Composition of cold-pressed hemp seed oils: key elements of quality and authenticity

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With the promotion of the agro-industrial chain of *Cannabis sativa* L., the presence on the market of food products obtained from industrial hemp has become more frequent and relevant. Among these, it is important to mention cold-pressed hemp seed oil, which, in relation to specific sensory quality and nutritional characteristics, is certainly one of the most widespread. In order to guarantee consumers regarding its safety, quality and authenticity, it is essential to know the key compositional parameters, their variability and the analytical methods that can be used to detect them. In this review article, all the evidence from the literature, useful to define a quality regulatory framework for the product category "cold-pressed hemp oil" according to the basic criteria of the Codex Alimentarius and some related considerations such as the seed conservation methods, the fundamental variables of the production process and the safety of the edible oil obtained, this last in relation to the legal limit (7.5 mg/kg), expressed as the sum of Δ^9 – tetrahydrocannabinol (acid plus neutral form), are discussed. Nowadays, apart this last legal obligation, there is no specific and harmonized EU legislation to define the quality and authenticity of cold-pressed hemp seed oil. In order to help achieve this objective, this review presents a discussion of the data from the literature and provides interpretative elements. The path and information described herein were keys in drafting the commercial standard entitled "Cold-pressed hemp oil obtained from *Cannabis sativa* L. seeds - Characteristics and methods of analysis" UNI-11876:2022.

Keywords: Hemp seed oils, cold-pressing, vegetables oils, *Cannabis sativa* L , authenticity

1. INTRODUCTION

As a consequence of the resumption of the traditional cultivation and supply chain of hemp (*Cannabis sativa* L.), given by Law n. 242 of 2nd of December 2016 [1], in the last decade there has been an increase in Italy from 27 to 603 dedicated hectares [2], especially in historically vocated areas. The varieties of industrial hemp allowed for cultivation are those registered in the Common Catalogue of Varieties of Agricultural Plant Species, as required by Directive 2002/53/EC [3]. Currently, 101 varieties of hemp [4] are registered in the catalogue, and from which it is permissible to produce food, in particular seeds, flour obtained from seeds, and oil obtained from seeds as defined in Annex 1 of the Decree of the Ministry of Health of 4 November 2019 and in the EU regulation [5,6]. For a certain period, hemp seeds were considered as a by-product of the fiber plant, and were often destined only for animal feed. Recently, thanks to the many research studies in this field, it has been shown that hemp seeds and the oil obtained from them are food products with a high nutritional and health value. The oil content and composition of the hemp seed are influenced by genetics and environmental factors. The seed contains over 30% oil, 25% protein, dietary fiber, vitamins, and minerals [7]. The most commonly used method for pressing the achenes is cold pressing.

The Codex Alimentarius defines cold-pressed oil as an oil obtained only through mechanical processes, such as extrusion or pressure without the application of heat and without altering its characteristics, which can only be purified by washing with water, decanting, filtration, and centrifugation [8]. Furthermore, cold pressing technology is advantageous as it is environmentally friendly, requires less energy than solvent-based systems/refining process, and represents a guarantee for the maintenance of nutrients [9] and specific sensory aspects that are peculiar and more intense in cold-pressed and not refined edible oils. In particular, hemp seed oil (HSO) has been described as dark green to light yellow-green in color, with a nutty flavor and sometimes with a slightly bitter aftertaste. However, for the evaluation of the sensory profile and quality, in order to describe positive attributes and defects, it is essential to refer to a common and shared vocabulary. For this purpose a specific lexicon with 45 descriptors was recently developed, together with a sensory wheel and a tasting sheet to allow training of different panels and to harmonize a procedure for sensory evaluation [10]. The lipid profile of cold-pressed HSO is characterized by the prevalence of polyunsaturated fatty acids (PUFA), followed by monounsaturated fatty acids (MUFA) and to a lesser extent by saturated fatty acids (SFA), mainly represented by linoleic acid, α-linolenic acid, oleic acid, γ-linolenic acid, and palmitic acid. The presence of small amounts of stearidonic acid is also peculiar in HSO, since this fatty acid is generally not present in common plant species cultivated for the production of vegetable oils. Typical sources of stearidonic acid derive from fish products, such as fish oils and microalgae, but recently, given the great interest, research has also highlighted significant amounts in some plant species, in particular the *Boraginaceae* family, such as Echium oil, extracted from the seeds of *Echium plantagineum* [11] and in small quantities in borage oil and black currant oil [12]. Cold-pressed HSO shows a ratio of ω-6/ω-3 fatty acids between 2.5:1 and 3:1, which is considered optimal from a nutritional point of view while, on the other hand, it is also extremely sensitive to oxidative degradation, auto-oxidation, photo-oxidation, and enzymatic oxidation with a risk of consequent rancidity of the product. In cold-pressed HSO there are also minor components such as phytosterols, tocopherols, chlorophylls, and carotenes which, due to their respective concentrations, can play an important role in exercising antioxidant or pro-oxidant activity. According to Reg. (EC) 1924/2006 [13], several nutritional and health claims are related to the fatty acid profile and in this paper the potential application to HSO will be discussed. For example, the claims are: *i*) source of omega-3 fatty acids, allowed if the product contains at least 0.3 g of alpha-linolenic acid per 100 g and per 100 kcal or at least 40 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal; *ii*) rich in omega-3 fatty acids, allowed if the product contains at least 0.6 g of alpha-linolenic acid per 100 g and per 100 kcal or at least 80 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal; *iii*) rich in polyunsaturated fats, allowed only if at least 45% of the fatty acids present in the product derive from polyunsaturated fats and provided that the polyunsaturated fats provide more than 20% of the energy value of the product; *iv*) rich in unsaturated fats, allowed only if at least 70% of the fatty acids present in the product derive from unsaturated fats and provided that the unsaturated fats provide more than 20% of the energy value of the product. To regulate the concentration of cannabinoids, there are legal restrictions, and lately the European Parliament has voted in favor of restoring the authorised Δ^9-tetrahydrocannabinol (THC) level on the field in industrial hemp from 0.2% to 0.3%, in hemp seeds Reg. (EU) 1393/2022 [6] establishes the limit of 3.0 mg/kg, while for HSO the limit raises to 7.5 mg/kg, a value that refers to the sum of delta-9-tetrahydrocannabinol (Δ^9-THC) and delta-9-tetrahydrocannabinolic acid (Δ^9-THCA). With reference to the synthesis of cannabinoids, some authors report their presence in the hemp seeds only as a consequence of cross-contamination deriving from contact with the glandular trichomes of cannabis inflorescences [14,15,16]. In particular, in the basal part of the glandular trichomes, the resin containing cannabinoids is synthesized and accumulates, above the cells of the spherical head, inside the outer membrane (cuticle) that covers the head. Sometimes this membrane opens allowing the resin to flow out and seep onto adjacent plant tissues, such as seeds [17]. Therefore, there would not be intrinsic presence of phytocannabinoids in hemp seeds [18,19,20], but some traces of these compounds can transfer to them through this mechanism, and consequently can be found in the oil extracted as a result of cross-contamination. It is also of fundamental importance to follow, during all stages of cold-pressed HSO production, careful and detailed technological practices aimed at: *i)* not triggering irreversible oxidative phenomena to preserve all the antioxidant components; and *ii)* avoiding possible contamination of unwanted substances to obtain a safe and high quality product. The quality, authenticity, and purity control of edible oils, even in a forensic view, is carried out through evaluation of a number of highly diagnostic parameters [21]. The composition in fatty acids, the content in minor components, such as phytosterols and tocopherols, are indicative and specific, both in terms of quality and quantity, of the botanical species of origin, while basic quality parameters such as free acidity and peroxide value allow evaluation of degradation status (hydrolytic or oxidative) of the oil. For evaluation of these parameters and their relative methods, references can be found in the current national and international regulations, being

Reg. (EU) 2022/2104, related to the characteristics of olive oils and olive pomace oils, and one of the cornerstones as well as the Codex Alimentarius [8]. In this work, an extensive bibliography has been collected on the characterization of cold-pressed HSO in order to collect and discuss all the useful data to define the quality parameters, as indicated and required by the general rules relating to vegetable oils, considering that, at present, a specific and harmonized regulation for cold-pressed HSO at the EU level is not present. Thus, the aim of the present paper is to provide a guideline for the main analytical reference parameters that are useful to define the quality and authenticity of cold-pressed HSO. Moreover, the references collected and discussed in this review were useful to draft a commercial standard UNI 11876:2022 [22], titled "Cold-pressed hemp oil obtained from the seeds of *Cannabis sativa* L. - Characteristics and methods of analysis".

2. HEMP SEEDS QUALITY FROM HARVESTING TO STORAGE

Seeds quality is essential to produce a good quality oil, whose value of free acidity and number of peroxides respect that indicated by the Codex Alimentarius [8]. In particular, it is very important to monitor selected environmental parameters, such as temperature, relative humidity, and storage time, as well as to check other parameters specifically related to seeds, i.e., moisture content and presence and percentage of impurities (dockage). These parameters are fundamental to obtain a low value of free acidity of the freshly produced oil (hydrolytic quality) and to avoid oxidation and fermentation or mold, which would also lead to sensory deterioration of the product. In general, hemp seeds are harvested when about 70% are ripe and the average moisture content is around 15-24%. Harvested seeds usually contain up to 15% impurities (green leaves, buds, stems, weed seeds, and foreign materials) and this dockage is often not cleaned in the pre-storage phase, which can last up to a year before processing. Due to the large presence of foreign bodies and the high moisture content at the time of harvest, hemp seeds can easily deteriorate and there can also be a greater presence of contaminating bacteria after harvest. In general, for different crops, in order to reduce storage loss, guidelines for more correct storage have been developed, e.g. rye [23], canola [24,25], durum wheat [26], and pinto beans [26], but, to date, there are no nationally or European guidelines for the correct storage of hemp seeds. Currently, several hemp growers use the guidelines for canola seeds, because hemp seeds have about 30-36% oil content, which is similar to that of some varieties of canola. However, high oil canola contains more than 42% [25,27], while the oil content of hemp seeds is about 36% [7]. In addition, it is necessary to consider that hemp seeds contain 15% of saturated fatty acids, which is lower than that of olive or canola oil, while polyunsaturated fatty acids are about 85% and, as is known, these compounds are easily prone to oxidation [28,29]. Additionally, other compounds, such as proteins, are present in hemp seeds and they are different than those in canola. These differences in the composition of hemp seeds compared to other oil seeds also affects their conservation. Finally, canola seeds usually have less than 5% of foreign bodies, which is lower compared to hemp seeds [30]. The main factors affecting the quality of stored oil seeds are temperature, moisture content or relative humidity, and storage time [29]. It is recommended to control the relative humidity inside the storage tanks, which must be equal to or less than 70% (water activity 0.7) because most fungi, molds, and bacteria do not develop in these conditions of low relative humidity. Seeds stored in lower relative humidity conditions have a longer shelf-life with little deterioration. In fact, when oil seeds are stored at high humidity, unbound (free) water is available for the development and multiplication of microorganisms. Seeds stored at higher temperatures always have higher rates of respiration than those stored at lower temperatures and therefore germinate more easily [31]. When temperatures are below 5°C, most molds do not grow [29]. Therefore, to correctly store hemp seeds temperature and humidity are the main factors to be monitored [23,26,32]. The deterioration can cause damage to the seeds, worsening their quality and, consequently, the quality of the oil produced. In particular, as mentioned before, lipid oxidation and the increase in the concentration of free fatty acids are linked to the deterioration of the seed [30]. Moreover, seed germination is the external biological expression of its physiological, biochemical, and morphological changes [33,34]. For this reason, 20% germination or the presence of visible mold is usually used as a cornerstone of guidelines for the safe storage of oilseeds [35]. In an intact, undamaged, and well-preserved seed, enzymatic hydrolysis does not occur or occurs to a very limited extent. In the oil industry, free acidity is one of the main oil quality parameters. This is because an increase in free acidity indicates an increase in the concentration of free fatty acids in the oil, often resulting from poor conservation of the raw material. Furthermore, these fatty acids, not bound in triglycerides, are more susceptible to oxidation, thus making the oil even more sensitive to oxidative phenomena. Moreover, these changes in the lipid composition determine a degradation of the sensory characteristics and the formation of unpleasant odors, as well as a decrease in its nutritional properties.

A study conducted by Jian and colleagues (2019) [30] showed that hemp seeds can be stored at 30°C for up to 10 weeks, regardless of the percentage of dockage, if the seeds have a moisture content of 9%. In order to store hemp seeds up to 6 months, it is essential to have a moisture content less than 8%.

Moreover, they determined an equation to calculate the storage time:
ɵ=a-b*T-(c-d*T)*MC

Where:
ɵ= is the storage time that does not cause deterioration of the seeds expressed in weeks;
T= temperature (°C);
MC= seeds humidity (%);
a,b,c,d= regression parameters.

This equation indicates that both T and MC have a linear influence on the storage time and the interaction between T and MC also has an influence on the correct storage period of the seeds. The predicted storage times may show a prediction error of approximately 1.14 weeks with respect to the storage times experimentally measured by the authors. Moreover, the method reported by ASTM established a protocol to determine the spoilage in hemp seeds based on their color [36].

3. COLD-PRESSED HEMP SEED OIL COMPOSITION

a. Fatty acid profile

The qualitative and quantitative determination of the total fatty acids defines the characteristic compositional profile of the oil, typical of botanical species, and therefore represent an important parameter of authenticity. Analytical determination is generally carried out by gas chromatography after derivatization of fatty acids in the corresponding stable and volatile methyl ester derivatives, as reported in the following methods: UNI EN ISO 12966 [37], Reg. (EU) 2022/2104 [38], NGD C41-1976 [39], and NGD C42-1976 [40]. Compared to other vegetable oils, HSO has the highest percentage, on average near 80%, of polyunsaturated fatty acids (PUFA) and linoleic acid (18:2 ω-6), with contents ranging from 50% at 70%; it is the most represented one, followed by α-linolenic acid (18: 3 ω-3), with percentages from 15% to 34% and γ-linolenic acid (18:3 ω-6) [7]. Peculiar, as reported in the introduction, is the presence of stearidonic acid (18:4 ω-3) in small amounts, from 0 to 2%. The ratio between the ω-6 and ω-3 series of fatty acids (Table I), currently between 2.5:1 and 3.4:1 in HSO, is perfectly in line with the dietary recommendations expressed by the FAO. According to Reg. (EC) 1924/2006 [13] relating to nutrition and health claims applicable to specific food products, HSO has a fatty acid profile that could bear the following claims: source of ω-3 fatty acids, rich in ω-3 fatty acids, rich in polyunsaturated fats, and rich in unsaturated fats.

b. Sterol composition

Similar to what was mentioned above for the determination of total fatty acids, the qualitative and quantitative profile of the sterol fraction also contained in the unsaponifiable fraction of the hemp seed oil is typical and related to the botanical origin, thus representing a very important parameter that can confirm the purity or authenticity of the product. The sterol determination method involves saponification, extraction of the unsaponifiable fraction, purification, and derivatization before instrumental gas chromatography analysis, as described in the methods indicated in UNI EN ISO 12966 [37], Reg. (EU) 2022/2104 [38] and NGD C 71 - 1989 [48], NGD C 72 – 1989 [49], and AOCS Ch 6-91 [50]. In cold-pressed HSO, total sterol ranges from 3425 to 6719 mg/kg, being β-sitosterol the most represented (70%), followed by campesterol (15%) and δ5-avenasterol (7%) [51,56] (Table II). In contrast to what is mainly cited in the literature, Schlag et al. (2022) [57] reported that the presence of lanosterol, as being not singularly quantifiable due its low concentration and the coelution with β-sitosterol, but, given the contradiction in the quantification (β-sitosterol in the author's table is reported as lanosterol); given that this evidence was not confirmed, the data of Schlang were not included in Table II. In order to compare the sterol profile with other vegetable oils, the qualitative profile of cold-pressed HSO could be similar to that of sesame oil, except for brassicasterol which was found in HSO (but less than 0.2%) only by Kostadinovic et al. (2015) [53]. While the acidic composition can be influenced by genetic manipulations, the sterol composition reports a lower variability, thus having great value in revealing mixtures with oils of different botanical origin or non-compliant, in particular in terms of total amount, as a consequence of refining treatments.

c. Stigmastadienes content

Refining impacts on the minor components in the case of sterols present in the unsaponifiable fraction, especially in the bleaching and deodorization phases, there is the elimination of the alcoholic group in position 3 and the formation of the double bond in position 3,4 with the consequent dehydration and elimination of a water molecule. This leads to the formation of a steradiene hydrocarbon. Cold-pressed vegetable oils, being not refined, should not contain steradiene hydrocarbons (i.e. stigmastadienes). Therefore, this analytical parameter represents an index of refining and/or possible mixture with refined oils to protect the genuineness of cold-pressed oils. The determination of stigmastadienes is carried out by gas chromatography, as described in methods UNI EN ISO 15788-1 [58], Reg. (EU) 2022/2104 [38]. Reg. (EU) 2022/2104 and Reg. (EU) 2022/2105 [59] indicate a limit of ≤0.05 mg/kg referring to extra virgin and virgin olive oils, in "cold-pressed oils" the natural presence of stigmastadienes is not justified and there is no legislation referable to this parameter. Therefore, a limit equal to ≤0.10 mg/kg could represent a reference, as already reported in some industry standards referring to pressure oils [60]. In our laboratory, we analyzed

Table I - Fatty acid composition of cold pressed hemp seed oil (the table indicates different significant digits, in accordance with each original publication)

Fatty acid	Leizer et al. (2000) [19]	Parker et al. (2003) [41]	Dubois et al. (2007) [42]	Callaway (2004) [7]	Tura et al. (2023) [43]	Rovellini et al. (2013) [44]	Siano et al. (2018) [45]	Tura et al. (2022) [46]	Occhiuto et al. (2022) [47]	
							Cv.Fedora	Cv.Futura 75	Cv.Futura 75	Cv.Futura 75
	%	%	%	%	%	%	%	%	%	%
Myristic acid (C14:0)	-	-	-	-	0.03-0.04	0.03	-	-	0.02	0.05
Pentadecanoic acid (C15:0)	-	-	-	-	0.01	0.02	-	-	-	-
Palmitic acid (C16:0)	5-7	6.26	6.3	5	4.95-7.12	6.07	7.15	7.68	6.95	8.67
Palmitoleic acid (C16:1)	-	-	-	-	0.10-0.37	0.14	-	0.14*	0.11	0.11
Heptadecanoic acid (C17:0)	-	-	-	-	0.03-0.05	0.03	-	0.05	0.03	0.05
Stearic acid (C18:0)	1-2	2.72	2.8	2	1.69-2.55	2.38	2.73	2.73	2.68	3.76
Oleic Acid (C18:1)	8-13	11.72	12.1	9	6.87-12.33	10.26	12.75	14.19*	12.31	16.73
Linoleic acid (C18:2)	52-62	59.96	55.9	56	38.48-52.16	55.75	56.08	55.84	56.16	51.39
Alfa-Linolenic acid (C18:3)	12-23	19.33	19.7	22	11.02-17.40	17.37	14.89	16.36	17.74	15.36
Gamma-Linolenic acid C18:3)	3-4	-	2.8	4	0.98-4.43	4.65	3.03	1.10	2.33	2.03
Stearidonic acid (C18:4)	-	-	-	2	0.20-1.50	1.48	-	0.77	0.81	0.56
Arachidic acid (C20:0)	0.39-0.79	-	0.7	-	0.03-0.06	0.87	0.89	0.32	0.50	0.73
Eicosenoic acid (C20:1)	0. 51	-	-	-	-	0.4	0.26	0.37	0.24	0.33
Eicosadienoic acid (C20:2)	-	-	0.8	-	0.03-0.06	-	1.03	-	0.14	0.24
Behenic acid (C22:0)	-	-	0.3	-	0.16-0.29	0.34	0.20	0.30	-	-
Erucic Acid (C22:1)	-	-	-	-	0.01	0.03	-	-	-	-
Lignoceric acid (C24:0)	-	-	-	-	0.07-0.13	0.17	-	0.15	-	-
Σ-SFA		8.98		7	6.89-9.47	9.86	10.97	11.23	9.68	12.53
Σ-MUFA		11.72		9	6.97-20.84	10.91	13.01	14.70	12.65	17.17
Σ-PUFA		79.29	79.1	84	59.11-70.38	79.25	75.03	74.07	77.67	70.31
Σ-PUFA/ Σ-SFA							6.84			
ω6		59.96	59.4	60		60.40			59.12	54.39
ω3		19.33	19.7	24		18.85			18.55	15.91
ω6/ω3			2.8	2.5	2.60-3.67	3.20		3.32	3.19	3.42

*Sum of the isomers

Table II – Sterol composition of cold pressed HSO. The table shows different expression of quantitative data and significant digits, in accordance with each original publication. Moreover, a conversion was made for all the reported data in mg/kg, apart for Rovellini et al. (2013) [44], for which the relative % was left. Data of Monserrat de la Paz et al. (2014) [51] are referred to a refined oil.

Sterol	**Matthäus & Brühl (2008) [52]**		**Rovellini et al. (2013) [44]**	**Monserrat de la Paz et al. (2014) [51]**	**Kostadinovic et al. (2015) [53]**	**Siano et al. (2018) [45]**	**Blasi et al. (2022) [54]**	**Gutièrrez Luna et al. (2022) [55]**
	average	**range**				**Cv.Fedora**		
	(mg/kg)	(mg/kg)	Relative%	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Cholesterol	34	22-74	0.3	0.92	14.78		8.60-13.03	-
Brassicasterol	-	-	-	-	1.72	-	-	-
24-metylencholesterol	40	25-81	0.7	7.05	29.57		-	-
Campesterol	709	257-1001	16	505.69	504.5	117.4	345.1-813.8	365.3
Campestanol	11	0-19	0.3	-	52.96		17.79-50.52	-
Stigmasterol	133	97-181	2.2	100.23	90.10	28.2	50-247.5	43.6
d7-Champesterol	26	12-39	0.6	-	33.36		-	-
d5 23 Stigmastadienol	-	-	-	-	-		-	-
Chlerosterol	36	23-74	0.9	58.39	21.32		10.43-52.19	-
β-Sitosterol	3191	2704-4434	67.8	1905.07	2311.35	530.4	1510-4010	2753.6
Sitostanol	76	39-127	0.9	-	52.96		19.77-67.60	-
d5 Avenasterol	336	209-572	7.4	142.80	219.06	72.6	142.56-528.44	243.3
d7-9(11)Stigmastadienol	-	-	-	-	-		-	-
d5-24Stigmastadienol	54	36-65	1.1	31.97	11.35		16.77-70.90	-
d7-Stigmasterol	30	19-47	-	21.74			-	-
d7-Stigmastenol	-	-	0.7	-	53.30		26.77-180.12	-
d7-Avenastrerol	51	32-79	1.1	19.87	28.89		42.96-93.02	27.0
Total sterols	**Matthäus & Brühl (2008) [52]**		**Rovellini et al. (2013) [44]**	**Monserrat de la Paz et al. (2014) [51]**	**Kostadinovic et al. (2015) [53]**	**Siano et al. (2018) [45]**	**Blasi et al. (2022) [54]**	**Gutièrrez Luna et al. (2022) [55]**
	average	**range**				**Cv.Fedora**		
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)		(mg/kg)
		4727,00	3922-6719	4393	2793.73	3425,22	-	2199-5891

the stigmastadienes in commercial cold-pressed HSO and found values around 0.09-0.14 mg/kg (data not published).

d. Tocopherols composition

Vegetable oils contain the presence of tocopherols and their corresponding tocotrienols which differ in the unsaturated lateral racemic chain. The various botanical species are characterized by the presence of almost all eight molecular species. The presence of these compounds is important as they have a strong antioxidant activity by acting as a "scavenger" against lipid oxidation. Tocopherols, in particular α-tocopherol, possess vitamin activity, thus further enhancing the nutritional value of the oily matrix. The analytical determination of tocopherols and tocotrienols is performed by applying chromatographic methods as indicated in UNI EN ISO 9936 [61], UNI/TS 11825 [62].The literature highlights that in cold-pressed HSO the most represented tocopherol is γ-tocopherol with content from 625.3 to 924.5 mg/kg, followed by α-tocopherol with 17-77.6 mg/kg, δ-tocopherol with 25-40.2 mg/kg, and β-tocopherol with 0.1-5.8 mg/kg (Table III). Liang et al. (2015) [56] reported that total tocopherols range from 800 to 1500 mg/kg with 85-91% represented by γ-tocopherol. Tocotrienols are not present, and only Blasi et al. (2022) [54] reported the presence of α-tocotrienol in some samples. However, there are also very distant values in the literature such as those reported by Siano et al. (2019) [45] who found in the Fedeora cultivar very low contents of α-tocopherol and γ-tocopherol equal to 2.7 and 5.0 mg/kg, respectively. The quali-quantitative determination of tocopherols is not a regulated parameter by national or international law for this oil. However, it represents an indicator of genuineness as it is known that the oils obtained from cold pressing keep the contents of the bioactive constituents almost unchanged; while refining treatments, especially due to temperatures reached of 110-120°C, determine their degradation. Thus, the presence of tocopherols in concentrations not in line with the characteristic profile can be attributable to refining or mixing treatments of different or refined oils. Furthermore, given that the presence of a profile in predominantly unsaturated fatty acids in the oily matrix causes a lack of stability, it is very important to preserve, during all stages of production, all the components that exert a strong antioxidant activity to extend the shelf-life of the product and maintain its nutritional properties.

e. Free acidity

This determination provides a measure of free organic acidity, highlighting the level of hydrolytic degradation. The analysis is carried out by titration as indicated in the methods described in UNI EN ISO 660 [63], Reg. (EU) 2022/2104 [38], NGD C 10 -1976 [64], and AOCS Cd 3d-63(03) [65]. The oil dissolved in a suitable solvent is titrated with a strong base in the presence of indicator. The result is expressed as an acid number or the mg of KOH necessary to neutralize the fatty acids present in 1 gram of oil. It is also possible to express the value as a percentage of fatty acid, usually the most representative of the matrix, e.g. oleic acid, palmitic acid. The Codex Alimentarius establishes an acidity limit of 4.0 mg KOH/g oil for cold-pressed and virgin oils [8]. The results reported in the literature are unexpected and show that this parameter, despite having a well-defined legal limit, is often widely exceeded (Table IV). Spano et al. (2020) [67] found that 3 of the 9 oils analyzed had contents beyond the limit, and Calzolari et al. (2021) [68] found 33 of 45 oils had values over the limit, reaching out-of-control results with values up to 17.24% if expressed as oleic acid. This parameter represents an evident criticality in the production of HSO. The consequence of these results can be attributable to critical aspects present in the production chain. Of primary importance is the good quality of the seeds, which must be harvested at the right degree of ripeness and humidity, well preserved and intact, in order to avoid, due to the presence of water or enzymatic substances deriving from the breakage of the seed, the triggering of unwanted reactions. During the ex-

Table III – Tocopherol composition of cold-pressed hemp seed oil (in the table different significant digits are indicated in accordance with what reported the authors and for uniformity of the results in the table all values have been converted into mg/kg).

Tocopherol	Rovellini et al. (2013) [44]	Kostadinovic et al. (2015) [53]	Siano et al. (2018) [45]	Blasi et al. (2020) [54]	Tura et al. (2022) [46]	Occhiuto et al. (2022) [47]	Tura et al. (2023) [43]
			Cv.Fedora		Cv.Futura 75		
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
α-tocopherol	37	17	2.7	3.5-77.6	38.73	39.2-47.7	14.6-53
β-tocopherol		2	-	3.7-5.8	-	0.1-2.1	-
ɣ-tocopherol	858(*)	649	5.0	625.3-1013.2	794.66	774.3-924.5	594-967
δ-tocopherol	33	25	-	14.0-35.1	29.22	3.2-40.2	19.6-50.3
Total (mg/kg)	928	697	-	655.0-111.08	-	816.9-1014.5	-

(*) β-tocopherol + ɣ-tocopherol

Table IV – Free acidity (different significant digits are indicated in accordance with what reported by the authors).

	(% Oleic acid)	(mg KOH/g)
Rovellini et al. (2013) [44]	0.49	
Tura et al. (2022) [46]		1.78
Occhiuto et al. (2022) [47]	0.58-0.65	
Kostadinovic et al. (2015) [53]	0.91	
Mikulcovà et al. (2017) [66]		0.7
Spano et al. (2020) [67]	0.40-17.24	
Calzolari et al. (2021) [68]	0.81-16.69	
Tura et al. (2023) [43]	0.45-2.31	0.89-4.58

traction phase, due to the high pressure exerted, a rise in temperature inevitably occurs, which can exceed 70°C. These reactions and thermal stress cause the triggering of hydrolytic degradation and its consequent evolution. Compliance with this parameter can be improved through careful selection and storage of seeds, followed by temperature control during the extraction process.

f. Peroxide value

The determination of the peroxide value (PV) expresses the degree of oxidative degradation of the oil. Oxidative rancidity is a phenomenon that mainly affects unsaturated fats and is strongly catalyzed by the action of light which acts, in the presence of oxygen, on the double bonds to form radical compounds that trigger reactions and lead to the formation of hydroperoxides, aldehydes, ketones, and short-chain carboxylic acids that give the characteristic rancid scent. The analysis is carried out through iodometric titration as indicated in the methods described in UNI EN ISO 3960 [69], Reg. (EU) 2022/2104 [38], NGD C 35 -1976 [70], and AOCS Cd 8b-90(03) [71]. Hydroperoxides, being primary oxidation products, react with a potassium iodide solution, and the titration of the developed iodine indirectly reports the concentration of the hydroperoxide content. The result is expressed as milliequivalents of active oxygen per kilogram of oil mEq O_2/kg. The Codex Alimentarius establishes a peroxide number limit of 15 mEq O_2/kg oil for cold-pressed and virgin oil. In the literature, PV values for HSO have ranged from 1.55 to 28.2 (mEq O_2/kg) [43,44,46,47,53,67], while Piskernik et al. (2021) [72] reported values between 23.7 and 77.2 (mmol/kg). Similar to what reported for the acidity parameter, for PV several authors also found values higher than the legal limit in the cold-pressed HSO sold on the market. Also in this case, all the critical points mentioned above (paragraph 2.5) contribute to triggering the oxidative phenomenon by promoting the formation of hydroperoxides in the presence of oxygen. It is possible to find very low peroxide values even in the presence of a marked manifest rancidity when the kinetics of the reaction has already evolved to the formation of secondary oxidation products, and in particular aldehydes and ketones. For this purpose, in order to have a accurate evaluation of the oxidative state of the oil matrix, it is advisable to consider the parameter of p-anisidine number. This could prove useful to evaluate the resistance to forced oxidation using Rancimat, not as a reference parameter, but as a useful element for producers and packers in order to not reduce shelf-life expectations too much.

g. Phytocannabinoid content

In HSO it is possible to find measurable quantities of Δ^9-THC resulting from the contact of the seeds with the bracts and leaves of the inflorescence. In oils that undergo refining processes, Δ^9-THC is removed, while for oils obtained by cold pressing this does not happen. Therefore, in order to keep Δ^9-THC levels below the legal limits, it is essential to clean the seeds. In fact, setting up a washing process of the seeds before extraction of the oil allows producing an oil with low amounts of THC, removing this cannabinoid from the surface of the seeds before pressing them. Furthermore, considering that the varieties of hemp admitted to cultivation (Common Catalog of Varieties of Agricultural Plant Species) are characterized by a low Δ^9-THC content, the risks of a cold-pressed HSO with a high content of these cannabinoids are reduced [73]. However, it is essential during quality control of cold-pressed HSO to monitor the cannabinoid content, in particular in relation to Δ^9-THC and tetrahydrocannabinolic acid (THCA). The method for the determination of Δ^9-THC, its precursors, and other cannabinoid compounds in food products containing hemp is described in EU Recommendation No. 2115/2016 [74], which provides for the chromatographic separation technique coupled with mass spectrometry (LC-MS or GC-MS) after purification treatment [liquid-liquid extraction (LLE) or solid phase extraction (SPE)]. However, given that not all laboratories have such sophisticated and expensive instrumentation, which also requires high professionalism for use, many validations of alternative methods that exploit GC-FID and HPLC techniques have been published. The German Pharmacopoeia has also adopted a Δ^9-THC quantification method based on extraction with solvent ethanol, followed by liquid chromatography with UV detector. Through these methods, it is possible to carry out the separation and quali-quantitative evaluation of all the main phytocannabinoids present. Reg.(UE) 1393/2022 defined a limit for the presence of Δ^9-THC in HSO equal to a maximum of 7.5 mg/kg, which is intended as the sum of the concentrations of the trans-Δ^9-THC substance and its inactive acid precursor (Δ^9-THCA-A). The literature highlights the presence of cannabinoids in HSO, and the most represented compounds are CBDA, CBD, Δ^9-THC, and Δ^9-THCA. Some authors have also indicated the presence of CBDA, CBG and CBN [15] (Table V). Regarding cannabinoid exposure assessment, in 2015 EFSA [76] established an acute reference dose (ARfD) for Δ^9-THC of 1 µg/kg body

Table V – Cannabinoid composition of cold-pressed hemp seed oil (different significant digits are indicated in accordance with what reported the authors and for uniformity of the results in the table all values have been converted into mg/kg).

Cannabinoids	Tura et al. (2023) [43]	Leizer (2000) [19]	Matthaus & Brühl (2008) [52]	Citti et al. (2018) [15]	Nigro et al. (2022) [75]
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
CBDA	4.25-91.6	-	-	<LOQ 821.1	180
CBD	ND-22.2	10	-	<LOD - 1056	
CBDV	-	-	-	<LOQ - 75.39	
CBG	-	-	-	<LOD - 1.381	
CBN	-	-	-	<LOQ -12.05	
Δ^9-THC	ND-5.29	ND	11-117	<LOD -1.804	
Δ^9-THCA	ND-5.0	-	-	<LOQ - 9.462	
Δ^9-THC+Δ^9-THCA	ND-10.30	-	-	-	

weight. Subsequently, the BfR (Federal Institute for Consumer Health Protection and Veterinary Medicine, 2021) [77] suggested a case-by-case toxicological assessment of foods containing HSO based on the ARfD. In 2020, in a scientific report from EFSA, for the scenario on HSO, [78], acute exposure to Δ^9-THC was estimated down, highlighting that for "high consumers" acute exposure to total Δ^9-THC in adults ranged from 3 to 21 µg/kg of body weight and in "other children" from 7 to 59 µg/kg of body weight. In the same report, the EFSA also recommended carrying out studies on the stability of Δ^9-THC and on the conversion of Δ^9-THCA during food processing (e.g. cooking). Nonetheless, with the limit of Δ^9-THC currently fixed at 7.5 mg/kg in HSO and assuming the consumption of an oil that reaches this limit, the maximum amount of this oil for a person weighing 75 kg would be 10 g/day. Kladar et al. (2021) [73] highlighted that cold-pressed HSO were the second most Δ^9-THC containing food product group and Steinmetz et al. (2022) [79] that only 4 of 102 HSOs samples analyzed had a low to moderate risk of inducing harm through typical dietary exposure to Δ^9-THC. However, assuming a reasonable usage equal to 38.21 g of HSO and 25.37 g of cold-pressed HSO (corresponding to 41.2 and 27.4 ml, respectively) an oil could be consumed daily without significant concern. The present scenario indicates that further investigation of Δ^9-THC in HSOs, guidelines for its production, and compliance with legal limits will be essential to protect consumer health and promote commercialization of a safe product, uniform in quality. Moreover, the European Commission has established that cannabidiol (CBD) can be considered as a novel food, although due to the significant uncertainties and data gaps related to certain effects on humans, the panel concluded that the safety of CBD as a novel food cannot be currently established.

h. Color

The evaluation of oil color is usually carried out by measurement of CIELab color space parameters, i.e. the color coordinates a* and b* and the psychometric index of lightness L*. These color parameters are frequently used as an index of oil quality. There is no reference legislation to define the color of seed oils, and the guidelines of the Codex Alimentarius (2021) [80] indicate that the color, odor, and taste of each product shall be characteristic of the designated product. The color of the oils mainly depends on the presence of two pigments, carotenoids and chlorophylls [81,82]. Their concentration in the raw material depends on the variety, degree of ripening of seeds, and climatic features during plant growth. Stress damage and senescence also affect their content [83]. Cold-pressed HSO has a color that varies from light green to very intense green. This color is a consequence of the considerable quantities of pigments, and in particular chlorophylls which are co-extracted during pressing. On the other hand, some authors reported that the the color of HSO can be yellow or green [84,85] in relation to the content of chlorophylls [86]. It is essential to highlight that several studies have reported that HSO contains a large amount of chlorophylls that can affect oxidative stability and lead to rancidity [15]. These natural pigments act as powerful prooxidants, increasing the susceptibility to photo-oxidation of the oils when exposed to light and promoting change from the intensive dark green color to yellow [83]. A recent study carried out on two cold-pressed HSO obtained from fresh and stored seeds of the Henola cultivar showed significant differences relating to the measurement of color; in particular, the parameter a* showed correlation between storage time of the seeds and a decrease in green color, precisely in line with the oxidation of chlorophyll [87]. The concentration of these pigments in the finished product, on the other hand, is mainly affected by the storage conditions; the oxidative and degradative processes that the oils undergo during storage alter the content of carotenoids and chlorophylls. Recent studies have shown that these compounds can be

considered as quality indicators for the finished product [88]. In addition, Matthäus & Brühl (2008) [52] reported that high-quality virgin HSO is distinguished by a light to dark green color, highlighting that the color turns yellow during storage. Moreover, previous published scientific papers showed that the value of yellowness and lightness of HSO increased during storage, which were related to changes in the color of oils during storage and effective indicators of difference in quality [89]. Several authors have investigated the color of HSO, in particular in relation to different pre-treatment on seeds (such as roasting of the seeds) [90], oil extraction process (e.g. ultrasound-assisted, supercritical extraction, etc.) [91,92], or oil oxidative stability (e.g. addition of essential oils to hemp seed oil) [89]. Moreover, color represents one of the most immediate sensory properties of food and is decisive in the consumers' choice; also, for this reason, the possibility of measuring color objectively is of great importance [88,93].

4. CONCLUSION

In this review, the aspects of quality and authenticity of cold-pressed HSO have been overviewed. The analytical parameters of acidity, number of peroxides, fatty acids, tocopherols, sterols, stigmastadienes, and phyto-cannabinoids represent a fundamental reference for their definition. It has been shown that many cold-pressed HSO exceed the acidity value foreseen by the Codex Alimentarius (4 mg KOH/g of oil), which is due to incorrect conservation of seeds or processing and present a detectable amount of Δ^9-THC. With reference to the latter parameter, the new legal limits recently introduced (7.5 mg/kg of oil) mitigate the concern for regulatory compliance in the market, but careful monitoring and updated research will be always necessary to guarantee any safety aspect. The data collected in this review have been used to draw up the UNI 11876:2022 standard, which represents a valid reference for those who produce and sell HSO. This information can be relevant to take care of any technological phase of production, and in particular the harvesting, storage, and extraction phase of seeds in order to preserve the sensory and nutritional characteristics of the final oil and to obtain high and harmonized quality on the market.

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