26.83 g/100 g) and fair amount of *in vitro* protein digestibility (66.69%) whereas, total phenolic content was very high (221.31 mgGAE/100 g) and a very little amount of α-tocopherol (0.05 mg/100 g) was present.

Keywords: Hemp seed, physicochemical properties, Himalayan foods, underutilized food, nutritional qualities

1. Introduction
Due to the growing population and rapid depletion of natural resources, it became essential to look into using newer indigenous plant resources. One of the world's most significant sources of renewable wealth today is agriculture. Numerous plant species are currently undiscovered and underutilized. As a result, researchers have concentrated their efforts on finding alternative or neglected plant species that may be used for various purposes. Plant species that have historically been used for food, fibre, fodder, oil, or medicinal purposes but have not yet been accepted by large-scale agriculturalists are referred to as "underutilized crops." Underutilized plants are those types of plants that exist as life support systems in hazardous environments and vulnerable ecosystems and have the genetic resilience to endure adverse situations and possess qualities of nutritional and/or industrial importance for a variety of purposes.

A member of the Cannabaceae family, *Cannabis sativa* L. (hemp seed) is an annual herb that is diploid (2n=20) and dioecious (male and female flowers grow on separate plants). It is readily available worldwide and is also referred to as marijuana in English and *bhang* in Hindi. The genetic heterogeneity and chemical constituents of the cannabis plant make taxonomy categorization exceedingly challenging. Cannabis was thought to have its origins in Central Asia around 500 BC (McPartland *et al*., 2000) [1]. Due to drug usage, the species is regarded as the most controversial plant in human history. Industrial hemp, however, is a versatile crop for farmers since all portions may be used in a variety of ways. Hemp is traditionally used to make a wide range of products, including rope, paper, clothes, sails, food, etc., due to its ability to grow well in both temperate and tropical climates. Commercial hemp is used as a fibre in the textile and paper industries as well as a substitute for plastic. *Indica* and *Sativa* are the two main subvarieties of hemp. The *Indica* species is known to have tetrahydrocannabinol, or THC, it is a chemical that accounts for most marijuana's psychological effects. The *Sativa* species has more cannabidiol, or CBD, which has both nutritional and therapeutic benefits. Hemp may be grown readily on barren terrain that enriches weak soil and thrives in environments with little access to resources like water. This plant should be encouraged for growth in the surrounding areas due to its significance in both traditional and industrial applications. Large-scale hemp production in areas where it is permitted may be a valuable source of income with positive effects on the nation's economy.

Global hempseed production peaked in the 20th century at 80,448 t. But starting in the 21st century, European nations particularly France, have taken the lead in producing hempseed, with a production peak of 82,707 t in 2017. In the past three decades, significant breeding efforts have been made to enhance hemp agronomy with the specific aims of lowering 9-tetrahydrocannabinol (THC) levels and reducing seed head cracking (Cherney and Small, 2016) [2].

Incidentally, Uttarakhand became the first Indian state to permit the commercial growing of hemp. The Indian Industrial Hemp Association (IIHA) received a license from the State Government in 2018 to cultivate hemp on 1000 hectares for fibre. Since a few years ago, hemp-based goods have been sold in Indian marketplaces, but they have mostly gone unchecked. This leads the Food Safety and Standards Authority of India (FSSAI) to issue a notification in November 2021 allowing the sale of hemp seed, oil, and flour as foods. When it comes to the utilization of hemp seeds, the state of Uttarakhand has also highlighted several fascinating aspects. For instance, people in the Kumaon area of Uttarakhand use hemp seed "chutney" in their daily meals since it has a rich flavor and keeps them warm during winters (Singh and Raghuvanshi, 2022) [3]. In some areas of the Kumaon's hilly terrain, rope made from hemp fibre is also used to bind livestock. *Cannabis sativa* seeds exhibit a high concentration of advantageous bioactive chemicals, antioxidant activity both *in vitro* and *in vivo*, and antimutagenic action. Additionally, hemp seeds are beneficial for health due to their high concentration of linoleic and linolenic fatty acids, which are necessary fatty acids. Hemp seed amino acids are also proven to enhance liver, pancreatic, and neurological system functioning (Wolfe, 2009) [4]. Despite of having numerous nutritional health benefits, hemp seed is consumed on a very limited scale due to the lack of information on its nutritional quality as well as the myth associated with narcotic effect of bhang. Therefore, the present work was undertaken to study the physicochemical and nutritional properties of hemp seed available in Uttarakhand.

2. Materials and Methods

Local varieties are available which have <0.3% THC (FSSAI, 2021) [5]. However, no varieties have been released to the best of our knowledge. Therefore, hemp seed have been taken from the local market of Pantnagar, Uttarakhand. Procured hemp seeds were cleaned manually and sorted for blemishes.

2.1 Evaluation of Physical Properties of Hemp Seeds

Thousand kernel weight, thousand kernel volume, hydration capacity and swelling capacity were determined using the method given by (Williams *et al*. 1983) [6]. Bulk density was determined by method given by (Narain *et al*. 1978) [7]. Color of the hemp seeds was determined using (Munshell Soil Colour Chart, 1954) [8].

2.2 Analysis of nutritional composition of hemp seeds

The sample's moisture content, total ash, crude protein, crude fat and crude fibre were evaluated using the technique (AOAC, 2010) [9]. The total carbohydrate content was calculated by subtracting the sum of the values on dry matter basis for total ash, crude protein, crude fibre, and crude fats from a hundred and reported g/100g of sample. The physiological energy of the sample was calculated by adding the product of crude protein %, crude fat %, and carbohydrate present in the sample by 4, 9, and 4, respectively. The physiological energy was expressed in Kcal/100g. The dietary fibre was estimated using the method given by (Asp and Johansson, 1981) [10]. *In vitro* protein digestibility was estimated by the procedure given by (Akeson and Stahman 1964) [11] and protein extraction method given by (De Groot and Slump 1969) [12]. Total phenolic content (TPC) was determined using Folin-Ciocalteu's reagent as reported by (Singleton, *et al*., 1999) [13]. The total antioxidant activity was determined by DPPH (2.2-Diphenyl-1- picrylhydrazyl) radical scavenging activity (Williams *et al*., 1995) [14]. α-tocopherol content in hemp seed flour was analysed using method of (Emmerie and Engel 1938) [15]. All analysis were carried out in triplicate and average values are reported.

3. Results and Discussion

3.1 Physical properties of hemp seed

Various physical properties of whole hemp seeds were determined, the pericarp of seed is of light olive brown color. Seed weight is an important parameter for the assessment of seed quality. In the present study, weight of 1000 seeds were found as 19.29 g which is similar to the value of 19.26 g reported by (Garavand *et al*. 2010) [16]. Seed volume of hemp seed was recorded 18.66 ml, hydration capacity was 0.03 g/seed, 0.07 ml of swelling capacity, 0.56 g/ml bulk density.

3.2 Nutritional composition of hemp seed: The clean and powdered hemp seeds had 5.6% moisture content. Other nutritional contents are analyzed and results are reported on dry matter basis in Table 1.

Table 1: Nutritional composition, in vitro protein digestibility and bioactive composition of hemp seed

Nutritional composition	Whole hemp seed
Moisture (%)	5.66±0.12
Total ash (%)	7.07±0.01
Crude protein (%)	20.40±0.02
Crude fat (%)	28.70±0.03
Crude fibre (%)	26.96±0.03
Carbohydrates (by difference)	16.87±0.02
Physiological energy (Kcal/100 g)	385.72±0.48
Dietary fibre (in g/100 g)	
Soluble dietary fibre	5.35±0.43
Insoluble dietary fibre	21.48±0.48
Total dietary fibre	26.83±0.87
In vitro protein digestibility (%)	66.69±2.62
Total phenol content (mgGAE/100g)	221.31±0.0.91
DPPH% inhibition	68.27±0.84
α-tocopherol (mg/100g)	0.05

*All values are mean±standard deviation of three replicates on dry weight basis

The moisture content of whole hemp seed was found to be 5.66% whereas, (Dobhal and Raghuvanshi, 2022) [17] observed 8.5% of moisture in black soybean. Hemp seed and black soyabean, both are native to Uttarakhand and both are having high fat and protein, therefore an attempt has been made to compare its nutritional qualities with black soyabean and both these results were compared on dry weight basis. The total ash content of whole hemp seed was higher (7.07%) than black soyabean (5.96%). Crude protein of whole hemp seed was found 20.40% which is slightly lower than black

soyabean. Hemp seeds contain 20–25% proteins of biological value equal to hen's egg white (Mikulec *et al*.2019) [18]. Hemp seed proteins contain all the essential amino acids for human health and the protein fraction of hemp is highly digestible (Farinon *et al*. 2020) [19]. Overall, whole hemp seed can be considered rich-protein source containing protein amount higher or similar than other protein rich products, such as quinoa (13%). (Leonard *et al*. 2019) [20] reported all nine of the essential amino acids that humans need is present in hemp protein. Its amino acid composition is distinguished by extremely high concentrations of arginine and glutamic acid and a considerable amount of amino acids containing sulphur. (Wang *et al*. 2008) [21] compared the amounts of sulphur-containing amino acids in hemp protein isolate to those in soy protein isolate and discovered that hemp protein isolate had greater ratios of essential to total amino acid content and improved enzyme digestibility. Compared to whole wheat (40), pinto beans (57), and lentils (52), the protein isolated from dehulled hempseed exhibited higher protein digestibility-corrected amino acid scores (61) but was still substantially lower than that from egg white (100) or beef (92) (House *et al*. 2010) [22].

3.3 *In vitro* protein digestibility: The IVPD value of whole hemp seed was estimated as 66.69% which is lower than black soybean, this may be due to the presence of hull and high crude fibre content in the seed negatively affects the digestibility of the hemp seed proteins. Removal of the hull fraction from the hemp seed led to an average increase in protein digestibility (House *et al*. 2010) [22].

Whole hemp seed has 28.70% crude fat content which is higher in comparison to black soybean and is reported to have only 17.86% (Sumangala and Kulkarni, 2019) [23] and 19.25% (Dobhal and Raghuvanshi, 2022) [17]. In comparison to other plant oils, hempseed oil has the highest proportion of polyunsaturated fatty acids (Callaway, 2004) [24]. Higher PUFA consumption has been linked to reduced risks of cardiovascular disease, cancer, rheumatoid arthritis, hypertension, inflammatory, and autoimmune diseases (Abedi and Sahari, 2014) [25]. Whole hemp seed was observed to have 26.96% of crude fibre which is very high in comparison to black soybean (8.23%). However, carbohydrate made up a good amount in black soybean (37.58%) and slightly lower in whole hemp seed (16.87%) significant portion of which are constituted in dietary fibre, mainly insoluble. Thus, based on all these parameters, more calorific value was observed in black soybean (415.05 kcal/100 g) than whole hemp seed (385.72 kcal/100g).

The results for the dietary fibre content of whole hemp seed have been presented in Table 1. The soluble and insoluble dietary fibre of whole hemp seed was 5.35 g/100g and 21.48 g/100g whereas, (Kumar *et al*. 2022) [26] reported 4.86 g/100g and 19.36 g/100g of soluble and insoluble dietary fibre in black soybean, respectively.

In plants phenolic compounds are responsible for providing structural support and protection against solar radiation, pathogens etc. However, from the consumers' point of view, they provide protection against non-communicable diseases *viz*; obesity, diabetes, cardiovascular diseases, cancer etc. not only by means of their antioxidant property but also by regulating numerous cellular processes including enzyme inhibition, protein phosphorylation and modification of gene expression (Lura *et al*. 2019) [27]. In the present study, TPC of whole hemp seed was observed as 221.31 mgGAE/100g. Similarly, other researchers found that a hemp seed oil total phenolic acid content of 188.23 mg/100 g GAE. In addition, phenolic content values ranging from 490 to 1194 mg/100 g GAE (defatted kernels) and 4080 to 10,920 mg/100 g GAE (defatted hull) were reported for seeds for seeds of two hemp varieties cultivated in China. Literature review also found TPC content of black soybean ranged between 19.87-46.25mgGAE/100g. Very limited studies have been conducted regarding the antioxidant activities of hemp seed. DPPH radical-scavenging activity of hemp seed was observed as 68.27% which is higher than value reported by (Chauhan *et al*. 2022) [28] for black soybean i.e, 27.69%. The α-tocopherol content in hemp seed was present in lower concentration i.e, 0.05mg/100 and this result were in agreement with (Matthäus and Brühl, 2008) [29].

4. Conclusion

Himalayan communities exercise a rich agriculture–medicine use system that not only provides adequate dietary diversity and nutrition but also delivers therapeutic security. Hemp seed have a good nutritional qualities *viz*. high protein with good *in vitro* bioavailability, high fat content and high dietary fibre. In some aspects, it is better than black soyabean from Uttarakhand. In Himalayan region, people consume hemp seeds in a limited way and know that it does not have narcotic effect, however in other parts of country this is widely misunderstood to have psychoactive components. Since the interest of consumers worldwide for ingredients derived from natural sources is ever-growing, the demand for hemp seeds protein is expected to proliferate. Consumption of foods fortified with whole hemp seed flour would be an important step toward relieving protein energy malnutrition in the poor countries of the world. Giving importance to this underutilized crop will not only enhance availability of oils and protein but also will be instrumental in increasing Farmers income.

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