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ORIGINAL PAPER

Full Characterisation of *Crambe abyssinica* Hochst. Seed Oil

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Abstract This work was dedicated to reporting the full chemical and physical characterisation of *Crambe abyssinica* Hochst. seed oil. The oil from the seeds was extracted using *n*-hexane. The seeds contain about 30 % oil. Density, refractive index, colour, smoke point, viscosity, acidity, saponification value, iodine value, fatty acid methyl esters, the relative position of fatty acids in C_1 and C_3 carbon glycerol, sterols, tocopherols, peroxide value, $E^{1\%}_{1cm}$ at 232 nm, and the susceptibility to oxidation measured by the Rancimat method were determined. The oil was found to contain high levels of unsaturated fatty acids, especially C22:1 (63.77 %). The dominant saturated acid was C22:0 (2.14 %). The oil was also found to contain high levels of β-sitosterol (51.93 %), campestanol (21.98 %), and brassicasterol (12.35 %). α-, γ-, and δ-Tocopherols were detected up to levels of 7.67, 125.04, and 3.99 mg/kg, respectively. The induction period (at 110 °C and 20 l/h) of the oil was 8.83 h. The relative position of fatty acids in C_1 and C_3 position was as follows: linoleic 0.45 %, oleic 8.84 %, and erucic 90.72 %. The thermal profile of the oil presented a single peak at −20.94 °C.

Keywords *Crambe abyssinica* seed oil · Oil characterisation · Relative position of fatty acids in triglycerides · Sterols · Susceptibility to oxidation · Thermal profile

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Introduction

Crambe (*Crambe abyssinica*) is an oilseed crop that belongs to the Brassicaceae family. Crambe is a plant distributed in the Abyssinian foothills and the North African plains. It grows as a weed in cultivated fields, under trees, and in the bush along the borders of field paths, generally singly and not forming continuous growth [1]. As indicated by the same authors, the fruit (about 530–1,840 fruits/plant) is of pale yellow colour, containing a single spherical seed, greenish-brown in colour, and about 0.8–2.55 mm in diameter.

Crambe seeds contain a high percentage of glucosinolates, which, unmodified or after enzymatic hydrolysis by myrosinase, can negatively affect the nutritional quality of defatted proteinic meal in livestock feeding. For these compounds and, in particular, their degradation products, there are some potential interesting applications after meal detoxification by glucosinolate removal and isolation [2]. Non-edible uses include addition to mineral lubricants, in the manufacture of greases, and as a mould lubricant in steel casting. Fully hydrogenated crambe oil is a white solid that has potential value as a component of wax compositions. However, a greater potential use exists in the industrial field for erucic acid products. Crude erucic acid is obtained by saponification of crambe oil. This erucic acid yields two primary products on ozonolysis, the 13-carbon atom dibasic brassylic acid and the 9-carbon atom monobasic pelargonic acid [2]. Brassylic acid is used in the manufacture of polyester, plasticizers, alkyd resins, lubricants, rubber additives, surface-active agents, new

types of nylon, and other polymers [1, 3]. Pelargonic acid finds use in the field of plasticizers, alkyd resins, vinyl stabilisers, hydrotropic salts, pharmaceuticals, synthetic flavours, and flotation agents [1]. Otherwise, the primary market for high-erucic acid oils is erucamide, a slip agent critical to the manufacture and use of polyolefin films. Films such as polyethylene are produced commercially for familiar products such as bread wrappers, shopping and garbage bags, shrink wraps, and plastic sheeting [3].

There are many studies concerning the cultivation of *Crambe abyssinica*. Its cultivation probably started in the 1930s in the former USSR [4]. Since then, the crop has extended to Poland, Sweden, the US, and The Netherlands. At the same time, new, improved varieties were introduced [4]. In 2000, *C. abyssinica* was cultivated in America, Canada, several European countries, Pakistan, and India, and it was introduced to China [5] and Italy [6], with a yield of up to 900–1,130 kg of oil per hectare [4, 6]. However, as indicated by Carlsson et al. [7], crambe is still under development as an agricultural crop and is not widely grown, but due to potential uses of the seed oil for the production of many valuable products (pharmaceuticals, lubricants, plasticisers, cosmetics, surfactants, etc.), many new countries (Austria, Belgium, Denmark, France, Germany, Italy, Portugal, the UK, and Argentina) have shown a scientific interest in it [8, 9].

This work was dedicated to reporting the full chemical and physical characterisation of *Crambe abyssinica* Hochst. seed oil. Specifically, oil content, density, refractive index, colour, smoke point, viscosity, acidity, saponification value, iodine value, fatty acid methyl esters, the relative position of fatty acids in C1 and C3 carbon glycerol, sterols, tocopherol content, peroxide value, $E^{1\%}_{1cm}$ (at 232 nm), and susceptibility to oxidation (by the Rancimat method) were determined. Since *C. abyssinica* is, to the best of our knowledge, rarely cultivated in Greece (despite its promising industrial uses), this study is dedicated to fully characterising the seed oil and determining any possible differences to those of the crops cultivated in other countries.

Experimental Procedures

Seeds and Standards

The seeds of *Crambe abyssinica* Hochst. were a kindly provided by VIORYL Chemical and Agricultural Industry Research S.A. (28th km National Road Athens—Lamia, Afidnes, GR-19014, Greece).

Tocopherol standards [dl-α-tocopherol, (+)-γ-tocopherol and (+)-δ-tocopherol] were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Taufkirchen, Germany). Sterol standards were purchased from Sigma-Aldrich. Lipozyme IM 20 was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark). All other reagents (analytical and HPLC grade) were purchased from Lab-Scan (Gliwice, Poland).

Oil Extraction

The oil from the seeds was extracted with *n*-hexane (by means of a Soxhlet apparatus) and analysed immediately. The oil content was determined after evaporation of the solvent using a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany).

Physical Characteristics

For the determination of the physical characteristics, the density (at 25 °C), refractive index (at 40 °C), colour by means of CIE $L \times a \times b$ (measured using a Lovibond tintometer, The Tintometer Ltd., Amesbury, UK), smoke point (as described by British Standards Methods of Analysis [10]), and viscosity (determined as mPa s at 24 °C using a LVT E3209 Brookfield viscometer, Brookfield Viscometers Ltd., Harlow, UK) were measured.

Chemical Characteristics

Determination of the chemical characteristics was carried out by means of acidity (determined as % of erucic acid according to IUPAC [11]), saponification value (determined as mg of KOH/g of oil according AOCS method Cd 3–25 [12]), and iodine value (determined by Wijs method as g of I/100 g of oil according to Egan et al. [13]).

Fatty Acid Composition

The determination of the fatty acid composition was carried out as reported earlier by Dourtoglou et al. [14]. Specifically, fatty acid methyl esters were prepared by reaction of the oil (200 μl) with a small amount of sodium methoxide in methanol at room temperature. The reaction lasted about 5 min with gentle agitation of the mixture periodically. After the reaction was completed, 10 ml of diethyl ether was added, and the organic layer was washed three times with water, dried over Na_2SO_4, and reduced to 1 ml by heating in a water bath (40 °C). The solution obtained was then subjected to GC analysis. Analysis of methyl and butyl esters was carried out with an Agilent 7890A gas chromatograph (Agilent Technologies, California, USA), equipped with a J&W (J&W Scientific, Köln, Germany) capillary DB-23 column (0.322 mm i.d., 30 m, 0.25 μm). Helium flow rate was set at 1.72 ml/min. The

column temperature program was: 150–212 °C at 2 °C/min and 212 °C (held for 1 min) to 240 °C (held for 5 min) at 10 °C/min. The injector and detector (FID) temperatures were maintained at 220 and 280 °C, respectively.

Relative Position of Fatty Acids in Triglycerides

The determination of the relative position of fatty acids in carbons 1 and 3 of glycerol in triglycerides was determined as described earlier by Dourtoglou et al. [14]. A mixture containing 200 µl of sample (oil) and 600 µl of *n*-butyl alcohol was placed in a test tube. Lipozyme IM (Novo Nordisk A/S) (250 mg) was added, and the whole mixture was agitated. The oil was allowed to react with the above alcohol for 10 min under gentle agitation using a Vortex Genie 2 (Scientific Industries Inc., Bohemia, NY). Then, 20 ml of pentane was added, followed by 10 ml of a saturated solution of NaCl. After gentle agitation and separation of the two phases, the water phase was removed. The organic phase was extracted twice with a saturated solution of NaCl and water and dried over Na_2SO_4. Most of the solvent was evaporated on a water bath at 40 °C. Finally, the products were subjected to GC analysis using the same equipment and conditions as described above for methyl esters.

Sterol Composition

The determination of sterol composition was carried out according to the method described by Tsaknis et al. [15]. The analysis was performed on an Agilent 5975B Gas chromatograph equipped with a J&W DB-5 capillary column (30 m × 0.25 mm × 0.25 µm). The pressure of the carrier gas (H_2) was 0.75 bar. Injector and FID temperatures were set at 280 and 300 °C, respectively. The column temperature was maintained at 260 °C, and the run time was 40 min.

Tocopherol Composition

The determination of the tocopherol composition was carried out as reported earlier by Lalas et al. [16]. Specifically, the analysis was carried out on a Shimadzu CBM-20A liquid chromatograph equipped with a SIL-20AC auto sampler and a CTO-20AC column oven. Detection was carried out using a Shimadzu RF-10AXL fluorescence detector set at 278 nm (excitation) and 339 nm (emission). The column used was a Waters μ-Porasil, (125 Å, 10 µm, 3.9 × 300 mm, Waters Corp., Waltham, MA). The preparation of the sample was done as follows: 1 g of lipid was accurately weighed into a 5-ml sample vial wrapped in foil paper to prevent oxidation. The oil was dissolved in 5 ml *n*-hexane before injection. A 20-µl sample was injected into the HPLC. 2-Propanol:*n*-hexane:absolute ethanol (2:97.5:0.5) at 1 ml/min was used as mobile phase.

Determination of the Oxidative State

Determination of the oxidative state was carried out by means of peroxide value (determined as meq O_2/kg of oil according to Lea [17]) and specific extinction ($E^{1\%}_{1cm}$) at 232 nm (carried out by the method of IUPAC [11]).

Determination of the Susceptibility to Oxidation with the Rancimat Method

The susceptibility to oxidation was determined according to a modification of the method described by Tsaknis et. al. [18]. Specifically, about 3 g of oil was accurately weighed into each of the reaction vessels, and the following procedure was carried out. The Rancimat 743 (Metrohm Ltd., Herisau, Switzerland) was switched on until the temperature of the oil batch reached 110 °C. Then, 80 ml of distilled water was placed into each of the conductivity cells, and the air flow rate was set at 20 l/h. The temperature was checked to ensure that it had a constant value. The air supply was connected to the tubes containing the oil samples, and the determination was carried out automatically until the conductivity reached the maximum value and the induction period was read. The induction period was compared to that of virgin olive oil (Elais S.A., Piraeus, Greece).

Thermal Profile (DSC profile)

The thermal characteristics of the seed oil was determined according to the method described by Nehdi [19] using a PerkinElmer Diamond DSC differential scanning calorimeter (PerkinElmer Inc., Shelton, CT, USA). The DSC was connected to nitrogen gas (flow rate at 20 ml/min) and a refrigerated cooling system (Intracooler 2P, PerkinElmer Inc). Oil samples of about 4 mg were weighed in open aluminum pans (no. 0219-0041, PerkinElmer Inc.). At the same time, an empty pan was used as a reference. The sample and reference pans were then placed inside the calorimeter and kept at −70 °C for 2 min. The temperature was increased from −70 to 70 °C at a rate of 5 °C/min.

Statistical Analysis

Results are displayed as means and standard deviation (in parentheses) of three simultaneous assays in all methods. Statistical significance (at $P < 0.05$) was assessed by ANOVA test.

Table 1 Physical and chemical characteristics, tocopherol content, oxidative state, and susceptibility to oxidation of *Crambe abyssinica* Hochst. seed oil

Physical characteristics	
Oil content (%)	30 (1.7)
Density at 25 °C (mg/ml)	0.900 (0.011)
Refractive index (n_D40 °C)	1.4646 (0.006)
Colour ($l \times a \times b$)	67.80 (0.33), −1.20 (0.15), 20.0 (0.21)
Smoke point (°C)	222 (1.7)
Viscosity (mPa s)	175 (1.7)
Chemical characteristics	
Acidity (% of erucic acid)	0.97 (0.07)
Saponification value (mg of KOH/g of oil)	175.52 (3.84)
Iodine value (g of I/100 g of oil)	88.67 (0.44)
Tocopherols content	
α-Tocopherol (mg/kg of oil)	7.67 (0.12)
γ-Tocopherol (mg/kg of oil)	125.04 (1.04)
δ-Tocopherol (mg/kg of oil)	3.99 (0.16)
Oxidative state	
Peroxide value (meq O_2/kg of oil)	0.806 (0.059)
$E^{1\%}_{1cm}$ at 232 nm	1.303 (0.006)
Susceptibility to oxidation (Rancimat method at 110 °C and 20 l/h)	
Crambe oil	8.83 (0.15)
Virgin olive oil	7.22 (0.35)

Values are means of triplicate determinations and standard deviation (in parentheses)

Results and Discussion

Crambe abyssinica Hochst. seed oil after extraction was submitted to full characterisation of its characteristics. Results are displayed in Tables 1, 2, and 3.

Physical Characteristics

Results of the physical characteristics of the oil are shown in Table 1. The extracted oil had a light yellow colour and a characteristic faintly nut-like odour. The results fell in the range of previously reported data (ranging from 25 to 40 %) [1, 20, 21]. The obtained values for density and refractive index were 0.900 and 1.4646, respectively. The colour values were expressed according to Hunter values ($L \times a \times b$) where L (measure of light reflectance), a (green when negative and red when positive) and b (blue when negative and yellow when positive). The color of crambe seed oil was 67.80 (0.33), −1.20 (0.15), and 20.0 (0.21). The results of the colour partially agree with those reported previously [3], which can be attributed to the different variety.

The oil is characterised by a high smoke point (222 °C), so it appears to retain stability and oiliness at high temperature. Its viscosity was 175 mPa s. The disagreement about viscosity in previously reported studies [2, 22] can be attributed to the different varieties used, the climatic conditions of the place of cultivation, and the different soils and procedure of collection.

Table 2 Fatty acid composition and relative position of fatty acids in C_1 and C_3 carbons of glycerol

Fatty acid composition %	
Palmitic (C16:0)	0.88 (0.22)
Stearic (C18:0)	0.53 (0.04)
Oleic (C18:1 ω-9)	15.07 (0.33)
Linoleic (C18:2 ω-6)	13.16 (0.30)
Arachidic (C20:0)	0.63 (0.14)
Eicosenoic (C20:1 ω-9)	2.40 (0.21)
Behenic (C22:0)	2.14 (0.13)
Erucic (C22:1 ω-9)	63.77 (0.96)
Lignoceric (C24:0)	0.44 (0.09)
Nervonic (C24:1 ω-9)	0.99 (0.07)
Relative position of fatty acids in C_1 and C_3 carbons of glycerol	
Erucic acid	90.71 (2.13)
Oleic acid	8.84 (1.01)
Linoleic acid	0.45 (0.20)

Values are means of triplicate determinations and standard deviation (in parentheses)

Table 3 Sterol composition

Sterols by GC	% of the sterol fraction
Cholesterol	0.81 (0.12)
Brassicasterol	12.35 (0.36)
Δ^7-Cholestenol	0.19 (0.01)
$\Delta^{5,25}$-Ergostadienol	0.16 (0.23)
24-Methylene cholesterol	2.21 (0.57)
Campestanol	21.98 (2.56)
Stigmasterol	1.38 (0.25)
Clerosterol	0.29 (0.050)
β-Sitosterol	51.93 (1.64)
Stigmastanol	0.49 (0.24)
Δ^5-Avenasterol	5.23 (0.34)
5,24-Stigmastadienol	0.38 (0.11)
Δ^7-Stigmastenol	0.28 (0.09)
Δ^7-Avenasterol	0.06 (0.03)

Values are means of triplicate determinations and standard deviation (in parentheses)

Chemical Characteristics

Results of the determination of the chemical characteristics are shown in Table 1. The obtained data for saponification

and iodine values compared with those previously reported [1, 2, 22, 23] and showed no significant differences. The iodine value was expected to be high because of the percentage of unsaturated fatty acids (see below). Acidity appeared to be higher than that reported in the literature [1], in any case lower than 1 %.

Fatty Acid Composition

The fatty acid composition of the *Crambe abyssinica* Hochst. seed oil is presented in Table 2. Total unsaturated fatty acids consisted of more than 95.3 % total fatty acids. As expected [1, 20–24], the major fatty acid was erucic (C22:1) in a concentration of 63.77 %, followed by oleic (C18:1) at 15.07 % and linoleic (C18:2) at 13.16 %. Erucic acid, a monounsaturated omega-9 fatty acid, is a constituent of crambe oil with great importance since it has a number of oleochemical uses (pharmaceuticals, lubricants, plasticisers, cosmetics, surfactants, etc.). Behenic acid (2.14 %) was found to be the dominant saturated fatty acid, followed by stearic acid (0.53 %). Both fell in the range of previously reported data [1, 20–24]. During the present work, C14:0, C16:1, C18:3, and C20:2 were not detected, while C16:0, C24:0, and C24:1 were detected at a lower concentration than previously reported [1, 20–24]. Differences can again be attributed to the different varieties used, the climatic conditions of the place of cultivation, and the different soils and collection procedures.

Relative Position of Fatty Acids in C_1 and C_3 Carbons of Glycerol

Table 2 shows the relative position of fatty acids in C_1 and C_3 carbons of glycerol. Erucic acid was determined at a concentration of 90.71 %, but oleic acid at 8.84 % and linoleic acid at 0.45 %. These results were expected since those three fatty acids (erucic, oleic, linoleic) comprise 92 % of the fatty acid profile of crambe oil.

Sterol Composition

The sterol composition of *Crambe abyssinica* seed oil is shown in Table 3. The sterol profile consisted mainly of campestanol, brassicasterol, β-sitosterol, and Δ^5-avenasterol, among which β-sitosterol was the most predominant (51.9 %), followed by campestanol (21.9 %) and brassicasterol (12.4 %), and accompanied by small amounts of 24-methylene cholesterol (2.2 %) and stigmasterol (1.4 %). Also, trace to minute amounts of the following sterols were detected: cholesterol, Δ^7-avenasterol, $\Delta^{2,25}$-ergostadienol, clerosterol, stigmastanol, 5,24-Stigmastadienol, Δ^7-stigmastanol, and Δ^7-avenasterol. To our knowledge, there are no previously reported data on sterol composition.

Tocopherol Composition

Table 1 shows the tocopherol composition as determined by HPLC. The tocopherol content consisted of α-, γ-, and δ-tocopherol. Most vegetable oils contain α-, β-, and γ-tocopherols. It is generally recognised that α-tocopherol is an efficient antioxidant, as a vitamin E homologue in vivo. However, some authors indicate a considerable discrepancy in its absolute and relative antioxidant effectiveness in vitro, especially when compared to γ-tocopherol [25]. Tocopherols present in *Crambe abyssinica* seed oil were expected to offer some protection during storage and processing. To our knowledge, there are no previously reported data on tocopherol composition.

Oxidative State and Susceptibility to Oxidation

The oxidative state of *Crambe abyssinica* Hochst. seed oil was determined using the peroxide value (PV) and $E^{1\%}_{1cm}$ at 232 nm and susceptibility to oxidation using the Rancimat method. The results are shown in Table 1. The PV was 0.806 meq O_2/kg of oil and fell in the range adopted as satisfactory. The $E^{1\%}_{1cm}$ at 232 nm was 1.303. To our knowledge there are no previously reported data on oxidative state. According to the results of the Rancimat method, the induction period of the oil was 8.83 h. The crambe oil had a longer induction period (about 22 %) compared to virgin olive oil (7.22 h) and hence higher resistance to oxidative deterioration. As indicated by the fatty acid determination, *Crambe abyssinica* Hochst. seed oil has a highly unsaturated profile (higher than olive oil). However, because of the higher tocopherol content (total tocopherol: crambe oil 136.7 mg/kg, olive oil 101.2 mg/kg)

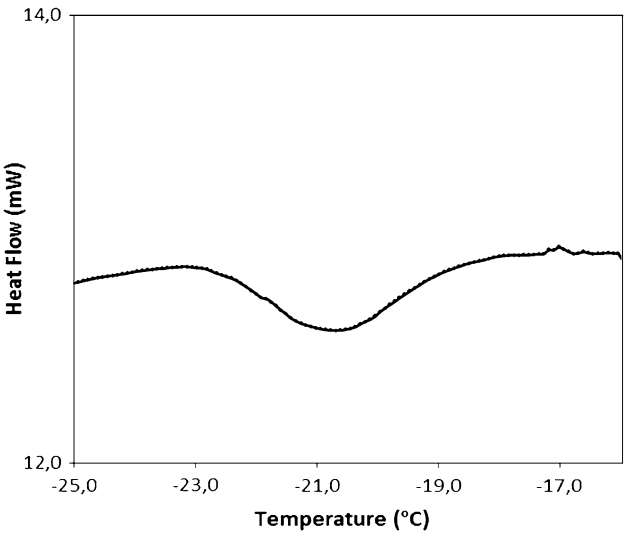

Fig. 1 DSC profile of *Crambe abyssinica*

and the presence of Δ^5-avenasterol, crambe oil appeared less susceptible to oxidation [26].

Thermal Profile

DSC provides information on the excess specific heat over a wide range of temperatures. Any endothermic or exothermic event is registered as a peak in the chart, and its area is proportional to the enthalpy gained or lost, respectively [19]. The thermal profile of crambe seed oil (Fig. 1) presented a single peak having the following characteristics: melting temperature −20.94 °C and heat flow 12.6 mW.

Conclusion

This work was dedicated to reporting the full chemical and physical characterisation of *Crambe abyssinica* seed oil. Oil content, density, refractive index, colour, smoke point, viscosity, acidity, saponification value, iodine value, fatty acid methyl esters, the relative position of fatty acids in C1 and C3 carbon glycerol, sterols, tocopherols content, peroxide value, $E^{1\%}_{1cm}$ (at 232 nm), and susceptibility to oxidation (Rancimat method) were determined. The seed oil was found to contain a high percentage of erucic acid, which has greater potential use in the industrial field. The oil was also found to contain high level of phytosterols (β-sitosterol, campestanol and brassicasterol) and γ-tocopherol. The Rancimat method proved resistance to oxidation that was higher than that of olive oil.

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