J Am Oil Chem Soc
DOI 10.1007/s11746-016-2886-6

ORIGINAL PAPER

Chemical Composition and Physical Properties of High Oleic Safflower Oils (*Carthamus tinctorius*, Var. CW88-OL and CW99-OL)

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Received: 5 August 2016 / Accepted: 9 August 2016

Abstract Chemical composition and physical properties of CW88-OL and CW99-OL cultivars of high oleic safflower seeds and their hexane-extracted oils were determined. Dry-based seed composition of CW88-OL and CW99-OL was: moisture = 4.29 and 4.23 %, oil = 42.29 and 46.44 %, Crude protein = 20.94 and 16.41 %, neutral detergent fiber = 28.11 and 28.49 %, ash = 1.55 and 2.01 %, phosphorus content = 2033 and 3995 mg/kg, respectively. Major fatty acids in oils were ~78 % oleic (O), ~13 % linoleic (L), ~5 % palmitic (P) and ~2 % stearic (St) acids, for both cultivars. The main triacylglycerols were OOO (~50 %), OOL (~20 %), SOL + OPO (~10 %), and LLP (~5 %). The oil composition of CW88-OL and CW99-OL in main minor components was: α-tocopherol = 582 and 551 mg/kg, total sterols = 3996 and 3362 mg/kg, phospholipids = 22 and 21 mg/kg and wax content = 70 and 74 mg/kg. For both cultivars, density and viscosity of the oils between 25 and 55 °C varied from 903.4 to 912.6 kg/m^3 and 63 to 23 mPa.s showing linear and exponential behaviors, respectively. The refractive index was 1.4694. The CIELab color parameters were: 89.69 and 89.53 (L^*), −3.72 and −3.07 (a^*), and 47.28 and 47.78 (b^*) (CW88-OL and CW99-OL, respectively). Thus, the high oil content of the seeds and nutritional quality of the oil accompanied by low levels of waxes and phospholipids makes the cultivars studied promising for producers and consumers.

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Keywords Safflower · Oil-seeds · Chemical composition · Physicochemical properties · Triacylglycerols · Waxes

Introduction

Safflower is an ancient crop and despite the uncertainty of its true origin, Egyptians used it 4000 years ago to dye cotton and silk. Nowadays, the selection to obtain cultivars with higher yields, oil content and disease resistance lead to a wider range of applications that goes from dye to cosmetics, pharmaceutics, edible oils and animal food products [1].

About 60 % of the world's production of safflower is concentrated in Kazakhstan, México, India and the United States. Safflower belongs to the Compositae (*Cynaraae*) family. It is an annual crop and since commercial cultivars are used it is not capable of establishing as a weed [1]. Safflower presents yellow, orange or red flowers and the fruit is an achene commonly known as the seed of safflower. The seed is similar to the sunflower but has approximately half of its size and the hull represents 33–45 % of the whole seed holding about 6–8 % of the total oil after extraction with expeller pressing [1]. The seed oil content ranges between 30 and 35 % and it is obtained using conventional extraction and refining methods [2].

As a general classification there are two safflower lines, the ones with a high content of oleic acid and those with high linoleic acid content. In this study, high oleic lines CW88-OL and CW99-OL were analyzed. Although several authors have already studied safflower seed composition and its oil [1, 3, 4], there is still missing information about some of its major and minor components. On the one hand, high oleic lines are much less studied than high linoleic ones. On the other hand, specifically CW88-OL and

Published online: 22 August 2016

CW99-OL are two cultivars that have been poorly studied, meaning that no complete characterization of these seeds or their oils was available up to now (that involves seed composition along with physical characterization and composition of major and minor components of the oil). Various authors have already determined the fatty acid profile in safflower oil from different cultivars [3–6] as well as tocols [3–6] and sterols [5, 7, 8] but less is known about triacylglycerol and wax profiles or phosphorus and phospholipid content. To the best of our knowledge, the wax profile was not available for either, high oleic or high linoleic oil and no triacylglycerol profile for high oleic lines could be found in the literature.

The aim of this study was to provide an integral and complete characterization of high oleic cultivars CW88-OL and CW99-OL including triacylglycerol, wax and phospholipid profiles along with several less common determinations such as phosphorus content, CIELAB color parameters, and variation of viscosity and density with temperature.

Materials and Methods

Materials

Two cultivars of commercial safflower seeds were analyzed: CW88-OL and CW99-OL. Due to their low humidity, seeds were stored in a dark and dry place at room temperature. All chemicals and solvents used in this study were analytical reagent grade, except chromatographic standards and solvents that were chromatographic or HPLC grade.

Methods

Seed Characterization

Seeds Conditioning Seeds were dried until constant weight in a vacuum oven at 55 °C. For all determinations previously conditioned seeds were used with the exception of moisture and volatile matter analyses.

Moisture and Volatile Matter Moisture content was determined using the AOCS Ca 2d-25 standard method [9] in which seeds are weighted and dried in a vacuum oven until constant weight. Results were expressed in percentage dry basis. The analysis was performed by triplicate for both cultivars.

Oil Oil content was determined using the IUPAC 1.122 standard method [10] by continuous extraction in a Soxhlet apparatus. For each extraction 10 g of grinded conditioned seeds were used. The solvent employed was *n*-Hexane. After the extraction, the solvent was evaporated in a rotary evaporator and traces of solvent were removed under a weak nitrogen stream. Four and five independent extractions of CW88-OL and CW99-OL seeds were performed, respectively. Results were expressed in percentage dry basis. Extracted oil was stored under a nitrogen atmosphere and refrigeration conditions in dark brown glass bottles until subsequent analyses.

Ash To determine the contents of ash the AOCS Ba 5a-49 standard method [9] was used. It consisted of a total calcination in a muffle furnace for 2 h at 600 °C. As a slight modification of the method, all samples were partially calcined though gradual heating with electric resistances before putting them into the muffle furnace to avoid material loss by the abrupt formation of vapors or flames. Results were expressed as a percentage of the dry basis. Four independent determinations were performed for each cultivar.

Crude Protein (CP) and Neutral Detergent Fiber (NDF) Analyses were performed on fat-free milled seeds and with a maximum particle size of 1 mm (standard sieve of 1 mm). CP was determined using macro Kjeldahl technique with a transformation factor of 6.25 to convert nitrogen into CP [11]. The sequential method described by Van Soest *et al*. [12] was used to obtain NDF. Results were expressed as a percentage of the dry basis. Two independent determinations were performed for each cultivar.

Total Carbohydrate Total carbohydrate in dry basis was obtained by subtracting all components described above (oil, ash, CP and NDF) to 100 %.

Phosphorus Content Analysis was performed from the seed sample in the same way that is described for oil characterization in de following section. Results are expressed as mg/kg seed. Three independent determinations were performed for each cultivar.

Chemical Characterization of Safflower Oil

Fatty Acids Fatty acid composition was determined by derivatization of the oil to fatty acids methyl esters (FAME) according to the AOCS Ce 2-66 official method [9]. Then, FAME were analyzed by GLC with a 4890D series gas chromatograph (Agilent, Hewlett-Packard), identified by comparing their retention times with Supelco standards (Supelco 37 Component FAME mix, Supelco Inc) and quantified as a percentage of total fatty acids according to AOCS Ce 1e-91 method [9]. FAME were separated on a fused-silica capillary column (SP-2380, 30 m × 0.25 mm i.d., film thickness 0.2 µm; Supelco Co., Bellefonte, PA) maintained at 170 °C for 15 min, then increased at 4 °C/

min to 210 °C and held at 210 °C for 10 min, using hydrogen as the carrier gas (22.987 cm/s). The injector was used in split mode with a ratio of 1:50. The injector and detector (FID) temperatures were 220 °C. Data acquisition and peak integration were performed using EC Chrom Elit 332. Two independent determinations were performed for each oilseed. For each determination sample was injected twice.

Triacylglycerols This determination was carried out using the AOCS Ce 5c-93 method [9] complementary to the AOCS Ce 5b-89 technique [9] in which individual triacylglycerols of oils and fats are separated, identified and quantified by HPLC (Varian Vista 5500) equipped with an IR detector (W2414, Waters). Triacylglycerols were separated on a RP-18 column (Hibar 25 cm × 4 mm i.d., LIChrospher 100, particle size 5 µm, Merck) with a constant flow of 1 mL/min using acetone:acetonitrile 60:40 vol/vol as the mobile phase. For identification and quantification authentic standards, soybean oil and the fatty acid composition of the sample were used. Chromatographic standards of triacylglycerols (1,2,3-trioctadecenoyl-glycerol (OOO), 1,2,3-trioctadecadienoyl-glycerol (LLL), 1,2,3-trioctadecanoyl-glycerol (StStSt), 1,2,3-trihexadecanoyl-glycerol (PPP), 1,2-distearoyl-3-palmitoyl-*rac*-glycerol (StStP), 1,3-dipalmitoyl-2-oleoylglycerol (POP), 1,3-dioleoyl-2-palmitoyl-glycerol (OPO), 1,2-dilinoleoyl-3-palmitoyl-*rac*-glycerol (LLP), 1,2-dioleoyl-3-stearoyl-*rac*-glycerol (OOSt), 1-palmitoyl-2-oleoyl-3-linoleoyl-*rac*-glycerol (POL), 1,2-distearoyl-3-oleoyl-*rac*-glycerol (StStO)) were provided by Sigma (Sigma Chemical Co., St. Louis, USA). Peaks' areas were obtained with Waters' Empower 2 software. Results were expressed as relative percentage of each triacylglycerol. Three independent determinations were performed for each oilseed.

Tocols Tocols content was determined from the lipid fraction by HPLC using the AOCS Ce8-89 method [9]. A standard of α-tocopherol with purity greater than 98 % was obtained from Sigma and used as external standard. The equipment used was a HPLC Varian (Vista 5500) chromatograph equipped with a Lichrosob Si 60, 25 × 0.4 cm and a particle size of 5 µm. The injection volume was 10 µL and the flow rate was 1 mL/min (mobile phase: isopropanol:hexane; 0.5:99.5 (vol/vol), filtered and degassed). Peak areas were obtained with Waters' Empower 2 software. Results were expressed as mg/kg oil. Though just one determination was performed for each cultivar, injection was performed in duplicate.

Sterols Sterol contents and profiles were determined by GLC with the European Union analytical method [13]. The oil sample containing 5-α-cholestan-3-β-ol (from Fluka Switzerland, purity 95 %) as internal standard was saponified with potassium hydroxide solution in ethanol. The unsaponifiable fraction containing sterols was removed with ethyl ether and then was separated by silica gel plate chromatography. Separation and quantification of the silanized compounds was carried out by GLC using a 30 m SE-54 column of 0.25 mm i.d. and 0.2 µm film thickness (Supelco Inc., Bellefonte, PA). The operation conditions were as follows: oven temperature, 260 °C (2 min)–1 °C/min–265 °C (39 min); injector temperature, 280 °C; FID temperature, 300 °C, injection volume, 1 µL; and hydrogen as the carrier gas (40.565 cm/s). Data acquisition and peak integration were performed using EZ Chrom Elit 332. The total sterol content was expressed as mg/kg oil and the sterol composition as the relative percentage of each sterol. Though just one determination was performed for each cultivar, injection was performed in duplicate.

Phosphorus Total phosphorus was determined by the AOCS Ca 12-55 official method [9]. This method determines phosphorus by ashing the sample in the presence of zinc oxide, followed by the spectrophotometric measurement of phosphorus as a blue phosphomolybdic acid complex at 650 nm. A calibration curve was made to correlate phosphorus content and absorbance. Phosphorus content was expressed as mg/kg oil and two independent phosphorus determinations were performed for each oilseed.

Phospholipids Phospholipids in the oil were concentrated using a diol solid-phase extraction (SPE) cartridge (J. T. Baker, USA) [14] and then quantified by the external standard method using HPLC according to the AOCS official method Ja 7b-91 [9]. The HPLC system consisted of a Varian Vista 5500 Chromatograph with an injection valve of a 10-µL sample loop, an UV detector (2998 Waters PDA) set at 206 nm and a Nucleosil 50-5 column (25 cm × 4.6 mm i.d., Macherey–Nagel). Separations were performed at room temperature by isocratic elution with hexane, 2-propanol, and acetate buffer in proportion 8:8:1 vol/vol/vol (filtered and degassed) with a flow rate of 2 mL/min. Standards for L-α-phosphatydilethanolamine (PE), L-α-phosphatidylinositol (PI), L-α-phosphatidylcholine (PC) from soybean, L-α-phosphatidylserine (PS), L-α-phosphatidic acid (PA) sodium salt from egg yolk lecithin with purities greater than 98 % were obtained from Sigma and used to obtain calibration curves. The phospholipid content, expressed as mg/kg oil, was calculated by: PL = 1000CPL V/M where CPL represents the phospholipid concentration obtained from the calibration curve in mg/mL, V is the volume in mL of the phospholipid concentrate that constitutes the sample to be injected into the HPLC system, and M is the weight in mg of oil in the SPE cartridge. Two independent determinations were performed for each oilseed. Injection was performed in duplicate.

Table 1 Chemical seed composition in g/100 g dry seed

Component	CW88-OL (%) dry basis	CW99-OL (%) dry basis
Moisture and volatile matter	$4.23^{a} \pm 0.16$	$4.29^{a} \pm 0.04$
Oil	$42.29^{b} \pm 0.91$	$46.44^{a} \pm 4.14$
Ash	$1.51^{b} \pm 0.01$	$2.24^{a} \pm 0.03$
Crude protein	$20.94^{a} \pm 0.19$	$16.41^{b} \pm 0.10$
Neutral detergent fiber	$28.11^{a} \pm 0.20$	$28.49^{a} \pm 0.22$

Data are mean values ± standard deviations, $n_{Moisture} = 3$, $n_{Oil} = 4$ and 5, $n_{Ash} = 4$, $n_{Protein} = 2$, $n_{Fiber} = 2$

Within the same row, values with different letters are significantly different ($p < 0.05$)

Waxes Wax composition was determined by a modified International Olive Council (IOC) method for wax determination involving a double-adsorbent layer of silica gel and silver nitrate impregnated silica gel as solid phase in the column chromatography followed by GLC [15]. Column chromatography was performed in a glass column (i.d. = 15 mm, length = 400 mm) with a double phase of silver nitrate-impregnated silica gel (3 g) placed in the bottom of the column and silica gel 2 % hydrated (12 g) placed on the top as a solid stationary phase. Sudan I dye was used to check the completion of the wax elution. The eluted wax fraction was evaporated to dryness, diluted with *n*-heptane and analyzed by capillary GC.

A Perkin Elmer Auto-System XL gas chromatograph equipped with a FID detector and a programmed-temperature on-column injector (Perkin Elmer, MA, USA) were used. The capillary column was an HP-5 (5 % diphenyl and 95 % dimethyl-polysiloxane), fused-silica, 15 m length 0.32 mm i.d., 0.25 μm film thickness (Hewlett-Packard, Palo Alto, CA, USA). The operating conditions were: hydrogen at 3 mL/min as carrier gas; oven temperature programming: initial temperature of 80 °C holding for 1 min, increasing at 20 °C/min to 240 °C, increasing at 5 °C/min to 325 °C, holding for 6 min; increasing at 20 °C/min to 340 °C holding for 27 min. On-colμmn injector was programmed from 80 to 320 °C at 20 °C/min and injection volume of 2 μL; FID at 350 °C. Data acquisition and peak integration were performed using Total Chrom Workstation v.6.3.1 data processor. The following wax standards of almost 99 % purity (Sigma Chemical Co., St. Louis, USA) were used for qualitative identification: C32 = lauric acid arachidyl ester ($C_{32}H_{64}O_2$), C36 = stearic acid stearyl ester ($C_{36}H_{72}O_2$), C38 = arachidic acid oleoyl ester ($C_{38}H_{74}O_2$), C40 = arachidic acid arachidyl ester ($C_{40}H_{80}O_2$), C42 = arachidic acid behenyl ester ($C_{42}H_{84}O_2$) and C44 = behenic acid behenyl ester ($C_{44}H_{88}O_2$). The C32 standard was also employed as internal standard. In addition, laboratory purified sunflower waxes from the filter cake of the dewaxing process of sunflower oil was used as standard for the identification of waxes with more than 44 carbon atoms. Though just one determination was performed for each cultivar, injections were performed in triplicate.

Physical Properties of Oil

Density was determined with a 25-mL pycnometer according to the AOAC Cc10c-95 official method [9] and viscosity with an Anton Paar Physica MCR 301 rheometer (GmbH, Austria). Viscosity was measured using concentric cylinder geometry (i.d. 26.658 mm, e.d. 28.925 mm with 26.658 mm length). Both determinations were conducted with temperature control between 25.0 and 55.0 °C. Oils' CIELAB color parameters were determined in a Hunterlab triestimulus UltraScan XE colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) with an optic glass cell (path 10 mm) in transmission mode. Total color transmitted through the sample was measured at 10° observer angle with D65 illuminant, while the refraction index was obtained with a refractometer (MISCO palm Abbe V. 05 II), both at 25 °C.

All physical determinations were made in triplicate for each cultivar.

Statistical Analysis

Results were expressed as mean values ± standard deviations. The number of independent determinations for each analysis was mentioned in the method description. In all cases, the differences between mean values were assessed with Student's *t* test, being considered statistically different at a significance level of 5 %.

Results and discussion

Chemical Composition of Seeds

Table 1 shows seed composition. Moisture content obtained for both cultivars was not only lower than those reported by Bozan *et al*. [3] but far away to the upper limit of the customary basis in Argentine trade in safflower seed [1]. No significant differences were found between these results ($p < 0.05$).

Likewise, no significant differences in fiber content were found between the two cultivars. However, CW99-OL showed higher values of oil content and ash content and lower protein content than CW88-OL ($p < 0.05$). When comparing these results with compositions found in other studies for other safflower cultivars, oil content (upper to 40 %, Table 1) was very well positioned with respect to

the literature in which a range between 19.6 and 47.7 % is reported [1, 3, 4]. After oil, the second most important component of seeds is protein since it enables its use for animal meal. These cultivars had regular protein content in agreement with several authors who reported a range between 15.44 and 22.5 % [1]. Fiber and ash content were similar to those found in the cited studies. Finally, total carbohydrates give a value in the range of 6–7 % (CW88-OL = 7.16 %, CW99-OL = 6.41 %).

Phosphorus content was significantly different between both samples (CW88-OL: 2033 ± 89 mg/kg seed d.b.; CW99-OL: 3995 ± 95 mg/kg seed d.b., $p < 0.05$) but both seeds could be considered a natural source of this nutrient taking into account the dietary reference intakes (>580 mg/day for men and women over 19 years old) [16]. On the other hand, phosphorus content of CW88-OL and CW99-OL seeds were in agreement with phosphorus content reported by Smith [1] (3670 mg/kg seed).

Oil Characterization

Fatty Acids

The relative percentages of fatty acids obtained for both cultivars were very similar (Table 2). The most abundant fatty acid was oleic acid (O, C18:1n-9c, ~78 %) followed by linoleic acid (L, C18:2n-6c, ~13 %) and palmitic acid (P, C16:0, ~5 %). The major polyunsaturated fatty acid was linoleic acid (n-6 PUFA) while only traces of n-3 PUFAs (C18:3n-3c; C22:6n-3c) were found. Major fatty acid composition in both cultivars, CW88-OL and CW99-OL, were in agreement with contents reported among literature for the high oleic line: *P* = 5–6 %, *O* = 74–80 %, *L* = 1.5–2 [17].

These cultivars of safflower show *O* and *L* content in the range that are present in olive oil (*O*: 55–83 %; *L*: 2.5–21 %, *P*: 7.5–20 %) but with a lower content of *P* [18] indicating a healthier profile of fatty acids since palmitic acid is hypercholesterolemic. When comparing high-oleic safflower with high-oleic sunflower oil, although they have similar content of *O* (75–85 %) [17], safflower had less content (~2 %) of stearic (St, C18:0; sunflower: 2.7–6.5 %) and higher content of *L* (sunflower: 8–10 %).

Triacylglycerols

Triacylglycerol fatty acids were distributed within glycerol molecules as shown in Table 3 (without distinguishing positional distribution). Major triacylglycerols found were the same for both oils. As expected from FAME analysis, triolein (OOO) was the major triacylglycerol representing approximately 50 % of the total. Much smaller proportions were found for OOL (20 %), StOL + OPO (10 %), LLP (5 %), PPL (4 %) and StLSt (4 %).

Table 2 Fatty acid composition (% GC area) of safflower oils

Fatty acid	CW88-OL (%)	CW99-OL (%)
C14:0	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
C16:0	$5.2^{a} \pm 0.1$	$4.8^{b} \pm 0.1$
C16:1	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
C18:0	$1.8^{b} \pm 0.0$	$2.0^{a} \pm 0.0$
C18:1n-9c	$77.5^{b} \pm 0.1$	$77.9^{a} \pm 0.1$
C18:2n-6c	$13.1^{b} \pm 0.0$	$13.2^{a} \pm 0.0$
C20:0	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.0$
C20:1	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.0$
C18:3n-3c	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
C22:0	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.0$
C24:0	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
C24:1n-9	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
C22:6n-3c	$0.1^{a} \pm 0.0$	Tr^{a}
Unidentified	$0.6^{a} \pm 0.1$	$0.4^{a} \pm 0.0$
SFA	8.0^{a}	7.8^{b}
MUFA	78.1^{b}	78.5^{a}
n-3 PUFA	0.2^{a}	0.1^{a}
n-6 PUFA	13.1^{a}	13.2^{a}

Mean values of duplicate analyses with double injection ± standard deviation. Means with different superscript letters in the same row were significantly different ($p < 0.05$)

Tr traces (≤0.05 %), *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *n-3 PUFA* and *n-6 PUFA* *n*-3 and *n*-6 polyunsaturated fatty acids, respectively

Since triacylglycerol composition was hard to find in the literature, there was not much information to compare with. O'Brien [17] reported 1.1 % of di-saturated (SUS), 15.9 % of mono-saturated (UUS) and 77.8 % of tri-unsaturated (UUU) triacylglycerols for the high oleic safflower lines. Comparing with these values, CW88-OL and CW99-OL reached a higher content of UUS and SUS and slightly lower content for UUU.

Sterols

Sterol distribution was similar in both cultivars of seeds (Table 4). The major sterols found were β-sitosterol, which represented 46 % of total sterols, followed by campesterol (10 %) and Δ-7-stigmastenol (10 %). In general terms, distribution of major sterols with respect to total sterols agrees with values reported by several authors [5, 6, 8]. Total sterol content is near the highest values commonly reported in the literature (1248–3619 mg/kg oil) [5, 8, 17]. In a recent study, Fernández-Cuesta *et al*. [8] assessed phytosterol content and profile of 20 safflower Spanish cultivars including CW88-OL and CW99-OL cultivars founding a

Table 3 Oils' triacylglycerols compositions

TAG	CW88-OL (%)	CW99-OL (%)
LLLn	$0.08^{a} \pm 0.13$	Tr^{a}
OLLn	$0.73^{a} \pm 0.07$	$0.61^{b} \pm 0.67$
PLLn	$0.01^{a} \pm 0.02$	Tr^{a}
LLP	$5.34^{a} \pm 0.43$	$5.24^{a} \pm 0.28$
OOLn	$0.55^{a} \pm 0.41$	$0.83^{a} \pm 0.04$
OOL	$21.17^{a} \pm 0.12$	$19.29^{b} \pm 0.04$
POL	$4.00^{a} \pm 0.014$	$3.65^{b} \pm 0.07$
OOO	$51.96^{b} \pm 0.42$	$53.92^{a} \pm 0.19$
StOL + OPO	$10.16^{a} \pm 0.43$	$9.68^{a} \pm 0.35$
PPP	$0.51^{a} \pm 0.06$	$0.42^{a} \pm 0.10$
PPSt	$0.58^{b} \pm 0.05$	$0.68^{a} \pm 0.03$
StLSt	$3.86^{a} \pm 0.14$	$4.04^{a} \pm 0.20$
StStP	$0.41^{a} \pm 0.09$	$0.41^{a} \pm 0.09$
StStO	$0.40^{a} \pm 0.21$	$0.26^{a} \pm 0.31$
Unidentified	$0.24^{a} \pm 0.15$	$0.96^{a} \pm 0.69$
UUU	$74.49^{a} \pm 0.17$	$75.44^{a} \pm 0.40$
UUS	$19.50^{a} \pm 0.31$	$18.57^{a} \pm 0.63$
SUS	$4.27^{a} \pm 0.14$	$4.04^{a} \pm 0.20$
SSS	$1.50^{a} \pm 0.02$	$1.52^{a} \pm 0.22$

Data are mean values ± standard deviations, $n = 3$. Within the same row, values with different letters are significantly different ($p < 0.05$)

Tr traces (≤0.005 %); Triacylglyceride nomenclature: *P* palmitic acid; *St* stearic acid; *O* oleic acid; *L* linoleic acid; *Ln* linolenic acid, *U* unsaturated fatty acid, *S* saturated fatty acid

Table 4 Sterol composition (%m/m Sterols) and total content (mg/kg oil) in safflower seed oils

Sterol	CW88-OL	CW99-OL
Cholesterol	$0.16^{a} \pm 0.01$	$0.26^{a} \pm 0.14$
24-Methylene cholesterol	$0.76^{a} \pm 0.02$	$0.62^{b} \pm 0.05$
Campesterol	$11.05^{a} \pm 0.42$	$10.85^{b} \pm 0.08$
Campestanol	$0.32^{a} \pm 0.05$	$0.32^{a} \pm 0.07$
Stigmasterol	$4.82^{a} \pm 0.12$	$5.39^{a} \pm 0.17$
Δ-7-Campesterol	$2.62^{a} \pm 0.20$	$3.05^{a} \pm 0.16$
Clerosterol	$0.91^{a} \pm 0.31$	$0.73^{a} \pm 0.14$
β-Sitosterol	$46.59^{a} \pm 0.35$	$46.19^{b} \pm 0.15$
Sitostanol	$3.36^{a} \pm 1.49$	$2.89^{a} \pm 0.07$
Δ-5-Avenasterol	$6.30^{a} \pm 0.16$	$4.64^{b} \pm 0.08$
Δ-7-Stigmastenol	$10.44^{a} \pm 0.31$	$13.02^{a} \pm 0.12$
Δ-7-Avenasterol	$5.60^{a} \pm 0.21$	$4.83^{b} \pm 0.05$
Unidentified	$7.48^{a} \pm 0.04$	$8.79^{a} \pm 0.92$
Total (mg/kg)	$3996^{a} \pm 24$	$3362^{b} \pm 73$

Data are mean values ± standard deviations, $n = 2$

Within the same row, values with different letters are significantly different ($p < 0.05$)

total sterol range between 2589 and 3619 mg/kg oil. The authors found a significant genetic variation and a positive correlation between total phytosterol content and the seed oil content. Although this correlation it is not observed in our samples, the samples show both a high total sterol content (>3300 mg/kg oil) and a high seed oil content (>40 %) (Tables 1, 4). Comparing the contents of these compounds with recognized hypocholesterolemic properties with those in olive oils (1053–3000 mg/kg oil) [18, 19] and high oleic sunflower (2500–5000 mg/kg oil) [21], these cultivars of safflower have a favorable sterol content.

Tocols

Tocol distribution is shown in Table 5. Both cultivars showed high content of α-tocopherol (>90 % respect to total tocols), followed by γ- and β-tocopherol. Content of β-tocotrienol and γ-tocotrienol is approximately 40 and 50 % higher in CW99-OL than in CW88-OL. In spite of this, total tocols content is similar for both cultivars.

When analyzing published studies from different sources there was a large variation between the magnitudes of individual and total tocols content. This may be due to the huge influence of multiple factors (cultivar of seed, previous treatments, sowing conditions, cultivation and storage) on the composition and contents of minority compounds in the oil. Total amount of tocols, especially α-tocopherol (major component) can be considered high when it is compare with values published by other authors for safflower oil (192–709 mg/kg oil) [3, 5, 6].

When comparing safflower oil with other healthy oils such as olive oil, the α-tocopherol value is still high. While safflower seeds studied in this paper presented around 565 mg/kg oil, Argentinean olive oils are in the range of 167–463 [19] mg/kg while high oleic sunflower oil could be in the range of 94–1860 mg/kg oil [17, 20]. This might be of interest due to the many healthy effects associated with this component such as potent antioxidant, prevention of some forms of cancer, anti-diabetics and anti-inflammatory.

Phosphorus and Phospholipid Content

Phosphorus content in the oil fraction was determined and results between cultivars were notoriously different (CW88-OL: 14.59 ± 0.70 mg/kg oil; CW99-OL: 5.09 ± 0.23 mg/kg oil). CW88-OL presented the highest concentration of phosphorus ($p = 0.03$). Phosphorus content in oil from CW88-OL and CW99-OL was much lower than values provided by Mihaela *et al.* [4] where a content of 52.1 ppm was reported. The phosphorus content in

Table 5 Tocols and Phospholipid content in safflower oils

Compound	CW88-OL	CW99-OL
Tocols (mg/kg oil)		
α-Tocopherol	$582.17^{a} \pm 16.76$	$550.51^{a} \pm 5.60$
β-Tocopherol	$2.55^{a} \pm 0.20$	$2.25^{a} \pm 0.52$
γ-Tocopherol	$4.05^{a} \pm 0.19$	$3.47^{a} \pm 0.08$
β-Tocotrienol	$13.24^{b} \pm 0.61$	$21.70^{a} \pm 0.10$
γ-Tocotrienol	$2.98^{b} \pm 0.15$	$6.93^{a} \pm 0.28$
Total	$604.99^{a} \pm 17.51$	$584.85^{a} \pm 6.38$
Phospholipids (mg/kg oil)		
PE	$19.67^{a} \pm 1.84$	$18.97^{a} \pm 1.40$
PA	$0.72^{a} \pm 0.33$	$0.95^{a} \pm 0.38$
PC	$1.76^{a} \pm 1.07$	$1.16^{a} \pm 0.54$
PI	ND	ND
Total	$22.15^{a} \pm 2.69$	$21.08^{a} \pm 1.42$

Data are mean values ± standard errors, $n_{Tocols} = 2$, $n_{Phospholipids} = 4$

Within the same row, values with different letters are significantly different ($p < 0.05$)

PE phosphatidylethanolamine, *PA* phosphatidic acid, *PC* phosphatidylcholine, *PI* phosphatidylinositol, *ND* not detected

Table 6 Wax content in safflower oils

Carbon number (C)	Wax content (mg/kg)	
	CW88-OL	CW99-OL
36	$2^{a} \pm 0.0$	Tr^{b}
40	$2^{a} \pm 0.0$	Tr^{b}
42	$5^{a} \pm 0.6$	$3^{b} \pm 0.0$
43	$3^{a} \pm 0.6$	Tr^{b}
44	$11^{a} \pm 1.2$	$12^{a} \pm 0.6$
45	$5^{a} \pm 0.6$	$2^{b} \pm 0.0$
46	$13^{b} \pm 0.6$	$17^{a} \pm 0.6$
47	Tr^{b}	$2^{a} \pm 0.0$
48	$9^{b} \pm 0.6$	$12^{a} \pm 0.6$
50	$5^{a} \pm 1.2$	$10^{a} \pm 0.0$
52	$6^{a} \pm 0.6$	$7^{a} \pm 0.6$
53	$2^{a} \pm 0.0$	$2^{a} \pm 0.0$
54	$4^{a} \pm 0.6$	$4^{a} \pm 0.0$
56	$3^{a} \pm 0.6$	$3^{a} \pm 0.0$
Total	$70^{a} \pm 4.6$	$74^{a} \pm 1.0$
Partially soluble waxes (C40–C43)	$10^{a} \pm 1.0$	$3^{b} \pm 0.0$
Crystallizable waxes (C44–C60)	$58^{a} \pm 3.6$	$69^{a} \pm 1.0$

Data are mean values ± standard errors, $n = 3$. Within the same row, values with different letters are significantly different ($p < 0.05$)

Tr traces (<2 mg/kg)

oils comes from the presence of inorganic phosphates and organic phosphatides that are extracted with the oil.

The total content of phosphatides in oils can be estimated by converting the total phosphorus content to the equivalent phosphatide content using a factor of 30 for soybean [9] and 24.7 for sunflower oil [22]. The use of the sunflower factor allows us to estimate a phosphatide content of 360 mg/kg oil for CW88-OL and 126 mg/kg oil for CW99-OL. These values are significantly higher than those determined by HPLC–UV (Table 5). It is supposed that these differences could be due to an important presence of inorganic phosphorus. In fact, Carelli *et al*. [22] found that experimental factors for crude sunflower oils were appreciably lower (17.6 for extracted crude oils, 9.1 for pressed oils); these differences were attributed to phosphorus from sources other than phospholipids and to the presence of minor phospholipids not quantified by chromatographic analysis. Moreover, the phosphatide content in the oil were significantly lower than the range reported in the literature (5000–12,000 mg/kg oil) for safflower oils [1, 17, 23]. Extracted oils with low phosphatide content, such as the ones analyzed in this study, are an advantage since the following degumming process (if necessary) could be done by simpler processes like water degumming [17].

Related to the distribution of phospholipids, phosphatidylethanolamine (PE) showed to be the major component in both cultivars (>90 %), while phosphatidylinositol (PI) was not detected in any of the samples. In contrast, Lee *et al*. [24] found that PI was the major phospholipid component of high linoleic safflower seed oil from roasted seeds and this component increased significantly as the roasting temperature increased while PE decreased. Anyway, roasting temperatures are much higher (140–180 °C) than those used in hexane extraction (68 °C).

Regarding reported phospholipid content in other oils, like sunflower or olive oils, it can be said that safflower oil had a lower content than crude sunflower oil (0.5–1.039 %) [14, 21, 22], but similar to olive oil (<0.1 %) [17].

Waxes

The total and individual wax contents are shown in Table 6. Total wax contents were lower than 100 mg/kg oil being majoritarian waxes in the range of 44–52 carbon atoms that belongs to the fraction named crystallizable (>C44). Although a different wax profile was observed between samples, waxes C44, C46 and C48 were prevalent representing about 50 % of the wax content in both cultivars. Anyway, both of them could be considered as low-wax-content oils (less than 500 ppm) [17] allowing soft dewaxing treatments (if necessary). Up till now, no safflower wax content or profile has been found in the literature. But, Smith [1] cites that one of the cost advantages of oleic safflower *versus* oleic sunflower oil is that oleic safflower oil does not require a winterizing step to produce salad grade oil, while oleic sunflower oil does.

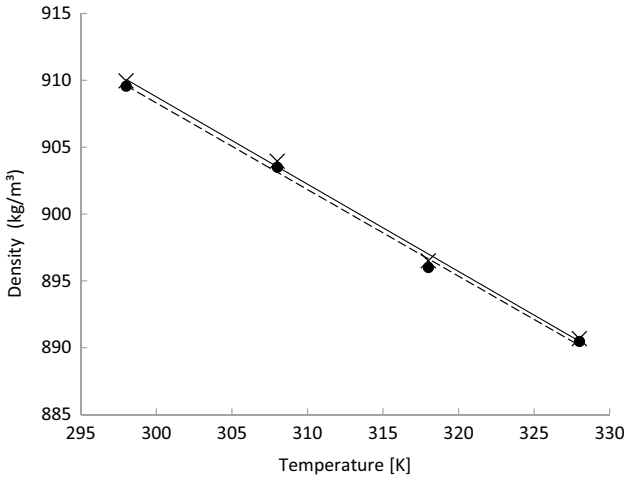

Fig. 1 Variation of safflower oils density (D) with temperature. (Experimental data: X, CW88-OL; ● CW99-OL. Mathematical models: — CW88-OL; --- CW99-OL)

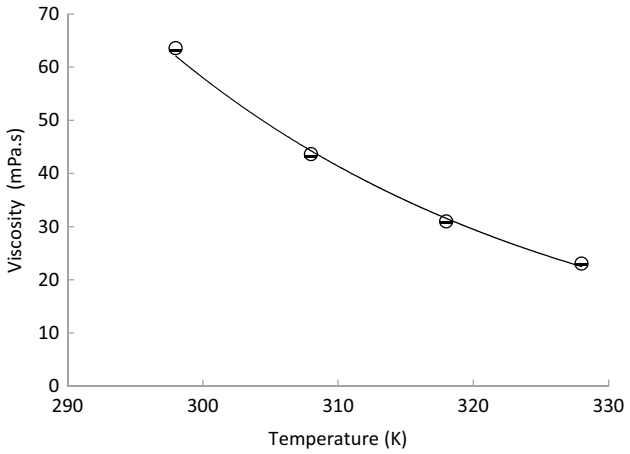

Fig. 2 Variation of safflower oils viscosity (V) with temperature. (Experimental data of both oils: O; mathematical model, —)

Based on these results, crude high oleic safflower oil presented substantial lower wax content than crude sunflower oil (460–900 ppm in oil from whole seed) [15, 17] being within the limit required for virgin olive oils (<150 mg/kg) [18] and in the order of wax content of refined high oleic sunflower oil [15].

Physical Properties

Densities (D) of oils are presented in Fig. 1. Although both oils showed statistically different D through all temperatures used ($p \ll 0.05$), D values were very similar showing a linear behavior ($D_{CW88} = -0.6503 \times T + 1103.8$ and $D_{CW99} = -0.6503 \times T + 1103.4$ with $R^2_{CW88} = 0.9978$ and $R^2_{CW99} = 0.9969$) within the range 298–328 K (with D between 912.6 and 903.4 kg/m^3) (Fig. 1). Both regressions were significant ($p < 0.005$) and despite the fact that they presented the same slope ($p > 0.95$), their y-intercepts were slightly different ($p < 0.05$). Density values reported in the literature at 25 °C (911.2–926 kg/m^3) were all in agreement with D obtained in this study [1, 22]. On the contrary, a single viscosity (V) regression (Fig. 2) was obtained for both cultivars ($V = 149 \times 10^4 \times \exp(-0.0338 \times T), R^2 > 0.99$) since no significant differences were found between measurements for a specific temperature. As can be seen in F2, values were between 63 and 23 mPa·s. The refractive index was 1.4964 ± 0.0001 for both cultivars. This value was in agreement with other published data [17]. Finally, CIELab color parameters were: 89.69 and 89.53 (L^*, $p > 0.05$), −3.72 and −3.07 (a^*, $p \ll 0.05$), and 47.28 and 47.78 (b^*, $p \ll 0.05$)] for CW88-OL and CW99-OL, respectively. These values indicate a marked trend toward green and yellow.

Conclusions

Results show both cultivars of safflower seeds have excellent properties to produce high-quality edible oils. Their high oil content rich in O along with their high levels of antioxidants turn them into suitable alternative for frying. Moreover, their light color, low phosphatide contents and low wax contents are also a remarkable feature since it reduces complexity and costs in the oil refining process. In addition, both favorable profiles of the fatty acids and amounts of nutraceutic compounds such as α-tocopherol and sterols contribute to making safflower oil attractive to consumers due to its nutritional value.

Acknowledgments This work was funded by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), ANPCyT (Agencia Nacional de Promoción Científica y Tecnológica) and Universidad Nacional del Sur, Argentina. The authors wish to acknowledge Clarisa Cicchelli, Pedro Forbito and Federico Delucchi for their kind assistance during the analytical work.

Compliance with Ethical Standards

Conflict of Interest The authors have declared no conflict of interest.

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