Research Paper

Physicochemical studies of hemp (*Cannabis sativa*) seed oil using enzyme-assisted cold-pressing

Sajid Latif and Farooq Anwar

Department of Chemistry & Biochemistry, University of Agriculture, Faisalabad, Pakistan

The effects of enzyme-assisted cold-pressing (EACP) on the physicochemical attributes of *Cannabis sativa* (hemp) seed oil were investigated using five enzyme preparations: Protex 7L, Viscozyme L, Kemzyme, Feedzyme, and Natuzyme. The oil contents (28.4–32.8%) offered by the enzyme-treated hempseeds were found to be significantly ($p < 0.05$) higher than that determined for the control (26.7%). The protein, fiber, and ash contents of the seeds were unaffected by the enzyme treatment. There were no significant ($p > 0.05$) variations observed for the values of iodine number, refractive index, density, unsaponifiable matter and fatty acid composition between the enzyme-extracted and control hempseed oils. The levels of saponification value, free fatty acids, iodine value and peroxide value were slightly varied between the oils tested. The color intensity of the enzyme-extracted oils was also higher than that of the control oil. A relatively higher level of tocopherols (724.4–788.8 mg/kg) was observed in the enzyme-extracted oils compared to the control (691.2 mg/kg), showing an enhancement of ca. 4.8–14.1% in the total tocopherols. The Rancimat profiles and sensory scores of the enzyme-extracted oils were noted to be improved compared to the control. The results of the present analysis (with respect to the control) showed that the enzyme added during the hempseed cold-pressing resulted in considerably higher oil yields, without adversely affecting the quality of the oil.

Keywords: Enzyme-assisted cold-pressing / Fatty acids / Hempseed oil / Oxidative stability / Physicochemical properties / Tocopherols

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1 Introduction

Cannabis sativa L. (hemp), a member of the Cannabaceae family, is an annual herbaceous plant. A native of Western and Central Asia (Russia, China, India, Pakistan, and Iran), the plant has long been grown commercially in Europe and many other parts of the world, mainly for its fiber and the oil extracted from its seeds [1, 2]. In view of the multiple potential uses, the global production and consumption of low δ-9-tetrahydrocannabinol (THC)-type industrial hemp is currently growing [3].

A number of nutritional, medicinal and pharmacological attributes of hemp have been described in the literature. The foliage and leafed branches of this plant showed sedative and narcotic properties [1, 2]. Hempseed has been reported to have positive health benefits such as the lowering of cholesterol and high blood pressure [3, 4]. The seeds have been employed as an important ingredient in foods and folk medicine. Nutritionally, the hempseed contains 20–25% protein, 20–30% carbohydrates, 25–35% oil, 10–15% fiber and is a rich source of minerals, particularly phosphorus, potassium, magnesium, sulfur, calcium, iron and zinc [5, 6].

Hempseed oil is a rich and balanced source of linoleic (*n*-6) and α-linolenic (*n*-3) fatty acids (FA) [7]. The potential health benefits of these two polyunsaturated FA (PUFA) are interesting owing to their anti-inflammatory, antithrombotic, antiarrhythmic and hypolipidemic properties [8]. Hempseed oil also contains appreciable amounts of tocopherols, which are reported to exhibit antioxidant activity [2, 9]. The presence of cannabidiol (CBD) in hempseed oil is generally linked with its anticonvulsive, anti-epileptic, and antimicrobial attributes [10, 11].

Cold-pressing does not allow an extraction yield equal to the solvent extraction technique, but has the advantage of

Correspondence: Farooq Anwar, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad 38040, Pakistan.
E-mail: fqanwar@yahoo.com
Fax: +92 41 9200764

minimizing degradation of the oil quality [5]. Enzymatic pre-treatment has emerged as a novel and effective means to improve the yield and nutritional quality of seed oils and residual meals. The use of enzymes in the oil extraction process has been studied by several researchers [12, 13]. The enzymes most frequently employed for oil extraction are cellulase, α-amylase, and pectinase [14].

There are two general approaches for enzymatic oil extraction: (i) enzyme-assisted aqueous extraction (EAAE) and (ii) enzyme-assisted cold-pressing (EACP). In the enzyme-assisted aqueous process, the enzymatic action is reported to improve the oil recovery by degrading the seed cell wall and rupturing the polysaccharide-protein colloid, which may cause emulsion formation, resulting in a low yield. However, in the EACP technique, the enzymes only facilitate the hydrolysis of the seed cell wall because in this non-aqueous system there is no polysaccharide-protein colloid [15].

To the best of our knowledge, there are no earlier literature reports available on hempseed oil extraction using EACP. The aim of the present study was to evaluate and quantify the physicochemical characteristics of EACP of hempseed oil. Five enzyme preparations with multi-enzyme activity (except for Protex 7L) were tried in the present experiments. The results of the enzyme-extracted hempseed oils were compared with those of a control oil (oil produced without enzyme treatment).

2 Materials and methods

2.1 Materials

Hemp (*Cannabis sativa*) seeds were purchased from a local market of Faisalabad, Pakistan. All reagents (of analytical and HPLC grade) used were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland). Pure standards of tocopherols [DL-α-tocopherol, (+)-δ-tocopherol, (+)-γ-tocopherol] and FA methyl esters (FAME) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Protex 7L (protease) was provided by Genencor (Rochester, NY, USA), and Viscozyme L (multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase) by Novozymes Bagsvaerd (Denmark), whereas Natuzyme (mainly cellulase, xylanase, phytase, α-amylase, pectinase activities) was provided by Bioproton (Pty Ltd., Australia), Feedzyme (mainly xylanase, β-glucanase, cellulase and hemicellulase activities) by Agil, UK, and Kemzyme (mainly α-amylase, β-glucanase, cellulase complex, hemicellulase complex, protease and xylanase activities) by Kemin Europa N.V., Belgium.

2.2 Enzyme treatment and pressing

Clean seeds were ground using a coffee grinder and passed through an 80-mesh sieve, followed by conditioning at 100 °C

for 20 min. Enzymatic hydrolysis was carried out under a predetermined and optimized set of conditions over a period of 6 h at 40 °C, with an optimized amount (% by seed weight) of each of the five enzyme preparations (Protex 7L, Alcalase 2.4L, Viscozyme L, Kemzyme and Natuzyme) at 45% moisture level. The hydrolyzed sample was dried in a petri dish at 100 °C in a vacuum oven (VOC-300 SD; EYELA, Tokyo, Japan) to inactivate the enzyme and to readjust the moisture to the desired level (3–4%) prior to pressing [16]. Pressing of the hydrolyzed and dried seed sample for oil extraction was done in a manual laboratory hydraulic press (Carver Press, USA) for 20 min at a pressure range of 29.4–49.0 MPa [17]. A control sample of hempseeds was also processed under the same set of conditions, except for the enzymatic pretreatment.

2.3 Analysis of oilseed residues

After oil extraction (with and without enzyme), the hempseed residues were analyzed for protein, fiber, and ash contents. Protein content ($N \times 6.25$) was determined according to the AOAC (1990) method 954.01 [18]. Fiber content was estimated according to the ISO (1977) method 5983 [19]. A finely ground sample (2.5 g) of meal was weighed and freed from fat by extraction with 15 mL *n*-hexane. The test portion was boiled with sulfuric acid (0.255 mol/L), followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol/L), followed by separation, washing and drying. The dried residue was weighed and ashed in a muffle furnace (TMF-2100; Eyela, Tokyo, Japan) at 600 °C, and the loss of mass determined.

Ash content was determined according to the ISO (1977) method 749 [19]. Of the test portion, 2 g was taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace (TMF-2100; Eyela) at 550 °C until a constant mass was achieved.

2.4 Analysis of extracted oil

2.4.1 Physical and chemical parameters

Determinations of density, iodine value, peroxide and *para*-anisidine (*p*-anisidine) values, free FA (FFA) content, saponification value and unsaponifiable matter of the enzyme-extracted and control hempseed oils were made following AOCS official methods [20]. The color and refractive index of the oils were determined by a Lovibond tintometer (Tintometer Ltd., Salisbury, UK) using a 1-inch cell and a refractometer (RX-7000α; Atago Co., Japan), respectively. Specific extinctions at 232 and 270 nm were determined using a spectrophotometer (U-2001; Hitachi Instruments, Tokyo, Japan). The oils were diluted with *iso*-octane, the absorbance values were recorded at 232 and 270 nm, and $\varepsilon^{1\%}_{1cm}(\lambda)$ was calculated following the standard IUPAC method [21].

2.4.2 Sensory analysis

Sensory analysis of the extracted oils was conducted by following the method described by Min [22]. The sensory qualities of the oils were evaluated using a hedonic scale of 1–10, where 1 indicates the poorest and 10 the highest flavor quality.

2.4.3 Oxidative stability

An automated Metrohm Rancimat apparatus, model 743, capable of operating over a temperature range of 50–200 °C, was used to determine the induction periods (IP) of the oils. Testing was carried out at 120 ± 0.1 °C, and oxidative stability was measured following a procedure described elsewhere [23]. Briefly, portions of oil (2.5 g) were carefully weighed into each of the six reaction vessels and analyzed simultaneously. The IP of the samples were recorded automatically and corresponded to the break point in the plotted curves.

2.4.4 Fatty acid composition

FAME were prepared according to IUPAC method 2.301 [21] and were analyzed on a Shimadzu (Kyoto, Japan) gas chromatograph, model 17-A, fitted with a methyl-lignocerate-coated (film thickness 0.20 µm) SP-2330 polar capillary column (30 m × 0.32 mm; Supelco, Bellefonte, PA, USA) and a flame ionization detector (FID). Oxygen-free nitrogen was used as the carrier gas at a flow rate of 3.0 mL/min. The column temperature was initially held at 180 °C for 2 min, and then increased to 220 °C at a linear ramp rate of 5 °C/min, followed by a 10-min hold-up period. The injector and detector temperatures were set at 230 and 250 °C, respectively. A sample volume of 1.0 µL was injected using the split mode. FAME were identified by comparing their relative and absolute retention times to those of authentic standards. A data handling program, Chromatography Station for Windows (CSW32; Data APEX, Prague, Czech Republic), was used for quantification. The FA composition was reported as relative percentage of the total peak area.

2.4.5 Tocopherol content

Tocopherols (α, γ, and δ) were analyzed using an HPLC system following the Current Protocols in Food Analytical Chemistry method [24]. Oil (0.1 g) and 0.05 g ascorbic acid were placed in a 16 × 125 mm test tube. Ethanol (90.2%, 5 mL) and 0.5 mL 80% aqueous KOH solution were added to the test tube and vortexed for 30 s. The test tube was flushed with nitrogen, capped and incubated in a water bath (70 °C) for 30 min with periodical vortexing. The tubes were placed in an ice bath for 5 min and then 3 mL deionized water and 5 mL *n*-hexane were added and vortexed for 30 s, followed by centrifugation for 10 min at 1000 × g (room temperature). The upper hexane layer was transferred to another test tube. The aqueous layer and the residue were re-extracted by

repeating the same procedure. The upper hexane layers from both the extractions were combined and evaporated to dryness under a nitrogen stream. Of mobile phase, 1 mL was added to the tube and vortexed for 30 s to redissolve the extract and then transferred to an HPLC sample vial. A 20-µL sample was injected into a Supelcosil LC-Si column (250 × 4.6 mm; Supelco). The chromatographic separation was performed by isocratic elution with a mobile phase consisting of ethyl acetate/acetic acid/hexane (1 : 1 : 198, vol/vol/vol) at a flow rate of 1.5 mL/min. Detection was monitored at 295 nm. Tocopherols were identified by comparing the retention times with those of pure standards of α-, γ-, and δ-tocopherols, and were quantified on the basis of peak areas of the unknowns compared with those of pure standards (Sigma Chemical Co.). Quantification was based on an external standard method. A D-500 Hitachi (Hitachi Instruments) chromatointegrator with a built-in computer program for data handling was used for quantification.

2.5 Statistical analysis

All the experiments were conducted in triplicate and statistical analysis of the data was performed by analysis of variance (ANOVA) using the statistical software Statistica 5.5 (StatSoft, Tulsa, OK, USA). A probability value at $p < 0.05$ was considered statistically significant. Data are presented as mean values ± standard deviation calculated from triplicate determinations.

3 Results and discussion

The yield of enzyme-extracted oil (28.4–32.8%) was found to be significantly ($p < 0.05$) higher than the control (26.7%), showing an enhancement of ca. 6–23% (Table 1). The highest oil content (32.8%) was found in the Viscozyme-treated samples, whereas Protex 7L produced the lowest oil yield (28.4%). The higher oil recovery with the Viscozyme L (multi-enzyme complex with a wide range of carbohydrases) can be ascribed to the relatively better solubilization of structural cell wall components of hempseed by this enzyme. Enzyme pretreatment facilitates the breakdown of the protein network surrounding the lipid bodies and also supports the conversion of the complex seed lipoprotein molecules into simple lipid and protein molecules, thereafter enhancing both the oil availability and extractability [25–27]. As expected, the different enzyme mixtures used in the present study exhibited varying extents of effectiveness towards improving the oil yield from hempseeds. This might be attributed to the different compositions of the enzyme mixtures tested. Our findings that enzyme mixtures with combined activity provide better results are also in agreement with those reported by Rosenthal *et al.* [14].

The analysis of the hempseed residues remaining after cold-pressing (with or without enzymes) revealed no signifi-

Table 1. Proximate composition of hempseeds.[§]

Parameter [%]	Enzyme-assisted					Control
	Kemzyme	Protex 7L	Viscozyme L	Feedzyme	Natuzyme	
Oil content	32.3 ± 0.5[a]	28.4 ± 0.4[c]	32.8 ± 0.3[a]	30.3 ± 0.4[b]	28.9 ± 0.5[c]	26.7 ± 0.6[d]
Protein content	24.8 ± 1.2[a]	25.2 ± 0.7[a]	24.7 ± 0.5[a]	24.9 ± 1.4[a]	25.0 ± 0.6[a]	24.8 ± 0.7[a]
Fiber content	17.1 ± 0.6[a]	17.4 ± 0.3[a]	17.3 ± 0.4[a]	17.2 ± 0.6[a]	17.1 ± 0.5[a]	17.5 ± 0.5[a]
Ash content	5.4 ± 0.5[a]	5.4 ± 0.2[a]	5.4 ± 0.3[a]	5.4 ± 0.3[a]	5.4 ± 0.4[a]	5.4 ± 0.3[a]

[§] Values are means ± SD, calculated as percentage on dry seed weight basis for three hempseed samples for each enzyme, analyzed individually in triplicate.
Mean values in the same row followed by the same superscript letter are not significantly different ($p > 0.05$).

cant ($p > 0.05$) variation in protein, fiber and ash contents from those of control. These results were in agreement with those of Concha *et al.* [28] who reported that enzyme-assisted extraction had no noticeable effects on the amounts of protein, crude fiber, and ash in rosehip seeds. The hempseed meal, due to its important amino acid and carbohydrate profiles, may be explored as a potential source of protein and other nutrients for human and poultry diets [5]. It has been reported that hempseed proteins, primarily composed of edestin and albumin (components of human blood plasma), are readily digestible [2, 5].

The physicochemical characteristics determined for the enzyme-extracted and control hempseed oils are given in Table 2. No significant ($p > 0.05$) differences were observed for the values of iodine number (152–158 g I/100 g oil), refractive index at 40 °C (1.4701–1.4703), density at 24 °C (0.93 mg/mL), and unsaponifiable matter (0.24–0.27%) between the enzyme-extracted and control hempseed oils. These findings were in agreement with those of Dominguez *et al.* [29] for enzyme-assisted hexane-extracted soybean oil. A slight variation was observed in the saponification number (181–185 mg KOH/g oil), FFA content (1.73–1.87%), and peroxide value (1.54–1.62 mEq/kg oil) of the enzyme-extracted oils relative to the control. Kemzyme-produced oils exhibited relatively lower FFA contents and iodine values, whereas Natuzyme-extracted oils were noted to be good in terms of the sensory attributes. The use of the sensory score for evaluating the nutritive quality and acceptability of an oil for human consumption is well accepted [22, 30].

As far as the color of the tested hempseed oils is concerned, the enzyme-produced oils exhibited notably ($p < 0.05$) higher values (5.3–5.5 R + 49.7–50.5 Y + 4.7–4.8 B) than those for the control (4.4 R + 39.8 Y). The intense color in the present analysis of enzyme-extracted hempseed oil might be in part due to the enzyme treatment [29]. In this context, it has been reported that enzyme-extracted oils are often characterized by higher levels of coloring pigments (*e.g.* chlorophyll, carotenoids, and xanthophylls). In fact, greater amounts of coloring pigments could be released from the seed tissues as a result of enzymatic hydrolysis which increases the color intensity [30].

The oxidation parameters of enzyme-extracted and control hempseed oils are depicted in Table 3. The specific extinctions at 232 and 270 nm, which revealed the oxidative deterioration and purity of the oils [2], for the enzyme-extracted hempseed oils (3.76–3.80 and 0.58–0.62, respectively) were almost comparable with those of the control (3.78 and 0.63, respectively). The IP (Rancimat: 20 L/h, 120 °C), which is an important feature to express the oxidative stability of oils and fats [23], was higher for the enzyme-extracted (1.44–1.71 h) hempseed oils than for the control (1.35 h). This might be in part due to the relatively higher concentrations of tocopherols in the enzyme-produced oils (see Table 5). The results of the present analysis are in accordance with those of Ranalli *et al.* [30] who reported a higher IP for enzyme-extracted olive oils. Of the enzyme treatments, Kemzyme-extracted oil was found to be the most stable (IP, 1.71 h), which may be due to the presence of higher levels of δ-tocopherol (the most potent antioxidant component among the tocopherol isomers) in this oil (Table 5). No previously reported data on the oxidation parameters of enzyme-extracted hempseed oils are available in the literature to make a comparison with the present findings.

The analytical data (with respect to the control) showed that enzyme-assisted extraction had no significant ($p > 0.05$) influence on the composition and contents of FA of hempseed oil (Table 4). These results were in agreement with the findings of Concha *et al.* [28] who reported that the FA composition of rosehip seed oil was not meaningfully affected by enzyme treatment. The tested hempseed oils mainly contained linoleic (18:2*n*-6) and α-linolenic (18:3*n*-3) acids, which contributed 54.22–57.89% and 18.29–18.73% of the total FA, respectively. Furthermore, the investigated oils also exhibited small amounts of γ-linolenic acid (18:3*n*-6), accounting for 1.84–1.91%. γ-Linolenic acid has recently gained much appreciation because of its health benefits [31]. As evident, hempseed oil is a rich source of essential FA for the human diet; however, a high degree of unsaturation renders it greatly prone to oxidative rancidity. As heat or light accelerates the oxidative degradation of oils, hempseed oil is not recommended for frying or baking, although moderate heat for short periods is probably acceptable [2, 5].

Table 2. Physiochemical properties and sensory score of hempseed oils.[§]

Parameter	Enzyme-assisted					Control
	Kemzyme	Protex 7L	Viscozyme L	Feedzyme	Natuzyme	
Refractive index, 40 °C	1.4703 ± 0.02^a	1.4702 ± 0.01^a	1.4703 ± 0.02^a	1.4701 ± 0.01^a	1.4702 ± 0.02^a	1.4699 ± 0.03^a
Density, 24 °C [g/mL]	0.93 ± 0.03^a	0.93 ± 0.02^a	0.93 ± 0.01^a	0.93 ± 0.02^a	0.93 ± 0.01^a	0.93 ± 0.02^a
Saponification value [mg KOH/g oil]	183 ± 3^{ab}	181 ± 2^b	184 ± 4^a	185 ± 7^a	182 ± 2^b	186 ± 3^a
FFA content [% as oleic acid]	1.73 ± 0.04^b	1.84 ± 0.05^a	1.87 ± 0.02^a	1.79 ± 0.09^{ab}	1.86 ± 0.06^a	1.75 ± 0.07^{ab}
Iodine value [g I/100 g oil]	152 ± 5^b	154 ± 4^{ab}	155 ± 6^{ab}	158 ± 4^a	157 ± 3^{ab}	155 ± 5^{ab}
Unsaponifiable matter [wt-%]	0.26 ± 0.01^a	0.25 ± 0.02^a	0.24 ± 0.01^a	0.27 ± 0.03^a	0.26 ± 0.02^a	0.27 ± 0.02^a
Panel test (score)	7.9 ± 1.2^a	8.1 ± 0.9^a	7.7 ± 1.1^a	7.9 ± 0.7^a	8.3 ± 0.9^a	6.5 ± 0.8^b
Color (1-in. cell)						
Red units	$5.3 \pm 0.2\ R^a$	$5.5 \pm 0.2\ R^a$	$5.3 \pm 0.1\ R^a$	$5.4 \pm 0.2\ R^a$	$5.4 \pm 0.3\ R^a$	$4.4 \pm 0.1\ R^b$
Yellow units	$50.3 \pm 1.5\ Y^a$	$50.4 \pm 2.6\ Y^a$	$49.7 \pm 2.2\ Y^a$	$50.3 \pm 1.6\ Y^a$	$50.5 \pm 1.9\ Y^a$	$39.8 \pm 1.2\ Y^b$
Blue units	$4.8 \pm 0.2\ B^a$	$4.7 \pm 0.1\ B^a$	$4.8 \pm 0.2\ B^a$	$4.8 \pm 0.1\ B^a$	$4.8 \pm 0.1\ B^a$	–

[§] Values are means $\pm$ SD of three hempseed oils, analyzed individually in triplicate.
Mean values in the same row followed by the same superscript letter are not significantly different ($p > 0.05$).

Table 3. Oxidative state of hempseed oils.[§]

Parameter	Enzyme-assisted					Control
	Kemzyme	Protex 7L	Viscozyme L	Feedzyme	Natuzyme	
Conjugated diene $\varepsilon^{1\%}_{1cm}$ (λ232)	3.77 ± 0.14^a	3.76 ± 0.17^a	3.78 ± 0.12^a	3.79 ± 0.09^a	3.80 ± 0.15^a	3.78 ± 0.11^a
Conjugated triene $\varepsilon^{1\%}_{1cm}$ (λ270)	0.62 ± 0.02^a	0.58 ± 0.01^a	0.61 ± 0.03^a	0.59 ± 0.02^a	0.62 ± 0.03^a	0.63 ± 0.01^a
Peroxide value [meq/kg]	1.54 ± 0.03^c	1.57 ± 0.05^a	1.59 ± 0.06^a	1.62 ± 0.03^b	1.56 ± 0.01^c	1.57 ± 0.01^a
p-Anisidine value	2.11 ± 0.03^a	1.86 ± 0.04^{bc}	1.83 ± 0.05^c	1.88 ± 0.02^b	1.87 ± 0.02^{bc}	1.89 ± 0.03^b
Induction period Rancimat method [h]	1.71 ± 0.18^a	1.44 ± 0.12^{ab}	1.52 ± 0.07^{ab}	1.58 ± 0.13^{ab}	1.62 ± 0.09^{ab}	1.35 ± 0.08^b

[§] Values are means $\pm$ SD of three hempseed oils, analyzed individually in triplicate.
Mean values in the same row followed by the same superscript letter are not significantly different ($p > 0.05$).

Table 4. FA composition (g/100 g FA) of hempseed oils.[§]

FA	Enzyme-assisted					Control
	Kemzyme	Protex 7L	Viscozyme L	Feedzyme	Natuzyme	
16:0	6.14 ± 0.14^a	6.16 ± 0.15^a	6.11 ± 0.17^a	6.07 ± 0.13^a	5.91 ± 0.19^a	5.95 ± 0.12^a
18:0	2.24 ± 0.10^a	2.21 ± 0.04^a	2.26 ± 0.08^a	2.19 ± 0.07^a	2.31 ± 0.06^a	2.25 ± 0.06^a
18:1	12.49 ± 0.24^a	12.63 ± 0.25^a	12.74 ± 0.27^a	12.87 ± 0.22^a	12.85 ± 0.28^a	12.55 ± 0.19^a
18:2	57.45 ± 4.47^a	55.18 ± 4.66^a	54.22 ± 3.82^a	55.19 ± 3.75^a	57.89 ± 4.84^a	56.61 ± 3.21^a
18:3α	18.31 ± 0.55^a	18.56 ± 0.38^a	18.65 ± 0.67^a	18.73 ± 0.45^a	18.29 ± 0.53^a	18.38 ± 0.43^a
18:3γ	1.91 ± 0.05^a	1.86 ± 0.03^a	1.85 ± 0.07^a	1.87 ± 0.05^a	1.84 ± 0.06^a	1.85 ± 0.07^a
20:1	0.91 ± 0.04^a	0.88 ± 0.02^a	0.92 ± 0.03^a	0.89 ± 0.05^a	0.88 ± 0.04^a	0.87 ± 0.03^a

[§] Values are means $\pm$ SD of three hempseed oils, analyzed individually in triplicate.
Mean values in the same row followed by the same superscript letter are not significantly different ($p > 0.05$).

The concentrations of α-, γ-, and δ-tocopherols in the enzyme-extracted oils ranged from 35 to 53 mg/kg, 654 to 725 mg/kg, and 24.1 to 35.8 mg/kg, respectively (Table 5). The contents of α-tocopherol, which has marked vitamin E potency, and δ-tocopherol, which exhibits greater antioxidant activity than either of γ-, β- or α-tocopherols [2], were found to be appreciably higher in Kemzyme-extracted hempseed oil (53 and 35.8 mg/kg, respectively) than in the other enzyme-

Table 5. Comparison of tocopherols in hempseed oils.[§]

Tocopherols [mg/kg]	Enzyme-assisted					Control
	Kemzyme	Protex 7L	Viscozyme L	Feedzyme	Natuzyme	
α	53 ± 8[a]	42 ± 5[b]	40 ± 3[c]	38 ± 6[c]	35 ± 4[c]	43 ± 3[b]
γ	685 ± 12[b]	654 ± 16[bc]	672 ± 14[bc]	693 ± 10[b]	725 ± 13[a]	615 ± 8[c]
δ	35.8 ± 2.1[a]	28.4 ± 1.6[b]	24.7 ± 1.4[c]	24.1 ± 1.7[c]	28.4 ± 0.9[b]	33.2 ± 1.5[ab]
Total	773.8 ± 28.6[a]	724.4 ± 35.4[ab]	736.7 ± 42.8[ab]	755.1 ± 38.1[ab]	788.4 ± 32.8[a]	691.2 ± 45.3[b]

[§] Values are means ± SD of three hempseed oils, analyzed individually in triplicate.
Mean values in the same row followed by the same superscript letter are not significantly different ($p > 0.05$).

extracted and control oils. On the other hand, the Natuzyme-produced oil, although lower in α-tocopherol (35.6 mg/kg) had the highest level of γ-tocopherol (725 mg/kg). We also observed that the values of γ-tocopherol were substantially higher in all the oils produced with enzyme adjuvant, revealing the efficacy of the tested enzymes towards a greater release of this particular tocopherol isomer.

Overall, the enzyme-extracted hempseed oils in the present analysis were significantly ($p < 0.05$) richer in total tocopherols (724.4–788.4 mg/kg) than the control (691.2 mg/kg), showing an enhancement of ca. 5–14% in the total tocopherols, which may be attributed to the enzymatic pretreatment. Our findings are in accordance with the investigations of Ranalli *et al.* [30] who reported that the use of enzyme during olive oil extraction resulted in a higher release of tocopherols. The use of enzymatic preparations containing cell wall-degrading enzymes during seed extraction results in the release of greater amounts of tocopherols and phenolics due to hydrolysis of the seed cell wall, resulting in a higher availability of such bioactive components in the oil [30, 32]. In some cases, the increase in the amounts of tocopherols and other bioactive components in enzyme-extracted oils could be attributed to a reduced complexation of such compounds with the seed polysaccharides and, consequently, enhanced partitioning into the oil phase [33]. As with many of the other traits, no previously reported data on the tocopherol contents of enzyme-extracted hempseed oils are available in the literature with which to compare the results of our present analysis.

4 Conclusions

The results of the present analysis, with respect to the control, showed that the oil yield obtained for hempseeds was meaningfully enhanced by the EACP method, without affecting the FA composition of the oil produced. Nevertheless, the tocopherol concentrations in the enzyme-extracted oils were improved. Although Viscozyme L proved to be the best enzyme to enhance the oil recovery, Kemzyme and Natuzyme also offered fairly good oil quality in terms of improved tocopherols levels, oxidative stability and sensory attributes. This

suggests the use of EACP as an alternative method to conventional cold-pressing for the extraction of oil from hempseeds.

Conflict of interest statement

The authors have declared no conflict of interest.

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