



Preparation and characterization of protein isolate and biodiesel from garden cress seed

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ABSTRACT

Garden cress seeds were analyzed for proximate composition and mineral content. The protein isolate (PI) was evaluated for chemical composition, mineral content, amino acid composition, biological values and some functional properties. The seed oil was evaluated for fatty acid profile; tocopherols and some of physicochemical properties were studied. Seeds were found to contain 29.11% oil and 25.12% protein. Phosphorus, magnesium and calcium constituted the major minerals of the seeds. The protein efficiency ratio (PER) of PI was 2.37. PI was found to possess relatively high values of water and fat absorption capacity, emulsification capacity and foam capacity. The fatty acid profile showed that linolenic acid (41.17%) and oleic acid (26.42%) were the major unsaturated fatty acids, whereas palmitic acid was the major saturated acid. Total tocopherol content of garden cress oil was 150.46 mg/100 g oil. The physicochemical characteristics of oil and biodiesel meet the norm specifications.

1. Introduction

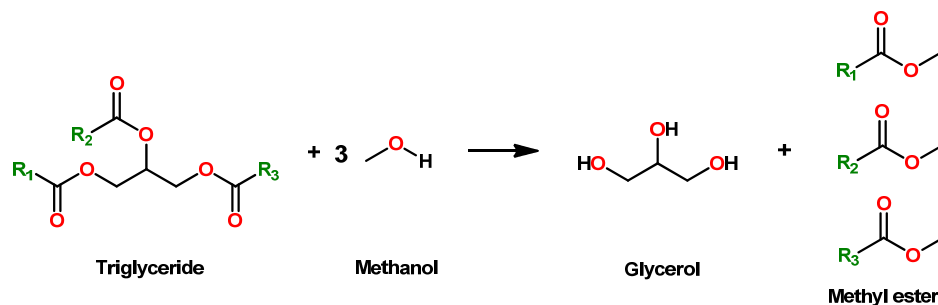
Garden cress (*Lepidium sativum* Linn.) is an annual herb, belonging to *Brassicaceae* family that is native to Egypt and west Asia but is widely cultivated in hot temperate climates throughout the world for various culinary and medicinal uses [1]. Garden cress (Gc) is a fast-growing edible plant. Seeds, leaves and roots of Gc are of economic importance; however, the crop is mainly cultivated for seeds. Garden cress is an important medicinal crop in India [2]. Garden cress seeds are brownish red in color and oval in shape. Morphologically, Gc seeds resemble that of an oil seed with the dicotyledonous endosperm accounting to 80-85% of the seed matter, the seed coat account for 12-17% and the embryo for 2-3% of the seeds, respectively. The Gc seeds are bitter, thermogenic, depurative, rubefacient, galactagogue, tonic, aphrodisiac, ophthalmic, antiscorbutic, antihistaminic and diuretic. They are useful in the treatment of asthma, coughs with expectoration, poultices for sprains, leprosy, skin disease, dysentery, diarrhoea, splenomegaly, dyspepsia, lumbago, leucorrhoea, scurvy and seminal weakness [3]. The seeds contain 25% of protein, 14-24% of lipids, 33-54% of carbohydrates and 8% of crude fiber [4,5]. The carbohydrates of the garden cress seeds comprise of 90.0% non-starch polysaccharides and 10% of starch. The seed bran has high dietary fiber content and also it has high water holding capacity. Gc bran can be used as a rich source of dietary fiber [1]. Garden cress seed contains 20-25% yellowish semidrying oil and the major fatty acid in it is alpha linolenic acid (32-34%) [6]. Mixing garden cress oil (GcO) with linseed oil led to significant increase in the oxidative stability of linseed oil [7]. GcO has a balanced amount of polyunsaturated fatty acids (PUFA) (46.8%) and monounsaturated fatty acids (MUFA) (37.6%) and also contains natural antioxidants viz., tocopherols and carotenoids which protect the oil from rancidity [6]. In this respect, [8] reported that erucic acid

(C22:1; 3.0 wt%), stearic acid (C18:0; 2.8 wt%), and arachidic acid (C20:0; 2.3 wt%) were among the minor fatty acids found in garden cress oil. The effect of erucic acid has been proven in the transient decrease in platelet counts and increase in platelet size in newborn piglets fed canola oil [9]. Similar findings regarding the effect of erucic acid on platelet counts has been reported in human studies on patients with adrenoleukodystrophy [10].

The idea to use vegetable oils as fuels is more than a hundred years old. Rudolf Diesel conducted an engine tests on plant oils at the World's Exhibition in Paris in 1900 [11]. Biodiesel, defined as mono-alkyl (methyl or ethyl) esters produced from plant oils and animal fats [12] by transesterification reactions, plays a very important role as an alternative to conventional diesel. Transesterification, also called alcoholysis [13-15], has been widely used to reduce the viscosity of triglycerides and produce biodiesel.

The biodiesel transesterification reaction is very simple (Scheme 1). This is an equilibrium reaction where an organic oil, or triglyceride, can be processed into biodiesel, usually in the presence of a catalyst, and alkali such as potassium hydroxide [15,16]. Biodiesel, which is produced from vegetable oils, animal fats or waste cooking oils has been shown to yield lower emissions of particulate matter (PM), unburned hydrocarbon, carbon dioxide, carbon monoxide, polycyclic aromatic hydrocarbons (PAH), and nitric polycyclic hydrocarbons (nPAH) than petrodiesel [17].

The main goal of the current investigation was to evaluate the physicochemical properties of garden cress oil and its biodiesel. Moreover, the quality of garden cress protein isolate was determined by evaluating the chemical composition, amino acid composition, biological values and some of functional properties of protein isolate.



Scheme 1

2. Experimental

2.1. Materials

Garden cress (*Lepidium sativum* Linn.) seeds were obtained from Harraz for Spices and Herbs Co. Cairo, Egypt. All reagents and chemicals were of analytical-reagent grade, and deionized water was utilized throughout this work.

2.2. Methods

2.2.1. Oil extraction

The extraction of oil of garden cress seeds was carried out by using chloroform:methanol (2:1, v:v) method according to the method described by Bligh and Dyer [18].

2.2.2. Biodiesel production and purification

Garden cress oil was used as the raw oil to react with methanol in a molar proportion of 1:6 to produce biodiesel. Sodium hydroxide of 1% on a mass base of the garden cress oil was used as the catalyst and pre-mixed with methanol by a mechanical stirrer. This mixture was thereafter added into a reacting tank to be mixed with the garden cress oil using a mechanical homogenizer in order to undergo transesterification reaction. The reaction temperature of the transesterification process was set at 60 °C in order to prevent the vaporization of the methanol from the reacting mixture during the biodiesel production process. The reaction was allowed to proceed for 50 min. At the end of the incubation, the mixture was transferred into a separatory funnel, left for 24 h and then the biodiesel separated from the glycerol. The biodiesel was then washed with hot deionized water (50 °C) five times to remove the glycerol, catalyst and other impurities [19-21]. The percent biodiesel yield was calculated by relating the biodiesel volume to that of the oil that underwent transesterification.

2.2.3. Preparation of garden cress protein isolate

The garden cress protein isolate was prepared by an isoelectric point precipitation technique as described by Ruiz and Hove [22] and Abbott [23]. The dried defatted seed suspended in distilled water containing 0.25% Na₂SO₃ at 1:100 (w:v) ratio. The mixture was magnetically stirred for 1 h while adjusting the pH to 9.0 with NaOH solution (4 M). Then, the mixture was centrifuged at 3500 rpm for 15 min at room temperature. The supernatant was transferred into a beaker, stirred for another 30 min, and the pH adjusted to 4.8 by the addition of HCl solution (1.0 N). The supernatant was left undisturbed for cold precipitation overnight at 4 °C. Then, the supernatant was carefully siphoned off and the protein slurry was washed three times with distilled water by centrifuging at

3500 rpm for 20 min. The precipitate was recovered by centrifugation at 3500 rpm for 30 min. The precipitate was washed by distilled water several times, and then neutralized by 1.0 M NaOH to pH = 7. The neutralized precipitate was dried at 50 °C for 48 h in an air convection oven, ground and passed through a 150 μm mesh sieve.

2.2.4. Analytical methods

Moisture, crude oil, crude protein (N x 6.25), crude fiber and ash of samples were determined as described in AOAC official methods [24].

The total carbohydrate content (on dry weight basis) was calculated by the difference (100 - (% protein + % lipid + % ash + fiber)).

The minerals, i.e., Ca, Fe, Zn, K, Cu, P, Mg and Na were determined in a dilute solution of the ashed samples by atomic absorption spectrophotometer (3300 Perkin-Elmer) as described in AOAC official methods [24].

Amino acids were determined according to method of Moore and Stein [25]. Hydrolysis of the samples was performed in the presence of 6 M HCl at 110 °C for 24 h under a nitrogen atmosphere. Sulfur-containing amino acids were determined after performic acid oxidation. Tryptophan was chemically determined by the method of Miller [26].

The amino acid score (AAS) was calculated for each essential amino acid using the FAO/WHO/UNU [27] reference protein as follows:

$$\text{AAS} = \frac{\text{Concentration of essential AA in the protein under test}}{\text{Concentration of essential AA in the FAO WHO/UNU standard}} \times 100 \quad (1)$$

Values for AAS lower than 100 indicate a deficiency of that amino acid. The limiting amino acid (LAA) was defined as that showing the lowest AAS value [28]. Protein efficiency ratio (PER) was estimated using the regression equation proposed by Alsmeyer [29]:

$$\text{PER} = -0.468 + 0.454 \times \text{Leucine} - 0.105 \times \text{Tyrine} \quad (2)$$

2.2.5. Protein properties

Water absorption (expressed as the amount of water retained by 100 g of protein) and fat absorption capacities (expressed as mL of oil absorbed by 100 g of protein) were estimated according to the procedure of Sosulski [30] and Sosulski [31], respectively. Emulsification capacity (mL oil/g protein at pH = 9) was determined as described by Beuchat [32]. The method of Lawhon [33] was used to determine the foam capacity at pH = 9.0 using 2% protein solution. The percentage increase in volume after 30 sec was recorded as foam capacity.

Table 1. Chemical composition (g) and mineral contents (mg) of garden cress seed (GcS), defatted flour (GcDF) and protein isolate (GcPI) per 100 g (dry weight basis).

Component (g/100 g)	(GcS) ^a	(GcDF) ^a	(GcPI) ^a	LSD (p = 0.05) ^e
Moisture	5.29±1.03 ^d	6.53±0.85	7.21±0.70	1.7392
Crude protein ^b	25.12±2.64	45.30±2.16	86.90±5.66	7.6226
Crude oil	29.11±1.91	1.60±0.18	0.65±0.08	2.2148
Crude fiber	5.70±1.02	9.68±0.45	1.90±0.16	1.2991
Ash	4.25±0.52	6.27±0.36	1.64±0.23	0.7415
Total carbohydrate ^c	35.82±4.65	37.15±3.99	8.91±0.87	7.1385
Mineral contents (mg/100 g)				
Potassium	1251.05±3.06 ^d	1671.10±2.78	366.11±1.85	5.2244
Phosphorus	610.63±2.15	815.42±0.98	165.00±3.14	4.5328
Magnesium	346.21±3.06	495.22±0.56	85.60±1.04	3.7835
Calcium	340.35±2.03	485.24±0.78	92.08±0.77	2.6610
Sodium	40.90±0.61	72.55±1.49	11.20±0.29	1.8870
Iron	18.31±1.12	31.25±0.60	6.28±0.41	1.5400
Copper	16.10±1.66	26.23±1.53	4.55±0.31	2.6207
Zinc	13.75±0.11	24.25±0.29	6.45±0.58	0.7586

^a p ≤ 0.05.^b Crude protein = N (%) × 6.25.^c Carbohydrate content obtained by subtracting the sum of moisture, crude protein, oil, crude fibre and ash from 100%.^d Data are expressed as mean ± SD. Values given represent means of three determinations.^e LSD: Least different significantly at p ≤ 0.05 according to Duncan's multiple range test.

2.2.6. Fatty acid composition

Capillary gas chromatography (HP 6890) was used for the qualitative and quantitative determinations of the fatty acids of the oil samples and reported in relative area percentages. Fatty acids were transesterified into their corresponding fatty acid methyl esters (FAMES) by shaking a solution of oil (ca. 0.1 g) in heptane (2 mL) with a solution of methanolic potassium hydroxide (0.2 mL, 2N). The FAMES were identified using a gas chromatograph equipped with DB-23 capillary column (60 m, 0.32 mm i.d., 0.25 µm thickness) and a flame ionization detector. The nitrogen flow rate was 3 mL/min; hydrogen and air flow rates were 40 and, 450 mL/min, respectively. The oven temperature was programmed from 150 to 170 °C at a rate of 10 min, then raised to 192 °C at a rate of 5 °C/min kept isothermal for 5 min and then raised again to 220 °C at a rate of 10 °C/min and kept at this temperature for 3 min. The injector and the detector temperatures were 230 and 250 °C, respectively. Fatty acid methyl esters FAMES were identified by comparing their retention times with a known fatty acid standard mixture. Peak areas were automatically computed by an integrator.

2.2.7. Tocopherol content

Tocopherols were quantified by HPLC according to AOCS official method Ce 8-89 [34]. Samples were dissolved in hexane to a concentration of 50-100 mg/mL, filtered through 0.45 µm centrifugal filters and analyzed by a Varian HPLC Pro-Star model 230 pump, model 410 autosampler, and model 363 fluorescence detector using excitation and emission wavelengths of 290 and 330 nm, respectively. The mobile phase consisted of hexane:2-propanol (99.5:0.5, v:v, made fresh daily) pumped at a rate of 1 mL/min. Samples were injected by autosampler using the full loop option (100 µL), and tocopherols were separated using an Inertsil (Varian) silica column (5 µm, 150A 250mm×4.6mm i.d.). Tocopherol peaks were identified by comparison to the retention times of known reference standards. A mixture of α, β, γ and δ tocopherol standards was injected on each day of analysis to verify HPLC response. Samples were quantified using external standard curves. Each determination was run in triplicate and mean values are reported.

2.2.8. Determination of the physicochemical properties of garden cress oil and biodiesel

Moisture content, acid value, peroxide value, iodine value, saponification value and refractive index were determined

according to AOAC official methods [24]. Viscosity was determined as described by Saguy *et al.* [35]. The specific gravity was measured in a 25 mL capacity specific gravity bottle at room temperature of 25 ± 2 °C [36].

2.2.9. Statistical analysis

Data are expressed as mean ± standard deviation (SD) of three replicates. The data were analyzed by analysis of variance (ANOVA) according to the procedures outlined by Gomez and Gomez [37]. Duncan's multiple range test was used to determine the differences among samples. Significant levels were defined as probabilities of 0.05 or less.

3. Results and discussion

3.1. Proximate composition of garden cress seed, meal and protein isolate (dry weight basis)

Data presented in Table 1 show the proximate composition of garden cress seed, defatted flour (meal) and protein isolate per 100 g (dry weight basis). The moisture content of seed flour was 5.29 ± 1.30%. This low level of moisture content is an index of stability, quality and increased shelf life of seeds [38]. Whole seeds had significantly (p < 0.05) the highest level of crude oil 29.11%, which was higher than that reported (22.7 wt%) in a previous study [8], while the lowest level of crude oil was observed in protein isolate 0.65%. On the other hand, garden cress meal and protein isolate had significantly the higher value of protein 45.30 and 86.90%, respectively, the process of defatting doubled the protein content. The protein content of the garden cress protein isolate was approximately 3.45 and 1.91 times as high as the protein content of the whole seeds and meal, respectively. The highest (p < 0.05) value of crude fiber was recorded for garden cress meal 9.68%. While protein isolate had significantly (p < 0.05) the lowest one 1.90%. Available literature on composition of garden cress seed show that the seeds contain 25% of protein, 14-24% of lipids, 33-54% of carbohydrates and 8% of crude fiber [4,5,39]. Proximate composition varies depending upon plant variety, agronomic practices, stage of collection of seeds and climatic and geological condition of area from where seeds are collected [40]. The obtained results indicate that garden cress seed had significantly the highest values of fat and protein component which motivated the author to undertake in-depth studies on both types of biomolecules.

Mineral contents of garden cress seed, defatted flour (meal) and protein isolate (mg/100 g dry weight basis) are presented in Table 1.

Table 2. Amino acid compositions and protein nutritional quality of garden cress seed protein isolate.

Essential amino acid	Protein isolate Amino acid content (g/100 g)	FAO/WHO/UNU (1985) *	Chemical score	Non-essential amino acid	Protein isolate g/100 g protein
Histidine	3.62	1.4	258.50	Arginine	3.64
Isoleucine	4.77	4.00	119.25	Alanine	5.20
Leucine	6.95	7.04	98.70	Aspartic acid	12.71
Lysine	6.86	5.44	126.10	Glutamic acid	25.01
Methionine	0.71	-	-	Glycine	5.00
Cystine	0.19	-	-	Proline	4.87
Total sulfur amino acids	0.90	3.52	25.56	Serine	4.19
Phenylalanine	5.63	-	-	-	-
Tyrosine	3.01	-	-	-	-
Total aromatic amino acid	8.64	6.80	127.05	-	-
Threonine	4.62	4.00	115.50	-	-
Valine	6.20	3.50	117.14	-	-
Tryptophan	1.82	0.96	189.58	-	-
Total essential amino acid	44.38	-	-	Total non-essential amino acid	55.62
Chemical score	25.56%				
1 st Limiting amino acid	Methionine + Cystine				
2 nd Limiting amino acid	Leucine (98.70)				
Protein efficiency ratio (PER)	2.37				

* FAO/WHO/UNU, 1985. Energy and protein requirements. WHO Tech. Rep. Series; No 724.

Table 3. Functional properties of garden cress seed protein isolate (GcPI) *.

GcPI	Water absorption (%)	Fat absorption (%)	Emulsification capacity at pH = 9 (mL oil/g protein)	Foam capacity at pH = 9 (%)
	229.0±2.89	350.2±0.99	65.00±3.20	39.50±1.57

* Means ± SD; values are means of three determinations.

Ash content indicates that garden cress seed is an appreciable source of minerals. Potassium, phosphorus, magnesium and calcium constituted the major minerals in garden cress seed 1251.0, 610.6, 346.4 and 340.3 mg/100 g dry weight basis, respectively. Sodium, iron, copper and zinc were found in low quantities in garden cress seed. Mineral content of garden cress is in agreement with those reported earlier for *L. sativum* [8,40]. These mineral values were generally within the range reported for most grain legumes [41,42], and similar to those reported for vegetables [43]. While grain legumes represent useful sources of desirable minerals, they are usually less biologically available than those from animal origin [44]. Defatting process resulted in significant ($p < 0.05$) increase in the mineral content as compared to full fat flour. The highest levels of mineral were found in garden cress meal, while the lowest concentrations of mineral were observed in protein isolate. These findings are in agreement with those obtained by Egbekun and Ehieze [45] who reported that defatting of beniseed (*Sesamum indicum L.*) increased mineral contents of defatted flour.

3.2. Amino acid composition and biological values of garden cress seed protein isolate

The nutritional quality of a protein is principally governed by its amino acid composition. Table 2 shows the amino acid composition and protein nutritional quality of garden cress protein isolate. Results indicated that total essential amino acids of garden cress protein isolate formed 44.38% of the total amino acid content. With the exception of sulfur containing amino acids, every essential amino acid of garden cress protein isolate were higher than their corresponding contents in the reference protein [27] pattern for children. The amino acid profile showed that valine, lysine and leucine had the highest values 6.20, 6.86 and 6.95 g/100 g protein, respectively; however, the lowest values were recorded for methionine and cysteine 0.71 and 0.19, respectively. Therefore, garden cress protein could very well complement those protein sources that are low in valine, lysine and leucine acids. Total amount of non-essential amino acids represented 55.62% of the total amino acid content. Glutamic acid and aspartic acid were found to be the major non-essential amino acids in garden cress protein isolate 25.01 and 12.71%, respectively. There is no previous

report on amino acid composition of garden cress protein isolate; however, these findings confirmed those reported for garden cress seeds by Sharma and Agarwal [39] and Zia-Ul-Haq *et al.* [40]. These results also agreed with those reported for some legume seed proteins and oil seeds [27,46] that plant proteins are limited by sulphur containing amino acids.

The results presented in Table 2 show that histidine scored significantly ($p < 0.05$) the highest chemical protein score 258.50, based on the FAO/WHO/UNU [27] reference. The results also indicated relatively high chemical protein scores 127.05, 126.10, 189.58, 119.25, 117.14, 115.50 and 98.70 for total aromatic amino acids, lysine, typtophan, isoleucine, valine, threonine and leucine, respectively. In contrast sulfur containing amino acids (Methionine + Cystine) had significantly the lowest score 25.56 and were predicted as the first limiting amino acids of garden cress protein isolate. These findings agreed well with those reported by Sharma and Agarwal [39] and Zia-Ul-Haq *et al.*, [40], who showed that methionine and cysteine recorded the lowest values of essential amino acids for garden cress seeds.

The protein nutritional quality parameters of garden cress protein are presented in Table 2. Sulfur containing amino acids and leucine were the first and second limiting amino acids, 25.56 and 98.70, respectively. This, therefore, implies that any dietary formulation involving this protein feed/food resource would be mindful of the dietary supply of these amino acids. Protein efficiency ratio (PER) of garden cress protein was 2.37. Similar PER values were reported by Saleh and El-Adawy [47] and Khattab *et al.*, [48] for chickpea and Egyptian cowpea. The high levels of essential amino acids of garden cress seed protein explains the high biological values of garden cress protein

3.3. Functional properties of garden cress seed protein isolate

The water absorption capacity (WAC) of garden cress protein isolate was of 229 mL H₂O/100 g (Table 3). This is likely due to the fact that the protein isolate has a great ability to swell, dissociate and unfold exposing additional binding sites, whereas the carbohydrates and other non-protein components may impair it [49].

Table 4. Fatty acid compositions and tocopherols of garden cress seed oil.

Fatty acid (%) *	Garden cress oil
C8:0 Caprylic acid	Not detected
C10:0 Capric acid	Not detected
C12:0 Lauric acid	Not detected
C14:0 Myristic acid	Not detected
C16:0 Palmitic acid	9.10
C16:1 Palmitoleic acid	0.16
C18:0 Stearic acid	4.40
C18:1 Oleic acid	26.42
C18:2 Linoleic acid	8.64
C18:3 linolenic acid	41.17
C20:0 Arachidic	3.57
C20:1 Arachidonic acid	Not detected
C22:0 Behenic acid	Not detected
C22:1 Erucic acid	6.56
SAFA	17.07
MUFA	33.15
PUFA	49.81
Tocopherols (mg/100 g)	
α -Tocopherol	18.49±0.86
β -Tocopherol	Not detected
γ -Tocopherol	115.47±2.79
δ -Tocopherol	16.50±1.14
Total	150.46

* SAFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

The WAC value obtained for garden cress protein isolate is similar to those values were obtained by El-Adawy *et al.*, [50] and Ogunwolu *et al.*, [51] for bitter lupin protein isolate and cashew nut, respectively (2.12 and 2.20 mL H₂O/g), processed by the same method. High WAC of garden cress protein isolate makes this protein a potential ingredient in meat, bread, and cakes industries. Oil absorption capacity (OAC) of garden cress protein isolate was found to be 3.50 mL oil/g (Table 3), and was similar to that was obtained by El-Adawy *et al.*, [50] for bitter lupin protein isolate (3.73 mL oil/g). While this OAC value was lower to that of cashew nut (4.42 mL oil/g), as reported by Ogunwolu *et al.*, [51]. According to Kinsella [49], the ability of protein to bind fat is very important for such applications as meat replacement and extenders, principally because it enhances flavour retention, and reputedly improves mouth feel. Also, high OAC of garden cress protein isolate makes it a good ingredient for the cold meat industry, particularly for sausages, where the protein can bridge the fat and water in these products. Proteins generally possess hydrophobic and hydrophilic properties due to different types of amino acids. This causes protein interaction with both oil and water molecules and the proteins can act as emulsifiers. Emulsion capacity depends on the hydrophobic-hydrophilic balance, which is affected by pH. At pH = 9 emulsifying capacity index (ECI) of garden cress protein isolate was found to be 65.0 mL oil/g protein (Table 3). The ECI value of Garden cress protein isolate was comparable to that of *Lupinusmutabilis* seed flours (55 mL oil/g) processed by the same method as reported by Sathe *et al.* [52]. While the ECI of the garden cress protein isolate is lower to that of bitter lupin protein isolate (91.6 mL oil/g), as reported by El-Adawy *et al.*, [50]. At pH = 9 foam capacity index (FCI) of garden cress protein isolate was found to be 39.50 % (Table 3). El-Adawy *et al.* [53], El-Adawy *et al.*, [50] and Ogunwolu *et al.*, [51] found a similar observation for foam capacity of citrus seed flours, bitter lupin protein isolate and cashew nut, respectively. Foam formation is governed by three factors; including transportation, penetration and reorganization of the molecule at the air-water interface. Therefore, to exhibit good foaming, a protein must be capable of migrating at the air-water interface, unfolding and rearranging at the interface [54]. According to Damodaran [55], the foam capacity and stability were enhanced by greater protein concentration, because this increases the viscosity and facilitates the formation of a multilayer, cohesive protein film at the interface.

3.4. Fatty acid composition garden cress oil

The results presented in Table 4 show the fatty acid (FA) composition (%) of garden cress oil. Eight FAs were identified and quantified in garden cress oil sample: palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (C20:0) and erucic acid (C22:1). Garden cress oil was characterized by the presence of high levels of poly and mono-unsaturated fatty acids of 49.81 and 33.15, respectively. The most prominent fatty acids in Garden cress oil were linolenic acid (41.17%) and oleic acid (26.42%). Moderate amounts of palmitic (9.10%), linoleic (8.64 %), erucic acid (6.56%) and stearic acid (4.40 %) were showed for garden cress oil. The oil also contained trace amounts of C16:1 (0.16 %) and C20:0 (3.57%) fatty acids. Saturated fatty acids (SFA) represented 17.06 % of total fatty acids for garden cress oil. The content of PUFA was significantly higher than MUFA and SFA content. The results showed that the chromatographic peak areas of linolenic (C18:3) and stearic (C18:0) fatty acids were markedly higher than those obtained by Zia-Ul-Haq *et al.*, [40]. In the same time, the presence of erucic acid (C22:1) in garden cress seeds was not reported by Zia-Ul-Haq *et al.*, [40]. On the other hand, the obtained fatty acid profiles for garden cress oil were similar to those reported by [6].

3.5. Tocopherol content

Tocopherols and tocotrienols are derivatives of 2-methyl-6-chromanol with a side chain of three terpene units attached at C2. The terpenoid side chain is in saturated form for tocopherol and in unsaturated form for tocotrienols. Both tocopherols and tocotrienols are further divided into individual compounds that are designated by the Greek letter prefixes α , β , γ , δ depending on the number and position of methyl substitution on the chromanol ring [56]. Tocopherols are recognised as the principal natural antioxidants in vegetable oils [57]. Table 4 shows the levels of tocopherols in Garden cress seed oil. Total tocopherol content of garden cress oil was 150.46 mg/100 g. The γ -tocopherol was found to be the predominant tocopherol in garden cress oil, represented 76.74 % of the total tocopherol content. γ -Tocopherol content in vegetable oil is positively correlated with the amount of α -linolenic present in it [58]. These findings are consistent with those obtained by Diwakar *et al.*, [6] who reported that γ -tocopherol is found to be the predominant tocopherol in garden cress seed oil.

Table 5. Physicochemical properties of garden cress (*Lepidumsativum* Linn.) oil and biodiesel^a.

Physicochemical parameters	Garden cress oil ^b	Garden cress biodiesel ^b	LSD ($p = 0.05$) ^c
Moisture content, %	Not detected	Not detected	-
Refractive index at 25 °C	1.4720±0.0004	1.4700±0.0007	0.0012
Specific gravity (g/mL)	0.90±0.11	0.85±0.16	0.3112
Viscosity (mPa/s at 30 °C)	57.4±2.10	8.50±0.60	3.500
Acid value (mg KOH/g Oil)	1.60±0.23	0.21±0.04	0.374
Peroxide value (meq. O ₂ /kg)	5.08± 0.28	0.90±0.10	0.470
Saponification value (mg KOH/g)	180.21±1.65	182.10±1.99	4.143
Iodine value (g I ₂ /100 g oil)	131.5±1.50	128.00±1.86	3.830
Biodiesel yield %	90.00±2.33	-	-

^a Data are expressed as mean ± SD. Values given represent means of three determinations.

^b $p \leq 0.05$.

^c LSD: Least different significantly at $p \leq 0.05$ according to Duncan's multiple range test.

3.6. Physicochemical properties of garden cress oil and biodiesel

Physicochemical properties of garden cress oil and Biodiesel are shown in Table 5. Moisture content of oil and biodiesel was not detected (Table 5), which reflects the quality of oil and biodiesel. Hydrolysis of ester bonds in lipids (lipolysis), resulting in the liberation of free fatty acids, may be caused by enzymatic action or by heat and moisture [57]. The refractive index shows the degree of unsaturation and presence of unusual components such as hydroxyl groups [58]. The refractive index of garden cress oil was 1.4720 which is within the range of edible oils [59].

Based on previous studies on storage stability of biodiesel [60-62], iodine value, peroxide value and the acid number can be used as the indicators of the storage stability of biodiesel. The acid value of garden cress oil was 1.60 mg KOH/g oil, this value is in conformity with the specifications of edible oils [59]. The maximum level of acid value of refined; cold pressed and virgin oils were 0.6 and 4.0 mg KOH/g oil, respectively [59]. Determination of total acid number is an important test to assess the quality of a particular biodiesel. It can indicate the degree of hydrolysis of the methyl ester, a particularly important aspect when considering storage and transportation as large quantities of free fatty acids can cause corrosion in tanks [63]. Low level of acidity was observed for biodiesel 0.21 mg KOH/g oil. Acid number (mg KOH/g oil) for biodiesel should be lower than 0.50 mg KOH/g in both EN 14214 and ASTM D6751 standard fuels. The high FFA content (>1%; w:w) will happen soap formation and the separation of products will be exceedingly difficult, and as a result, it has low yield of biodiesel product [64]. The iodine value can be used to represent the amount of unsaturated fatty acids in oil. A higher iodine value indicates a higher content of unsaturated fatty acids in oil. The iodine value of the garden cress oil, which was used as the raw oil for the biodiesel production, was 131.5 g I₂/100 g oil. The iodine value of the biodiesel obtained from garden cress oil was 128.0 g I₂/100 g oil. No statistically significant ($p > 0.05$) differences were found between the oil and its biodiesel for iodine values. Since the iodine value is only dependent on the origin of the vegetable oil, the bio-diesel esters made from the same oil should have similar iodine values [19]. The peroxide value is an indicator of the extent of peroxidation of fatty acid oil at its initial stage of storage. Peroxide value (PV) of Garden cress oil was 5.08 meq. O₂/kg, which is less than 10 meq O₂/Kg, and therefore within the acceptable value range for fresh oil [59]. The peroxide value of the biodiesel obtained from garden cress oil was 0.90 meq O₂/kg, the low PV of garden cress biodiesel indicates that it is less prone to oxidative rancidity. The saponification value indicates the amount of saponifiable units (acyl groups) per unit weight of oil. A high saponification value indicates a higher proportion of low molecular weight fatty acids in the oil or vice versa [6]. Saponification value is used for measuring the average molecular weight of oil and expressed in milligrams of potassium hydroxide (mg KOH/g oil). The saponification value

of garden cress oil and biodiesel were 180.21 and 182.10 mg KOH/g, respectively, indicating that the oil and biodiesel of garden cress seed contained high molecular weight mass fatty acids (Table 5). Saponification value is related to the average molecular weight of the sample, but the acids that are present in glycerides or in methyl esters are the same. Only the change of glycerol by methanol is produced. In consequence, the average molecular weight does not change significantly and so it may not be observed changes in the saponification value. Viscosity value of garden cress oil was 57.4 mPa.s, while the biodiesel obtained from garden cress oil had significantly ($p < 0.05$) the lower value 8.50 mPa.s. Converting vegetable oils to biodiesel decreases the viscosity and increases the volatility allowing them to be used in diesel engines [65]. The specific gravity of garden cress oil was 0.90 g/mL, while that of the biodiesel obtained from garden cress oil was 0.85 g/mL, which confirms with ASTM D6751 standard.

The significant ($p \leq 0.05$) decreases in viscosity, acid and peroxide values for biodiesel could be explained by the positive effects of purification and washing processes during the preparation of biodiesel and by the fact that polar molecules dissolve best in polar solvents. Polar molecules can hydrogen bond with polar solvents, such as water, hence increasing their solubility. As shown in Table 5, the biodiesel yield from garden cress oil was 90%. This was similar to those reported by Galadima *et al.*, [66]; Kywe and Myao, [67]. The results of quality assessment tests performed on the biodiesel obtained from garden cress oil revealed that most of the fuel specifications comply with ASTM (American Society for Testing Materials) D 6751 requirements (Table 5).

4. Conclusions

In conclusion, the results of this study showed that garden cress (*Lepidumsativum* L.) was rich in oil and protein and, considering its fatty acid profile, it lies in linolenic-oleic group. Moderate amounts of erucic acid (6.56%) were showed for garden cress oil. The oil can be considered as potential, alternate and non-conventional oil and can be used as a new and valuable source of biodiesel. Protein isolated from garden cress seed showed high protein content (86.90 %). High WAC of garden cress protein isolate makes this protein a potential ingredient in meat, bread, and cakes industries. Also, high OAC of garden cress protein isolate makes it a good ingredient for the cold meat industry, particularly for sausages, where the protein can bridge the fat and water in these products. Therefore, the protein isolated from garden cress seed could be a desirable food ingredient and can be used as nutrient substitution or supplementation and as functional agent in food systems.

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