

Review

# Nutritional and Industrial Insights into Hemp Seed Oil: A Value-Added Product of *Cannabis sativa* L.

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**Abstract:** Industrial hemp is mainly cultivated for its fibers aimed at the production of textiles, paper, and cordage; the inflorescences for medicinal purposes; and the seeds are used by the food industry due to their high nutritional and functional matrix of protein, fiber, lipids, and microelements. Hemp seed oil (HsO) is a unique source of polyunsaturated fatty acids, with a phenomenal  $\omega 6:\omega 3$  ratio of 2.5–3.0, significantly enhancing human health when consumed daily. HsO is mostly obtained through cold pressing due to minimal thermal treatment, and although of lower yield compared to solvent extraction, it presents higher quality lipid fractions and organoleptic characteristics such as color, taste, flavor, and density. Although HsO is a powerful source of polyunsaturated fatty acids, antioxidants, and phytosterols, its production lacks standardized quality control parameters, except for THC, which is subject to EU legislation. Therefore, it is essential to build up a quality protocol system for standardizing seed conservation, oil extraction methods, and quality parameters. This review aims to display an overall nutritional framework of the HsO and encourage further research into its use in the food value chain.

**Keywords:** *Cannabis sativa* L.; hemp seed; hemp seed oil; quality characteristics; fatty acids content



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## 1. Introduction

Hemp is the common name of *Cannabis sativa* L., which belongs to the Cannabaceae family [1,2] and due to its very long history and cultivation, its exact origin is not well established. Numerous researchers agree that it probably originates from Eastern or Central Asia and presumably from China, where it was cultivated mostly for its medicinal properties. Hemp was introduced and cultivated in many European regions approximately from the 22nd until the 16th century BC, although there is a possibility it was an indigenous species for Europe as well [3]. It was primarily cultivated for fiber, textiles, and animal feed; however, it gradually abounded during the first half of the 20th century due to legal issues regarding the content of psychotropic substances. It was re-introduced in the new millennium, initially in Canada, with Europe and the United States soon to follow up, resulting in the rearrangement of their legislations [4].

Hemp was cultivated as a multi-use crop aiming at the production of different types of products such as textiles, paper, biofuel, functional foods, bio-composites, cosmetics, personal care products, and even construction. Many researchers mention the versatility of the hemp plant, which provides valuable products like fiber, essential oils, and seeds deriving from different parts of the plant [5–7]. Currently, it has been estimated that over fourteen countries cultivate hemp for both research and marketable reasons [8], and Kaur and Kander [9] added that thousands of hemp-based products exceed while the market

interest increases rapidly, implementing the hemp seed by-products in numerous fields. The present review aims to present the nutritional value of hemp seeds and hemp seed oil and encourage further research for their use in the food value chain along with the establishment of a protocol system aiming to standardize their quality parameters.

## 2. Hemp Seeds and Hemp Seed Oil

Initially, hemp seeds were considered a by-product of *Cannabis sativa* cultivation for the fiber production industry, but this thought was re-orientated and arose in the interest of the food industry because of their high nutritional and functional properties for human consumption, let alone the very low tetrahydrocannabinol (THC) content, which was reported to be lower than 0.3% [10,11]. According to the livestock industry, hemp seeds and their by-products are mainly used as animal feed, but their products (oil, meal, flour, and protein powder) are gaining in the market with a growing interest in their usage for human nutrition [12,13].

Focusing on the nutritional profile of the hemp seeds (Hs), it is proven that they are a great matrix of protein, dietary fiber, carbohydrates, and fat, alongside minerals and antioxidant compounds for animal and human consumption [11,14,15].

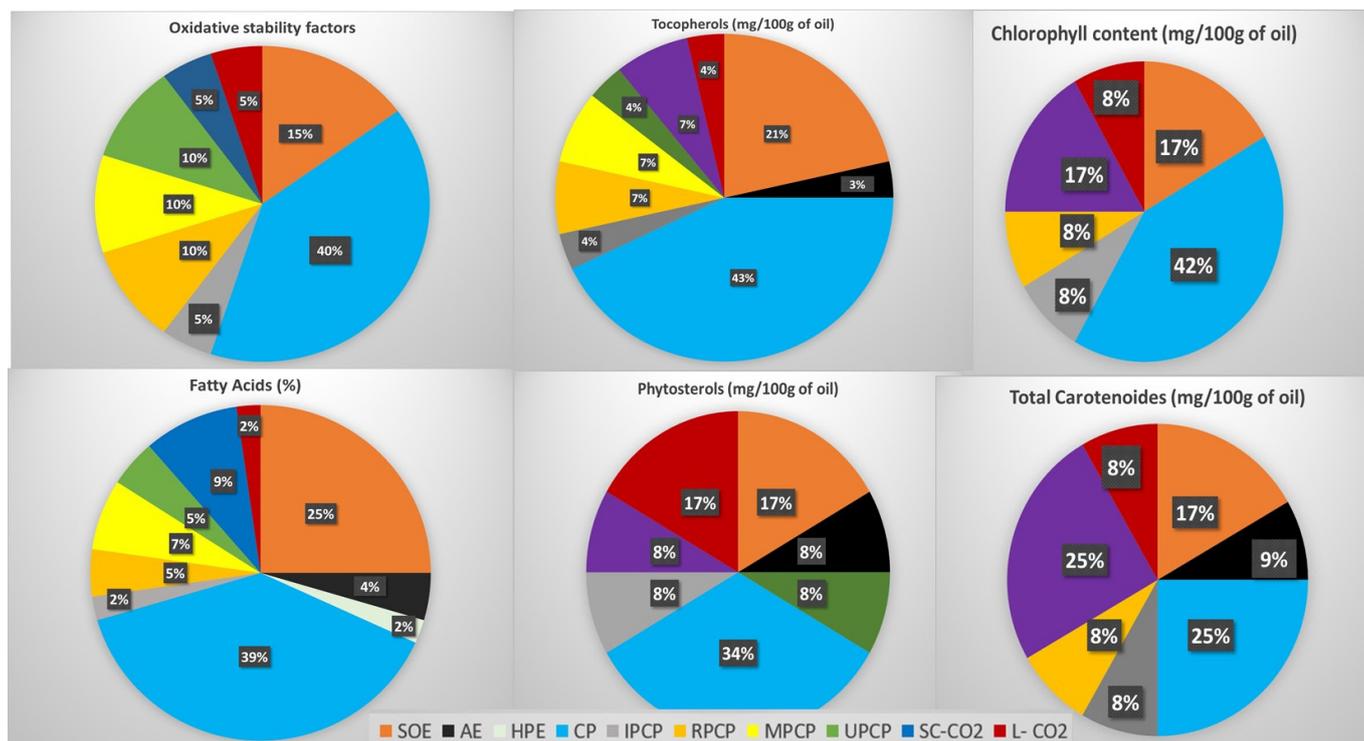
The Hs contains 20–30% carbohydrates, 10–15% insoluble fiber, 20–25% protein, and 25–35% fat [6,8,14]. Furthermore, it is considered a unique source of essential amino acids and phenolic complexes corresponding to a promising health promoting product [11,15]. In general, the Hs were investigated and several research studies were performed to evaluate the nutritional and functional properties of its by-products, such as protein, flour, meal, cake, and finally oil [5,16–18].

Hemp seed oil (HsO) is one of the derivatives of seeds obtained after HsO extraction. HsO is a powerful source of polyunsaturated fatty acids (PUFAs), tocopherols, and phytosterols and low-saturated fatty acids (SFAs) that play a key role in human health preservation [8,14,19]. It is obtained either from whole or dehulled hemp seed, mainly using organic solvent extraction techniques, hydraulic pressing techniques, and recently through supercritical CO<sub>2</sub> methods, with or without pretreatment of the Hs, aiming for a final product of fine quality [7,20–22]. Figure 1 presents, in the form of pie charts, the extraction methods used for each key parameter of hempseed oil quality. These parameters include oxidative stability factors, fatty acid composition (%), tocopherols, phytosterols, chlorophyll content, and carotenoid content (mg/100 g of oil).

In general, the organic solvent oil extraction (SOE) is performed by pre-processing the seeds (cracked or ground) and washing them with the solvent, following a separation oil–solvent procedure under high temperatures of at least 63–70 °C, using distillation and evaporation techniques [23].

Various researchers [8,24–26] agree that the SOE is considered a commonly used method, simple and easy. Thus, due to the large amounts of organic solvents used, the long extracting periods, the eco-unfriendly profile, and the toxic residues released in the environment, the industry is seeking new methods for oil extraction for HsO.

To enhance this statement, Aiello et al. [22] reported that HsO samples extracted using organic solvents were susceptible to oxidation, presented higher peroxide values, and released unpleasant odors during sensory evaluation. Likewise, Burton et al. [8] pointed out that the high temperatures during SOE lead several thermosensitive compounds susceptible to high temperatures to deterioration. However, it is important to note that solvent extraction methods result in a non-edible oil that requires refining in order to meet the standards for edible oils.



**Figure 1.** Different extraction methods used for comparative analysis of oxidation stability factors, fatty acids (%), tocopherols, phytosterols, chlorophyll, and carotenoid contents (mg/100 g of oil) for hempseed oil. SOE = solvent extraction; AE = aqueous extraction; HPE = hot-pressed extraction; CP = cold-pressed extraction; IPCP = infrared pretreatment cold-pressed extraction; RPCP = roasting pre-treatment cold-pressed extraction; MPCP = microwave pretreatment cold-pressed extraction; UPCP = ultrasound pretreatment cold-pressed extraction; SPF-CO<sub>2</sub> = supercritical CO<sub>2</sub> extraction; L-CO<sub>2</sub> = liquid CO<sub>2</sub> extraction.

On the other hand, cold pressing (CP) techniques prevail towards SOE simply by being more sustainable, cost-effective, and time-saving, with no health hazard either for workers or consumers due to lack of organic solvents [7,20,21,27]. The CP extraction is a complex technique combining high pressures and significantly low temperatures in comparison to SOE [27] that allows the preservation of thermolabile compounds and minimizes any possible oil degradation, resulting in a final product of higher quality with no unwanted solvent residues [7,22,25]. Cold pressing oil extraction results in a significantly lower yield compared to solvent extraction, leaving up to 35% potentially available oil bound to the hemp seed cake by-product [20,22,26,27].

The need to increase the Hs yield efficiency led to a new extracting method using supercritical conditions (32 °C and 74 bar) for fluid carbon dioxide (SPF-CO<sub>2</sub>). This method is used for oil extraction in both food and pharmaceutical industries, yielding higher amounts of oil and of higher quality [20]. This method is considered innovative, as this is where CO<sub>2</sub> is used to replace organic solvents due to its dominant properties, like being non-toxic and inflammable, inexpensive, recyclable, and leaving no residues [20,22]. Moreover, according to research presented by Aiello et al. [22], liquid and supercritical CO<sub>2</sub> extraction methods minimize both hydrolysis and oxidative reaction, resulting in increased bioactive and aromatic compound concentrations by allowing the extraction of heat-sensitive compounds like PUFAs.

Furthermore, Devi and Khanam [24] reported that currently several modified methods were developed, aiming to enhance oil extraction; thus, Latif and Anwar [28] indicate that a proper treatment of the hemp seeds prior to oil extraction is considered a crucial step

towards obtaining oil of high quality and of increased efficiency. In general, the majority agree that an efficient extraction process should be cost-effective, with a small carbon footprint, using smaller solvent amounts, lower temperatures, and shorter extraction times.

Recently, ultrasound and microwave pretreatments for solvent and cold press extraction techniques were demonstrated. These methods can increase extraction efficiency and, in parallel, decrease the time of extraction by breaking down the cell matrix of the oil bodies and extracting the essential compounds [7,22]. Oil bodies are susceptible to high temperatures, high frequencies, and time exposure conditions; therefore, both ultrasound and microwave pretreatment should be performed under strict conditions; otherwise, the HsO quality would deteriorate [7,24,25]. Ultrasound is considered an eco-friendly technique using low levels of energy and natural solvents and produces high-quality products [28]. Soroush [25] reports that the microwave-assisted extraction increases the efficiency of the oil extraction significantly and reduces the extraction time but still exposes the oil to high temperatures, which results in oils of different qualities.

Mansouri et al. [27] proposed roasting as a pretreatment for hemp seeds to enhance yield oil extraction, mainly in cold-pressed techniques. If given proper attention to roasting temperatures and exposure time (optimal conditions of 163 °C and 15 min), this procedure can also improve certain organoleptic attributes of the oil (color, flavor, and taste) and contribute positively to the phenolic content and antioxidant capacity. The authors point out that thermal treatment can actually prohibit lipase activation and retard both oxidation and rancidity processes through storage time.

Devi and Khanam [24] report two more assisting extraction techniques involving high temperatures but for laboratory-scale uses only, percolation (PER) and pyrolysis (PYR). Finally, a promising pretreatment application of the hemp seeds prior to extraction is mentioned [25], called enzyme-assisted cold-pressing (EACP), with improved oil yield, oxidative stability and organoleptic characteristics (color, viscosity, flavor, and taste).

Cold pressing technique is well known to deliver lower yield if compared to solvent extraction and thermal treatment, thus the oil quality is superior with specific organoleptic properties. Therefore, it is consequential to perform quality and composition analysis of the HsO alongside economic analysis prior to commercialization to indicate whether the HsO is suitable for human use and consumption, depending on the extraction method [7,29].

### 3. Hemp Seed Oil Quality Characteristics

#### 3.1. Peroxide Value

Free fatty acid oxidation negatively impacts oil quality [27,30–32]. Following the EU legislation [33], peroxide value (PV) is used to evaluate the oxidation stability of oils, and according to Codex Alimentarius [34], the limits for vegetable oils, and therefore for HsO also, are set at 15 meq O<sub>2</sub>/kg of oil, and acid value (AV) is mainly used for oil deterioration with limits given at 4 mg KOH/g of oil [27,35]. Both PV and AV are dependent on seed storage, cultivation, harvesting manipulation, and drying conditions [6,22,35] and certainly on extraction conditions like pressure, temperature, and time of extraction [32,35]. The PV demonstrated a wide range from 1.31 meq O<sub>2</sub>/kg of oil [31] to 50.20 meq O<sub>2</sub>/kg of oil [36], and in general, all extraction methods exhibited values below 15 meq O<sub>2</sub>/kg of oil, indicating low oil oxidation (Table 1). Solvent extraction HsO presented the highest PV, although there were cold-pressed samples that had even higher values, but the authors explained that these differences may be due to storage conditions, different cultivars, and climate factors [35,36]. According to Rezvenkhah et al. [32], HS pretreatment prior to extraction under mild temperature and power conditions (ultrasound, microwave) can decrease PV, though according to Aiello et al. [22], high temperatures and light exposure can accelerate photo-oxidation due to high chlorophyll content.

Furthermore, although FA is an essential factor for oil quality evaluation, the literature review shows very poor findings. In general, the result ranges from 0.90 mg KOH/g of oil [35] to 4.58 mg KOH/g of oil [35], with values below the above-mentioned limits provided by Codex Alimentarius, although a value of 19.67 mg KOH/g of oil was recorded, which may have been influenced by factors such as drying and storage [37].

### 3.2. Fatty Acid Composition

The HsO lipid profile is characterized by a high PUFA content followed by monounsaturated fatty acids (MUFAs) and SFAs [11]. PUFAs are considered essential because they are responsible for a vast variety of biological and physiological processes (cardiovascular, metabolic, inflammatory, dermatological, etc.) [11,38], and their powerful representative is linoleic acid, followed by  $\alpha$ -linolenic [6,35]. According to Table 1, total SFAs content ranges between 4.1% of oil [7] to 12.95% of oil [39], although lower values were reported [40,41]. Additionally, the MUFA content ranges between 2.94% of oil [41] and 20.84% of oil [35] and PUFAs between 21.15% of oil [41] and 84.0% of oil [19]. The most dominant fatty acid is linoleic, 15.0% of oil [41] to 65.4% of oil [7], followed by  $\alpha$ -linolenic with values ranging from 3.0 [10] to 28.5% of oil [3], which are the optimum contents for a healthy diet but also are responsible for oil oxidation susceptibility and rancidity due to their contribution to high FA values [6,35]. Moreover, oleic, palmitic, stearic, and  $\gamma$ -linolenic acids are also considered valuable acids [40,42]. The  $\omega$ 6: $\omega$ 3 ratio of HsO is also an important factor that has an optimum ratio between 2.5 and 3.0, which is ideal for human consumption [17,35], contributing to the prevention of chronic diseases such as cholesterol reduction, high blood pressure control, and anti-inflammatory effect control [11,37]. Most of the samples presented at Table 1 respond to the general  $\omega$ 6: $\omega$ 3 optimum ratio. In reference to the results evaluated, the FFA content variability and  $\omega$ 6: $\omega$ 3 ratio are influenced by several factors like climate, cultivation, harvesting procedures, light and temperature exposure during storage [11,35,37], alongside the extraction methods, conditions, and refining procedures prior to extraction [37]. Nevertheless, several authors point out that the genotype, the variety used, and the origin of the HS also interfere with the FFA content [3,37,38]. According to Galasso et al. [3] and Izzo et al. [37], in terms of common varieties and genotypes (e.g., Finola, Futura, Fedora, etc.) that were equally treated, like post-harvest treatment, fertilization, and exposure to the same environmental conditions and obtained from certified gene banks, both variety and genotypes may significantly influence the quality and quantity of the oil extracted, indicating the potential of their improvement.

**Table 1.** Fatty acid (%) profile, peroxide value (meq O<sub>2</sub>/kg of oil), and acid value (mg KOH/g of oil) of hemp seed oil samples with different extraction methods.

Method <sup>1</sup>	Oxidation Parameters		Fatty Acids (%)										References	
	Peroxide Value (meqO <sub>2</sub> /kg Oil)	Acid Value (mg KOH/ g Oil)	Saturated		Monounsaturated			Polyunsaturated						
			Total SFA	(16:0)	(18:0)	Total MUFA	(18:1ω9)	Total PUFA	(18:2ω6)	(18:3ω3)	(18:3ω6)	(18:4ω3)		Ratio ω6:ω3
SOE	-	-	9.60–10.30	6.66–6.98	2.08–2.82	-	9.38–13.00	-	55.56–56.58	14.69–17.27	2.56–4.49	-	3.50	[40]
	-	-	9.40–12.80	5.980–8.60	2.26–4.61	9.70–16.90	9.20–16.80	71.00–80.70	46.10–58.20	12.40–28.40	0.49–3.85	0.16–1.54	1.63–4.60	[3]
	-	-	10.90 ± 0.70	5.60 ± 0.50	2.31–3.96	-	12.21–18.78	71.99–78.58	53.86–58.99	12.28–18.88	3.51–6.22	-	3.16–5.03	[16]
	-	-	7.10–9.10 <sup>2</sup>	2.10–2.80 <sup>2</sup>	3.90 ± 0.40	17.50 ± 6.30	16.20 ± 3.20	72.00 ± 4.30	54.70 ± 4.10	16.20 ± 4.00	-	0.50 ± 0.50	-	[43]
	-	-	9.24–9.46	5.76–5.77	2.40–2.41	10.60–10.95	10.50–10.12	79.76–80.16	57.13–57.51	17.78–18.09	3.25–3.30	-	3.18–3.21	[44]
	-	-	7.74	5.37 ± 0.13	1.56 ± 0.04	11.66	11.51 ± 1.05	80.60	56.16 ± 0.85	17.96 ± 0.23	3.48 ± 0.15	-	3.29	[20]
	6.40 ± 0.55	-	-	6.17 ± 0.10	2.63 ± 0.10	16.11	15.78 ± 0.05	74.20	55.07 ± 0.10	18.50 ± 0.15	0.60 ± 0.02	-	2.97	[32]
	6.4 ± 0.55	-	9.69	6.17 ± 0.10	2.63 ± 0.10	16.11	15.78 ± 0.05	74.20	55.07 ± 0.10	18.50 ± 0.15	0.60 ± 0.02	-	2.97	[31]
	19.95 ± 0.50	-	10.95 ± 0.10	6.50 ± 0.07	2.64 ± 0.01	13.39 ± 0.03	12.56 ± 0.04	75.66 ± 0.13	56.85 ± 0.10	15.70 ± 0.02	2.99	-	3.81	[22]
	-	-	9.40–10.00	5.53–6.11	2.58–2.72	14.0–16.1	12.70–14.90	70.60–72.40	51.20–52.60	14.88–18.97	1.59–2.95	0.70–0.94	2.68–3.51	[18]
AE	-	-	-	6.20	2.90	-	13.60	-	52.40	18.10	2.44	-	2.90	[45]
	-	-	-	6.10 ± 0.02	2.91 ± 0.03	-	15.10 ± 0.06	-	60.74 ± 0.32	11.66 ± 0.33	0.039 ± 0.005	-	2.60	[46]
HPE	-	-	11.48–12.81	6.61–6.28	3.19–3.60	12.17–12.82	10.56–11.62	75.01–76.26	54.66–54.86	16.03–17.81	3.61–4.25	-	3.28–3.68	[39]
CP	-	-	-	5.00–7.00	1.00–2.00	-	8.00–13.00	-	52.00–62.00	12.00–23.00	3.00–4.00	-	-	[14]
	-	-	-	5.00	2.00	-	9.00	84.00	56.00	22.00	4.00	2.00	2.50	[19]
	-	-	-	6.00	2.00	-	9.00	82.00	54.00	22.00	4.00	2.00	-	[47]
	-	-	11.24–12.94	6.91–8.04	2.47–3.28	-	-	66.02–72.43	51.94–55.74	12.35–15.39	0.8–2.46	-	3.50–4.23	[48]
	-	-	10.9 ± 0.47	7.15 ± 0.42	2.73 ± 0.25	13.01 ± 1.10	12.75 ± 1.10	75.03 ± 3.31	56.08 ± 3.05	14.89 ± 1.18	3.03 ± 0.43	-	4.00	[10]
	1.55–1.88	1.15–1.29 <sup>3</sup>	9.68–12.53	6.95–8.67	2.68–3.76	12.65–17.17	12.31–16.73	70.31–77.67	51.39–56.16	15.36–17.74	2.03–2.33	0.56–0.81	3.19–3.42	[6]
	1.83–12.64	-	9.43–11.41	5.43–6.46	1.92–2.58	9.83–16.64	7.06–13.10	70.85–78.90	46.24–51.25	10.91–17.03	0.37–3.68	0.13–1.21	3.10–4.51	[49]
	3.97–23.89	0.90–4.58	6.89–9.47	4.95–7.15	1.69–2.55	6.97–20.84	6.87–20.47	52.59–70.38	38.48–52.16	11.02–17.40	0.98–4.43	0.20–1.50	2.61–3.67	[35]
	-	-	11.64–12.95	6.25–6.52	3.10–3.53	11.91–12.89	10.34–10.67	74.84–76.45	54.56–54.86	16.02–18.29	3.25–4.054	-	3.18–3.67	[39]
	2.99 ± 0.06	2.97 ± 0.32	10.13 ± 0.40	7.65 ± 0.37	2.48 ± 0.10	19.34 ± 0.26	19.34 ± 0.26	70.53 ± 0.45	52.50 ± 0.31	16.09 ± 0.21	1.94 ± 0.34	-	-	[27]
	8.90–50.20	3.40–3.60	-	5.70–6.30	3.00–3.20	-	13.30–13.60	-	54.80–56.90	16.00–18.50	1.30–2.80	-	3.00–3.70	[36]
	-	-	11.92 ± 0.07	7.06 ± 0.11	2.77 ± 0.09	10.55 ± 0.19	10.55 ± 0.19	77.53 ± 0.09	55.48 ± 0.12	21.51 ± 0.14	-	-	-	[50]
	-	0.81	4.10 ± 0.40	-	-	12.40 ± 1.00	12.40 ± 1.00	75.03 ± 3.31	56.08 ± 0.70	18.10 ± 1.00	-	-	3.60	[7]
	1.94 ± 0.15	1.76 ± 0.05	9.80–10.20	5.60–6.24	2.65–2.78	14.60–16.00	13.30–14.70	71.80–73.20	52.20–53.40	15.22–18.58	1.72–3.09	0.72–1.00	2.79–3.48	[18]
1.8–8.8	3.77–19.67 <sup>3</sup>	-	6.29 ± 0.17	2.51 ± 0.19	-	-	-	-	18.76 ± 0.78	4.76 ± 0.14	-	3.29 ± 0.14	[30]	
-	-	10.17 ± 0.32	6.31 ± 0.30	2.83 ± 0.02	16.36 ± 0.10	15.12 ± 0.04	72.98 ± 0.20	54.85 ± 0.38	18.13 ± 0.16	0.52 ± 0.04	-	3.00	[37]	
-	-	-	-	-	-	-	-	-	-	-	-	-	[38]	
IPCP	2.89 ± 0.05	3.74 ± 0.33	9.76 ± 0.01	5.82 ± 0.02	2.91 ± 0.01	15.72 ± 0.01	15.72 ± 0.01	74.25 ± 0.10	54.61 ± 0.07	16.23 ± 0.03	2.62 ± 0.75	-	-	[51]
RPCP	2.98 ± 0.10	2.95 ± 0.31	9.74 ± 0.21	7.29 ± 0.16	2.45 ± 0.06	19.73 ± 0.11	19.73 ± 0.11	70.53 ± 0.25	52.59 ± 0.48	16.22 ± 0.44	1.71 ± 0.35	-	-	[27]
	1.33–3.09	-	9.99–10.15	6.18–6.32	2.85–2.87	16.25–16.40	15.00–15.12	73.09–73.19	54.80–55.03	18.11–18.29	0.52–0.54	-	3.00	[38]
MPCP	2.50 ± 0.05	-	-	6.60	2.70	-	-	-	-	19.10	3.60	-	-	[52]
	-	2.81	9.24	5.78 ± 0.13	2.56 ± 0.06	16.45	15.98 ± 0.08	74.26	55.50 ± 0.15	18.13 ± 0.07	0.63 ± 0.03	-	3.06	[32]
-	-	9.50 ± 0.40	-	-	-	8.30 ± 0.30	-	63.6 ± 0.10	-	18.60 ± 0.17	-	3.40	[7]	
UPCP	-	2.64	8.90 ± 0.20	-	-	-	10.40 ± 0.10	-	57.00 ± 0.13	23.90 ± 0.20	-	2.40	[7]	
	1.31 ± 0.05	-	9.43	6.18 ± 0.10	2.70 ± 0.05	16.17	15.88 ± 0.05	74.40	55.30 ± 0.05	18.09 ± 0.04	1.01 ± 0.06	-	3.05	[31]
SC-CO <sub>2</sub>	-	-	-	3.00–10.08	0.40–3.99	-	8.04–13.62	-	48.85–63.66	16.71–26.20	0.11–0.95	-	2.11–3.17	[42]
	-	-	7.51–7.77	5.05–5.36	1.45–1.75	11.12–11.63	10.99–11.46	80.85–81.35	59.23–59.77	17.95–18.20	3.39–3.58	-	3.25–3.31	[20]
	-	-	2.65–3.54	1.59–2.19	0.61–0.91	2.94–5.06	2.46–4.63	21.15–28.31	15.00–19.89	5.15–8.24	0.32–1.15	0.20–0.45	-	[41]
5.50 ± 1.00	-	10.81 ± 0.12	6.36 ± 0.05	2.68 ± 0.02	13.35 ± 0.05	12.67 ± 0.03	75.84 ± 0.06	57.04 ± 0.02	15.68 ± 0.04	2.99 ± 0.03	-	3.82	[22]	
L-CO <sub>2</sub>	8.9 ± 0.20	-	8.23 ± 0.23	5.46 ± 0.14	1.68 ± 0.07	12.51 ± 0.09	11.96 ± 0.10	78.34 ± 0.32	58.04 ± 0.27	15.86 ± 0.04	4.09 ± 0.04	-	3.91	[22]

<sup>1</sup> SOE = solvent extraction, AE = aqueous extraction, HPE = hot-pressed extraction, CP = cold-pressed extraction, IPCP = infrared pretreatment cold-pressed extraction, RPCP = roasting pretreatment cold-pressed extraction, MPCP = microwave pretreatment cold-pressed extraction, UPCP = ultrasound pretreatment cold-pressed extraction, SPF-CO<sub>2</sub> = supercritical CO<sub>2</sub> extraction, L-CO<sub>2</sub> = liquid CO<sub>2</sub> extraction. <sup>2</sup> The author expressed the results as mg GAE/100 g of seed. <sup>3</sup> The value was initially expressed as FA (% oleic acid), and was converted to AV (mg KOH/g of oil) by multiplying the FA with the factor that equals ten times the molecular weight for KOH (56.1) and dividing by the molecular weight of oleic acid (282.4) to ensure consistency.

### 3.3. Tocopherol Content

Tocopherols are naturally occurring compounds with significant antioxidant activity. Their profile consists of four isomers,  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols, and  $\gamma$ -tocopherols is the most dominant one [32,40,45,52]. Due to their antioxidant activity, they are strongly related to HsO stabilization, preventing its oxidation [38,41,50] during extraction and storage, enhancing the HsO's shelf life and nutritional profile [27,35,51,53]. Moreover, it also mentioned their health efficacy effects for cancer, cardiovascular diseases, Alzheimer's, and neurodegenerative diseases [30,50,53].

It is evident from Table 2 that the total tocopherols of the HsO range from 3.47 mg/100 g of oil [37] to 562.8 mg/100 g of oil [36], with the majority of the HsO demonstrating values between 80 and 100 mg/100 g of oil. Furthermore,  $\gamma$ -tocopherol ranged between 0.50 mg/100 g of oil [10] and 516 mg/100 g of oil [12], characterized as the most abundant isomer. Table 2 shows remarkable differences occurred among different extraction methods or pretreatment of the seed's prior extraction. Nevertheless, according to Aiello et al. [22], HsO obtained through solvent extraction may present considerable reduction in the tocopherol content due to refinement for solvent removal. On the other hand, Liang et al. [53] and Mendoza et al. [18] mention that factors such as hemp variety, agronomic conditions, seed processing methods, and oil storage may affect both the quality and quantity of the tocopherols. Izzo et al. [37] also point out the need for better management in order to reduce differences in bioactive component contents.

**Table 2.** Total tocopherol and tocopherol isomer content (mg/100 g of oil, except for the values followed by superscript 2–4) of hemp seed oil with different extraction methods.

Methods <sup>1</sup>	Tocopherols (mg/100 g of Oil)					References
	Total Tocopherols	$\alpha$ -Tocopherol	$\beta$ -Tocopherol	$\gamma$ -Tocopherol	$\delta$ -Tocopherol	
SOE	-	3.30–5.20	-	49.80–71.80	-	[18]
	-	-	-	212.10–294.90 <sup>2</sup>	62.00–115.70 <sup>2</sup>	[40]
	83.26	0.79 $\pm$ 0.15	-	79.28 $\pm$ 0.20	3.18 $\pm$ 0.34	[31]
	83.26	0.79 $\pm$ 0.16	-	79.28 $\pm$ 0.21	3.18 $\pm$ 0.35	[32]
	-	-	-	21.10–51.36 <sup>3</sup>	2.74–5.78 <sup>3</sup>	[15]
	-	4.93 $\pm$ 0.09	-	96.71 $\pm$ 1.72	-	[22]
	79.70	3.40	0.60	73.30	2.50	[52]
AE	84.40 $\pm$ 0.80	0.30	0.20	96.30	3.00	[45]
CP	-	traces	-	468 <sup>4</sup>	-	[14]
	-	2.56 $\pm$ 0.06	0.596 $\pm$ 0.01	59.79 $\pm$ 1.21	3.97 $\pm$ 0.15	[50]
	-	2.78 $\pm$ 0.01	-	56.41 $\pm$ 0.02	-	[30]
	-	0.27 $\pm$ 0.05	-	0.5 $\pm$ 0.08	-	[10]
	562.80	41.60	48.60	-	-	[36]
	-	16.1 $\pm$ 5.33	-	516 $\pm$ 400	12.00 $\pm$ 4.00	[12]
	81.69–101.45	3.92–4.77	0.01–0.21	77.43–92.45	0.32–4.02	[6]
	-	2.12–109.56	-	37.60–90.70	-	[49]
	-	1.46–5.30	-	59.4–96.7	1.96–5.03	[35]
	48.31 $\pm$ 0.61	3.18 $\pm$ 0.09	1.13 $\pm$ 0.04	42.69 $\pm$ 0.50	1.31 $\pm$ 0.05	[27]
-	3.90–9.90	-	58.50–67.80	-	[18]	
3.47–13.25	-	-	-	-	[37]	
79.88 $\pm$ 0.22	5.29 $\pm$ 0.07	-	70.75 $\pm$ 0.24	3.85 $\pm$ 0.05	[38]	
IPCP	12.81 $\pm$ 0.097	3.198 $\pm$ 0.02	-	122.33 $\pm$ 0.07	2.53 $\pm$ 0.01	[51]
RPCP	46.98 $\pm$ 0.48	3.58 $\pm$ 0.12	0.69 $\pm$ 0.04	41.70 $\pm$ 0.43	1.01 $\pm$ 0.05	[27]
	66.00–74.00	3.50–4.80	-	60.0–66.0	2.50–3.50	[38]
MPCP	92.97	4.712 $\pm$ 0.54	-	84.105 $\pm$ 1.74	4.15 $\pm$ 0.44	[32]
	77.6–86.0	3.00–3.30	0.50–5.6	71.90–74.90	2.50–2.80	[52]
UPCP	97.13	4.32 $\pm$ 0.65	-	89.26 $\pm$ 1.92	3.55 $\pm$ 0.25	[31]
SPF-CO <sub>2</sub>	-	0.78–3.05 <sup>3</sup>	0.07–0.30 <sup>3</sup>	12.58–29.38 <sup>3</sup>	0.61–2.49 <sup>3</sup>	[41]
	-	3.96 $\pm$ 0.07	-	77.01 $\pm$ 1.07	-	[22]
L-CO <sub>2</sub>	-	3.92 $\pm$ 0.52	-	77.42 $\pm$ 0.98	-	[22]

<sup>1</sup> SOE = solvent extraction; AE = aqueous extraction; HPE = hot-pressed extraction; CP = cold-pressed extraction; IPCP = infrared pretreatment cold-pressed extraction; RPCP = roasting pretreatment cold-pressed extraction; MPCP = microwave pretreatment cold-pressed extraction; UPCP = ultrasound pretreatment cold-pressed extraction; SPF-CO<sub>2</sub> = supercritical CO<sub>2</sub> extraction; L-CO<sub>2</sub> = liquid CO<sub>2</sub> extraction. <sup>2</sup> The author expressed the results as mg/100 g of seed. <sup>3</sup> The author expressed the results as mg/100 g of dry matter. <sup>4</sup> The author expressed the results as g/lit of oil.

### 3.4. Phytosterols Content

Phytosterols are endogenous antioxidant compounds that play a key role in oil rancidity inhibition, enhancing their stability during storage and cooking temperatures [51,53]. Phytosterols are mainly composed of plants resembling cholesterol molecules, acting either as their substitute in a healthy diet [43] or inhibiting cholesterol, leading to hypercholesterolemia reduction and cardiovascular health risks [10,14,53]. Among the phytosterol group,  $\beta$ -sitosterol, stigmasterol, and campesterol are the most dominant, showing antifungal, anti-inflammatory, and antiviral properties [10,14]. Total phytosterol content ranges between 195 mg/100 g of oil [54] and 341.5 mg/100 g of oil [45] even when thermal treatment occurred (Table 3).  $\beta$ -Sitosterol is the most abundant sterol, ranging from 53.04 mg/100 g of oil [10] to 228.4 mg/100 g of oil [45], and alongside campesterol (6.2 mg/100 g of oil [22]–29 mg/100 g of oil [54]) and stigmasterol (2.82 mg/100 g of oil [10]–9.77 mg/100 g of oil [51]), highlighting HsO as functional food with supplementary healthful and profitable properties [14].

**Table 3.** Total phytosterol and phytosterol isomer content (mg/100 g of oil, except for the values followed by superscript 2–3) of hemp seed oil with different extraction methods.

Methods <sup>1</sup>	Phytosterols (mg/100 g of Oil)				References
	Total Phytosterols	$\beta$ -Sitosterol	Stigmasterol	Campesterol	
SOE	-	79.70 $\pm$ 0.10	3.40 $\pm$ 0.90	7.30 $\pm$ 0.70	[43]
	-	90.75 $\pm$ 0.42 <sup>2</sup>	2.88 $\pm$ 0.17 <sup>2</sup>	6.20 <sup>2</sup>	[22]
AE	341.50	228.40	8.90	53.60	[45]
HPE	197.00–206.00	125.00–128.00	3.00–5.00	29.00–33.00	[54]
CP	-	100–148 <sup>3</sup>	-	-	[14]
	195.00–231.00	53.04 $\pm$ 2.54 123.00–135.00	2.82 $\pm$ 0.21 4.00–5.00	11.74 $\pm$ 0.93 29.00–33.00	[10] [54]
IPCP	236.20 $\pm$ 0.22	198.35 $\pm$ 0.098	9.77 $\pm$ 0.04	28.04 $\pm$ 0.08	[51]
SPF-CO <sub>2</sub>	-	85.83 $\pm$ 0.54 <sup>2</sup>	4.00 $\pm$ 0.06 <sup>2</sup>	9.69 $\pm$ 0.08 <sup>2</sup>	[22]
L-CO <sub>2</sub>	-	80.76 $\pm$ 0.35 <sup>2</sup>	5.06 $\pm$ 0.28 <sup>2</sup>	14.19 $\pm$ 0.63 <sup>2</sup>	[22]

<sup>1</sup> SOE = solvent extraction; AE = aqueous extraction; HPE = hot-pressed extraction; CP = cold-pressed extraction; IPCP = infrared pretreatment cold-pressed extraction; SPF-CO<sub>2</sub> = supercritical CO<sub>2</sub> extraction; L-CO<sub>2</sub> = liquid CO<sub>2</sub> extraction. <sup>2</sup> The author expressed the result as %w/w. <sup>3</sup> The author expressed the results as g/Lit of oil.

### 3.5. Total Phenolic Content

Phenolic compounds are plant-derived metabolites with major antioxidant, antimicrobial, and anti-inflammatory activity that reduces the risk of chronic diseases [15,30,55]. The presence of phenolic compounds of the HsO also highlights a powerful positive influence on oxidative stability and sensory attributes of the oil [6,40,53,56], but the total phenolic content of the HsO depends on the extraction method. Cold-pressed HsO shows concentrations ranging from 1.208 mg GAE/100 g of oil [49] to 440.0 mg GAE/100 g of oil [55] in contrast to solvent-extracted oils that exhibit values reaching up to 5160 mg GAE/100 g of oil [40] (Table 4). It is indicated that the method used plays a key role in the antioxidant activity; however, it is claimed [40] that the significantly high TPC content may also be enhanced by the variety and origin of the seeds.

**Table 4.** Total phenolic content (mg GAE/100 g oil, except for the values followed by superscript 2–3) of hemp seed oil with different extraction methods.

Methods <sup>1</sup>	TPC (mg GAE/100 g Oil)	References
SOE	1368–5160 <sup>2</sup>	[40]
	381.80–779.80 <sup>2</sup>	[15]
CP	188.23 $\pm$ 2.51	[30]
	440.00 $\pm$ 0.10	[55]
	44.00–188.00	[53]
	267.50 $\pm$ 8.84	[56]
	123.00 $\pm$ 69.00	[12]
	290.32–384.52 1.21–18.68	[6] [49]

**Table 4.** *Cont.*

Methods <sup>1</sup>	TPC (mg GAE/100 g Oil)	References
CP	6.44 ± 0.45	[27]
	32.50–160.80	[37]
	10.22 <sup>3</sup>	[38]
RPCP	12.12 ± 0.54	[27]
	11.14–26.58 <sup>3</sup>	[38]

<sup>1</sup> SOE = solvent extraction; CP = cold-pressed extraction; IPCP = infrared pretreatment cold-pressed extraction; RPCP = roasting pretreatment cold-pressed extraction. <sup>2</sup> The author expressed the results as mg GAE/100 g of seed. <sup>3</sup> The author expressed the results as mg GAE/100 g of defatted seed.

### 3.6. Chlorophylls and Carotenoids Content

According to Tura et al. [57], sensory evaluation of HsO reveals that the positive characteristics in the oil color, ranging from dark green to light yellow, are based on the presence of pigments [30,44,53]. The most dominant pigments responsible for the color are chlorophylls (a, b) and carotenoids. Chlorophylls are thermo-sensitive and light-sensitive lipid-soluble compounds that accelerate FA degradation when exposed to inappropriate conditions during oil extraction and storage, resulting in intense rancidity, quality degradation, and shelf-life deterioration [35]. On the other hand, carotenoids, the second pigment derived from oil extraction responsible for the yellowish shades, exhibit a positive antioxidant role [22], reducing rancidity risks and preventing color change during storage [53]. Table 5 presents the total chlorophyll content, which ranges from 0.078 mg/100 g of oil [35] to 7.521 mg/100 g of oil [30], depending on the extraction method. Total carotenoid content is demonstrating a wide fluctuation from 0.25 mg/100 g of oil [35] to 8.3 mg/100 g of oil [45], influenced also by the extraction method, with aqueous and solvent extraction presenting the highest amounts (Table 5, Figure 1). Cold press allows the extraction of chlorophylls that deliver the green color, but due to their susceptibility to oxidation, they are considered undesirable and so must be reduced. Except for refining and bleaching for chlorophyll control [35,39,53], roasting treatment of the seeds prior to cold pressing is suggested to stabilize both color and chlorophyll activity [27].

**Table 5.** Total chlorophyll, chlorophyll isomers, and total carotenoid content (mg/100 g of oil, except for the values followed by superscript 2) of hemp seed oil samples with different extraction methods.

Methods <sup>1</sup>	Chlorophyll Content (mg/100 g of Oil)			Total Carotenoids (mg/100 g of Oil)	References
	Total Chlorophyll	Chlorophyll-a	Chlorophyll-b		
SOE	1.61–6.12	0.83–3.25	0.78–2.87	0.44–1.72	[44]
	-	12.55 ± 0.35	1.79 ± 0.12	6.065 ± 0.26	[22]
AE	-	-	-	8.30 ± 0.30	[45]
CP	7.521 ± 0.004	-	-	-	[30]
	9.86	5.92	3.95	3.36–5.34	[53]
	0.078–7.57	-	-	0.25–3.39	[35]
	3.91 ± 0.022 <sup>2</sup>	-	-	1.06 ± 0.08 <sup>2</sup>	[27]
	0.15	0.04–0.48	-	-	[37]
IPCP	4.52 ± 0.01	-	-	1.24 ± 0.01	[51]
RPCP	3.654 ± 0.04 <sup>2</sup>	-	-	1.01 ± 0.08 <sup>2</sup>	[27]
SPF-CO <sub>2</sub>	-	-	-	12.5	[53]
	-	19.53	3.54	0.82–12.54	[42]
	-	10.72 ± 0.28	23.29 ± 3.99	6.1 ± 0.10	[22]
LCO <sub>2</sub>	-	1.28 ± 0.19	1.46 ± 0.08	0.96 ± 0.12	[22]

<sup>1</sup> SOE = solvent extraction; AE = aqueous extraction; CP = cold-pressed extraction; IPCP = infrared pretreatment cold-pressed extraction; RPCP = roasting pretreatment cold-pressed extraction; SPF-CO<sub>2</sub> = supercritical CO<sub>2</sub> extraction; L-CO<sub>2</sub> = liquid CO<sub>2</sub> extraction. <sup>2</sup> The authors expressed the results as mg/100 g seed.

## 4. Conclusions and Future Purposes

Industrial hemp is a multipurpose sustainable cultivation complying with circular economy requirements, and currently, among the numerous products derived from different parts of hemp, HsO is a product with high nutritional value but of questionable quality background. As referenced previously in the text, HsO is a powerful food product

of unique  $\omega_6:\omega_3$  ratio, of high tocopherol, phytosterol, and phenolic content that elevates its nutritional value as an asset for chronic disease prevention. Although according to EU regulation 2022/1393, HsO is defined as food derived from hemp seeds with a maximum level of  $\Delta^9$ -THC of 7.5 mg/kg, and for hemp seeds the relevant level is 3 mg/kg [57,58], there are several other hemp products available on the global market with no certified quality parameters. The scientific concern is increased for HsO regarding the essential parameters, extraction methods, conservation methods of the seeds, and safety variables [59]. Cold-pressed HsO, up until now, lacks a quality and authenticity framework harmonized with EU legislation and the Codex Alimentarius, which only regards cold-pressed oils, clarifying that they are obtained only through mechanical processes (pressure, extrusion, expelling, etc.) without other applications that may change or modify the characteristics of the oils [34,57,59]. It is supported worldwide, the importance along with the necessity for optimized standard methods and quality parameters for HsO quality, and it is suggested that AV, peroxide value, nutritional profile, and sensory characteristics should be evaluated, aiming to promote a unique and health-supportive product, following maybe other oil compliance procedures.

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