RESEARCH ARTICLE

Canola Oil Extraction

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Hexane-Free Green Solvent Extraction of Canola Oil From Microwave-Pretreated Seeds and of Antioxidant-Rich Byproducts

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The ethanol extraction from microwave-pretreated and untreated canola seeds is studied. Two process are used to obtain the oil: in a control process (P1), the solvent-free total extract (E) is washed with hexane, obtaining an oil fraction (OF). The second process (P2) consists of the partial evaporation of the solvent of the total extract (E), cooling, centrifugation, and separation by decantation of the generated phases: oil-rich phase (OF + Ethanol), solvent-rich phase (EthF + Ethanol), and solid phase (SF); and then evaporating the solvent to obtain OF and an extract of soluble in the phase rich in ethanol (EthF). No significant differences due to microwaves are detected in the yields. P2 gave mean yields of 32.8%db of OF, lower than obtained with P1 (42.1%db), 4.2%db of precipitated solids, and 7.7%db of EthF, which present a mean content of hexane-solubles of 5.1%db. However, the quality analysis shows a smaller oxidative damage and an increase in canolol content due to the microwaves. P2 also generates an antioxidant-rich byproduct, allowing to recover the canolol prior to a refining stage of the oil.

***Practical Applications*: The use of organic solvents for the extraction of vegetable oils have some disadvantages, such as health and safety problems. At the same time, ethanol has begun to be studied as an alternative solvent due to its lower production costs than other alternative solvents, and to the fact that it is recognized as a "bio-renewable" solvent. Numerous studies have shown the extraction of oil and impurities obtained with ethanol. However, no method have been developed for obtaining an insoluble-free canola oil from the extract obtained with ethanol without using hexane. In addition, a favorable effect of the canolol content pretreated with microwaves in the canola oil is reported, nevertheless, the canolol is eliminated during the refining stage of the crude oil. The hexane-free development process allows to obtain canola oil and an antioxidant-rich byproduct, allowing to recover the canolol prior to a refining stage of the oil.**

1. Introduction

Canola oil is the third most consumed vegetable oil in the world. Known as the "oil of the heart," it has an optimal and distinctive ratio of ω-6:ω-3 fatty acids (2:1), it is low in saturated fat and high in tocopherols and other bioactive compounds.[1] The presence of canolol (a potent antioxidant) has been detected in the canola oil from seeds subjected to thermal treatments.[2] Canolol accounts for 85% of total phenolics in crude canola oil.[3] Several authors studied the effect of microwave pretreatment on oilseed extraction and the tocopherol content of canola oil extracted with hexane,[4,5] and by cold-pressing[6,7] reported an increase in the canolol concentration of canola oils due to a microwave pretreatment. In previous works, a content of 27 mg kg^{-1} of canolol was reported for canola oil extracted with hexane, and of 168 mg kg^{-1} for canola oil extracted with hexane from microwave-pretreated seeds,[5] while a content of up to 532 mg kg^{-1} was observed in canola oil extracted by pressing from seeds subjected to combined hydrothermal-microwave pretreatments.[8] Increases in the oil yields from microwave-pretreated seeds extracted with hexane have also been reported.[4,5] As for canola meal, it is rich in phenolic compounds (6.3–18.4 mg g^{-1} of defatted meal[9] and has a protein content of 38–43% with a suitable amino acid composition, which is used for the

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production of protein concentrates for human and animal consumption.[10,11]

The oil extraction process is carried out by pressing and/or solid-liquid extraction, where hexane is the most commonly used solvent in the industry due of its stability, high solubilizing power, and convenient boiling point that favors its recovery.[12,13] However, this solvent obtained from petroleum is highly flammable, thus increasing the costs of industrial safety systems and causing negative effects on health and the environment, in addition to the negative image that consumers have of solvents,[14,15] thus being of interest to replace them by benign and more environmental friendly solvents.

Works on the oil extraction of oilseeds with alternative processes have been reported. Stahl et al.[16] carried out the extraction of sunflower and canola oil with supercritical carbon dioxide, while Zhang et al.[17] extracted canola oil by an enzymatic method in an aqueous medium.

Ethanol has begun to be studied as an alternative solvent[18] because of its lower costs of production than other alternative solvents, and the fact that it is recognized as a "green biosolvent," since it can be produced by fermentation from plants growing widely over the world, it generates no environmental pollution and presents a lower volatility than hexane, which makes it a safer solvent. In turn, the ethanol 99.5% production from azeotropic ethanol has been studied by extractive distillation using glycerol as extractive agent[19] finding lower energy costs than conventional methods, glycerol being a byproduct of biodiesel production, an important industry in Argentina. Due to its polar nature, the extraction of minor nutritional compounds such as tocopherols[20] could be achieved, increasing the quality of the oil obtained. However, at the same time it could produce the extraction of insoluble compounds such as some phosphatides, pigments, and sugars that would need to be removed from the oil.[21]

Recently, the extraction of soybean oil was studied using different ethanol-water proportions as solvent, resulting in the extraction of a protein fraction in addition to the oil.[22] Baümler et al.[20] studied the extraction of sunflower oil from collets using ethanol as solvent, reporting the extraction of oil insolubles such as sugars, among others, and separating them by hexane fractionation. In previous works, canola oil extraction was analyzed in an exploratory way using ethanol as solvent, and later by fractionating the extract with hexane.[23] Hron and Koltun[24] developed an oil extraction process for cottonseed using a mixture of ethanol and water, separating the oil from the obtained extract by cooling and decantation and Kulkarni et al.[25] extracted flaxseed oil by a three-phase partitioning process using t-butanol. Linnet[26] describe a process of extraction of vegetable oils using ethanol as a solvent, showing the potential use of this type of green processes at the industrial level. In turn, Carré et al.[18] evaluated the economic feasibility of a rapeseed oil extraction process with ethanol, finding higher costs in the pretreatment of the seeds but higher values in the products obtained than the conventional processes. In this context, the implementation of a microwave pretreatment could represent not only an increase in the canolol content but also a reduction in energy and reduction of times in the previous seed drying stage. The aim of this work was to prove that it is possible to obtain canola oil by means of a solid-liquid extraction process using ethanol as solvent and without the use of hexane, maintaining high yields and obtaining high antioxidant extracts due to the application of a microwave pretreatment.

2. Experimental Section

2.1. Raw Materials

A batch of 10 kg of winter canola Hornet variety (harvest year: 2015, stored for 7 months at 4 °C) supplied by AL HIGH TECH S.R.L., colza 00 breeder (Argentina) was used.

The characterization of the raw material according to its proximate composition was carried out by Sánchez et al.,[5] presenting a moisture content of 8.2 ± 0.3 percent on dry basis (%db), 46.3 ± 0.3%db of oil, 24.9 ± 0.8%db of nitrogen-free extract, 20.3 ± 0.1%db of proteins, 5.0 ± 0.1%db of crude fiber, and 3.5 ± 0.1%db of ash.

2.2. Microwave Pretreatment

The methodology proposed by Ramos et al.[4] was used, subjecting the canola seeds to radiation for 5 min at 607 W of power (optimum values determined by Ramos et al.[4]) in a BGH Quick Chef model 36960 microwave oven (Argentina).

2.3. Extractions

For each extraction, 20 g of canola seeds were ground in a coffee grinder (Moulinex, Argentina), the resulting granulometry was analyzed with vibratory sieves (Zonitest, Argentina) for both the microwaved sample and the untreated one. The sample brought into contact with 99% ethanol in a stirring batch system (magnetic stirrer) with a thermostatically controlled bath at a constant temperature of 60 °C for 4 h, using previously determined optimal conditions (unpublished data). A ratio of 17 mL of solvent/g of meal was used.[5,27,28] After the set time, the contents were centrifuged in a Presvac MSP-4650 R Plus refrigerated equipment (Argentina) for 5 min at 5438 G. Then the sample was filtered and the micelle was collected in a flask (Et). The oil was obtained by two different methods.

2.4. Oil Extraction

Figure 1 shows the schematics of the two methods (P1 and P2) applied to obtain the oil. P1 involves a hexane washing step similar to that used in other works[20,23] in order to perform a control comparison with P2, which is hexane-free. The complete process to obtain the oil (extraction and separation of oil insolubles) was carried out in duplicate both for P1 and P2.

2.4.1. Process 1: Hexane Washing (P1)

In method P1, the overall solvent of the total extract (Et) obtained in the ethanol extraction was evaporated in a R-3000 Büchi rotary evaporator (Switzerland), thus obtaining the total solvent-free

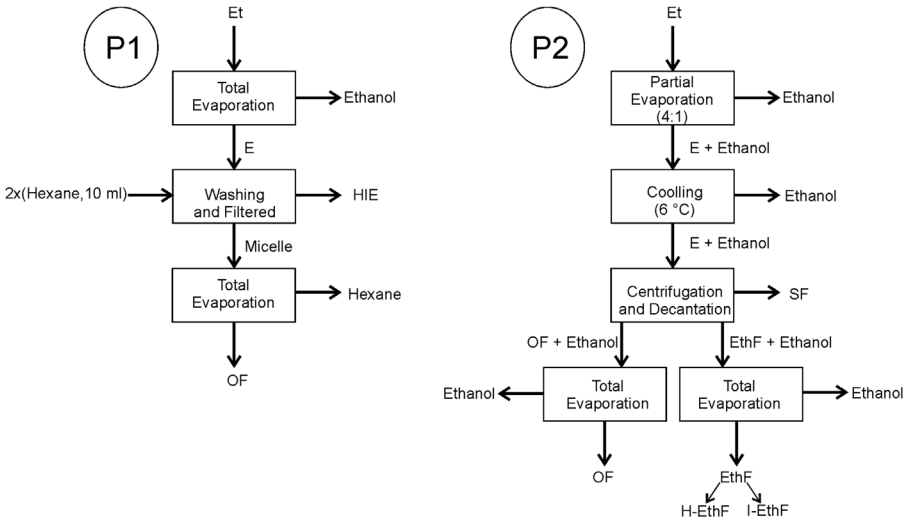

Figure 1. Schematic view of the methods used to obtain oil. P1: Process 1: Hexane washing. P2: Process 2: Phase separation. Et: Total extract. E: Total solvent-free extract. HIE: Fraction of hexane-insoluble extract for P1. OF: Fraction of hexane-soluble extract for P1 and P2. SF: Solid phase obtained by P2. EthF: Solubles in the ethanol-rich phase. I-EthF: Hexane-insolubles of the ethanol-rich phase by P2. H-EthF: Hexane-solubles of the ethanol-rich phase by P2.

extract (E), which was washed by adding 10 mL of hexane and filtered with white-band quantitative filter paper JP40 (Prolab, Sao Pablo/SP/BRA). The procedure was repeated once. The residue retained on the filter is the fraction of hexane-insoluble extract (HIE). The hexane was evaporated from the collected solution to obtain the fraction of hexane-soluble extract, the oil fraction (OF). Quantification of all fractions was performed gravimetrically. Assays were performed in duplicate.

2.4.2. Process 2: Phase Separation (P2)

The method P2 (similar to that used by Hron and Koltun,[24] involved the partial evaporation of the solvent (to an approximate solvent:oil ratio of 4:1) of the total extract (Et), resulting in the generation of three phases: an oil-rich phase (OF + ethanol), a solvent-rich phase (EthF + ethanol), and a solid phase (SF). Given the dependence of the solubility of oil in ethanol with temperature[18] and in order to decrease the final content of oil in the phase rich in ethanol, the concentrated extract was cooled to 6 °C and centrifuged at 5438 G for 15 min. Then the three phases were separated by decantation, and the ethanol was evaporated from each phase, thus obtaining separately oil and solubles in the ethanol-rich phase (EthF). Quantification was performed gravimetrically. In turn, fractionation of EthF with hexane was performed to determine the content of hexane-solubles. All assays were performed in duplicate.

2.5. Properties of the Obtained Products

The oils were characterized by the acid value according to IUPAC 2.201,[29] peroxide index (PI) according to AOCS Cd 8,[30] p-anisidine value (pAV) according to Cd 18–90 AOCS,[30] fatty acid composition (determined by gas chromatography).[31] Methylation of fatty acids 1 mL of sample, 1 mL of cloroforme (99.0–99.4%, Merck), and 1 mL of methanolic acid (cetyl chloride (≥98.0%, Merck) in methanol (99.9&, Sintorgan) were added in s glass tube. The tubes were shaken for 1 min and placed in a water bath at 70 °C for 1 h. After cooling to room temperature, 4 mL of 6% K2CO3 was added and stirring for 1 min. After phase separation upper phase (aqueous phase) was removed and 2 mL of cloroforme was added to lower phase (oil phase). After phase separation, 1 mL of phase with methyl esters was injected into a Shimadzu GC − 2014 Chromatograph (China), with FID detector. The GC was equipped with a HP-23 capillary column (cis/trans FAME Column, D 0.25 mm). Temperatures of the oven, detector, and injector were 210, 240, and 300 °C, respectively. The split relation was 160.4. Fatty acid methylesters were identified by comparison with known AOCS #3 standards: Methyl myristste (99.8%), Methyl palmitate (99.8%), Methyl stearate (99.9%), Methyl oleate (99.7%), Methyl linoleate (99.5%), Methyl arachidate (99.6%), Methyl behenate (99.6%), Methyl erucate (99.4%), Methyl lignocerate (99.5%) (Restek, Reagents) with peak area used for quantification).

Total oxidation index (Totox) according to (1):

$$\text{Totox} = 2.PI + pAV \quad (1)$$

In turn, the canolol content and tocopherol composition were determined for all fractions following the technique described by Sánchez et al.,[5] and the total carbohydrate content of the hexane-insoluble fractions was determined by the phenol-sulfuric method described by DuBois et al.[32] with an external standard curve using glucose (99%, Merck). A solution of 10 μg mL^{-1} of the sample in water was prepared, 1 mL of the solution and 1 mL of 5% aqueous solution of phenol was added in a test tube. A 5 mL of concentrated sulfuric acid was added rapidly to the mixture. After 10 min, the tubes are shaken for 30 s and placed for 20 min in a water bath at room temperature for color development. The blank and the standard solution were prepared according to the procedure described above, using glucose. Finally, the samples was measurement in UV 1800 PC

mapped spectrophotometer (China) with a wavelength of 490 nm. Assays were performed in duplicate.

2.6. Statistical Analysis

Results are reported as mean ± standard deviation. In order to detect differences between the yields, analysis of variance (ANOVA) with Tukey's test was used, considering that the means were significantly different if $p \leq 0.05$, using the Infostat software.[33]

3. Results and Discussion

3.1. Granulometry

Figure 2 shows the distribution of particles for pretreated and untreated samples. The pretreatment with microwaves allowed obtaining a granulometry of smaller size in comparison with the sample without pretreatment

3.2. Oil Yields

The yields (expressed as percentage on dry basis and relative to the original sample,%db) obtained for the different fractions using methods P1 and P2 are shown in **Table 1**.

The yield values obtained by the free-hexane P2 were similar to those obtained by other hexane-free processes. Stahl et al.[16] reported an oil yield of 32.8% for the supercritical extraction of canola oil with carbon dioxide. Zhang et al.[17] obtained up to 76% of total oil by the aqueous enzymatic extraction of rapeseed oil.

For both methods P1 and P2 and for all the obtained fractions, no significant effect of the microwave pretreatment was observed on the oil yields ($p \geq 0.05$). Ramos et al.[4] did not detect significant differences as of 4 h of hexane Soxhlet extraction in the oil yields obtained from microwave-pretreated and unpretreated seeds, and the same behavior was observed in the extraction with ethanol.

Since ethanol can extract compounds such as carbohydrates and proteins,[34] the mean HIE for P1 (4.5%db) can be attributed to these compounds. Baümler et al.[20] obtained 9.98%db of hexane-insolubles from sunflower collets processed by Soxhlet extraction using ethanol (95%) as solvent, reporting that they mostly consisted of sugars and phospholipids.

In the case of method P2, the oil yields (OF) were significantly lower than that obtained by P1 (average difference 9.3%db), however the sum of mean oil and EthF obtained by P2 (OF + H-EthF, 37.8%db) presented a difference of 4.2%db points with respect to the mean OF yield obtained by P1. In turn, 41% of the oil-insoluble solids present in E (HIE for P1) could be separated by P2 as precipitated fraction (SF). It is worth noting that a solid in oil (soluble in hexane) was observed for the OF obtained by P1, which was not detected in the oil extracted with hexane.[5,27,35] This solid could be attributed to phospholipids and other minor lipids with structural functions in the fatty bodies and in other organelles of vegetable cells, which could be extracted with ethanol (polar compound) but not with non-polar solvents such as hexane.[36] However, once the structures formed by these compounds are dissolved, they could be solubilized in hexane. Further studies are necessary to confirm the composition of this solid fraction in the OF. On the other hand, the presence of these compounds was not observed in the oil obtained by P2; these could be included in the EthF. The results of this work are consistent with those reported by Yatsu and Jacks,[37] who extracted oil with hexane from isolated fatty bodies, and observed by electron microscopy that the membranes were not extracted by the hexane. In turn, Tzen and Huang[38] separated the interfacial material from fatty bodies by extracting the oil with diethyl ether and dissolving the interfacial material (proteins, phospholipids, glycolipids, etc.) with mixtures of chloroform and methanol (polar compounds).

It was observed that the solvent-free hexane-soluble fraction of EthF (H-EthF) was in the solid state, which is consistent with the findings stated above.

3.3. Quality Analysis of the Obtained Fractions

3.3.1. Hexane-Soluble Fractions

Tables 2–5 present the quality characteristics of the hexane-soluble fractions.

No differences were detected in the fatty acid composition for the samples extracted with ethanol (P1 and P2) and with hexane (**Table 2**).

As for Total Tocopherol Content (TTC), no significant effect of the microwave pretreatment was detected, and similar results were reported by Sánchez et al.[5] and Ramos et al.[4] for canola oil extracted from microwave-pretreated seeds using hexane. In turn, it was observed that the TTC content in the OF obtained by

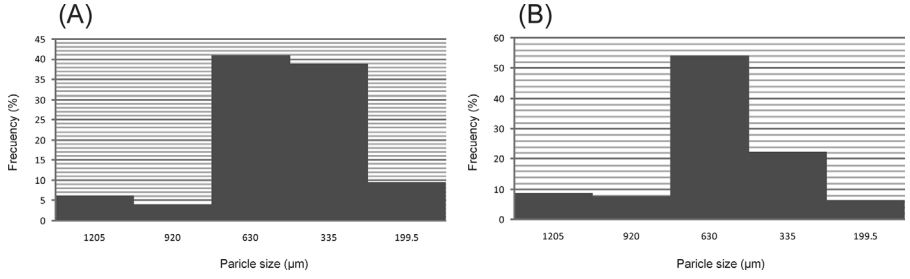

Figure 2. Particle size distribution. A) Microwave-pretreated sample. B) Unpretreated sample.

Table 1. Fraction yields obtained by P1 and P2 extraction methods.

	Ethanol				
	Untreated sample		Microwave pretreated sample		Hexane
Solvent	P1	P2	P1	P2	Untreated sample
OF (%db)	$41.7^{a} \pm 0.8$	$32.4^{b} \pm 0.2$	$42.4^{a} \pm 1.1$	$33.1^{b} \pm 1.3$	$39.1^{a} \pm 0.8$
HIE (%db)	$4.7^{a} \pm 0.2$	–	$4.3^{a} \pm 1.4$	–	–
SF (%db)	–	$4.5^{a} \pm 0.2$	–	$4.0^{a} \pm 0.2$	–
H-EthF (%db)	–	$5.0^{a} \pm 0.1$	–	$5.1^{a} \pm 0.3$	–
I-EthF (%db)	–	$3.0^{a} \pm 0.4$	–	$2.3^{a} \pm 0.2$	–

P1: Process 1, Hexane washing; P2: Process 2, Phase separation; Hexane, Hexane extraction; OF, Oil fraction; HIE, Fraction of hexane-insoluble extract for P1; I-EthF, Hexane-insolubles of the ethanol-rich phase by P2; SF, Solid phase obtained by P2; H-EthF, Hexane-solubles of the ethanol-rich phase by P2. Different letters in same line indicate significant differences (Tukey's test, $p < 0.05$).

P1 was higher than that obtained by P2, but it was not significantly different from that of the oil obtained with hexane, showing to be independent of the type of solvent used in the extraction of tocopherols. In general, the same behavior was observed for the different tocopherol isomers (**Table 3**). The H-EthF fractions presented a total tocopherol content up to 467% higher than that of the P2 OF, indicating a concentration of these compounds in the H-EthF phases (**Table 4**). These results show that during the formation of two phases in the P2 process, part of the extracted total tocopherols remained in the ethanol-rich phase. It should be noted that the HIE and SF fractions are incompatible with the determination technique of tocopherols and canolol contents.

Canolol exhibited an increase of up to 317% by effect of the microwave pretreatment, indicating the production of this compound with microwave radiation,[5–7,39] and of up to 2564% in the H-EthF phases with respect to the oils obtained by P2, presenting a larger concentration thereof (Table 4). These results show that an antioxidant-rich byproduct was obtained, containing 83% of the canolol of the OF obtained by P1. The activity of the investigated phenolic compounds was related to the number of hydroxyl groups and methoxyl substituents in the aromatic ring.[40] In this context, canolol has a greater number of methoxyl substituents in the aromatic ring than tocopherols, while both compounds have a hydroxyl group in the aromatic ring. In turn, Cortese et al.[41] reported a significant increase in the oxidative stability of canola oil obtained by pressing due to hydrothermal-microwave pretreatments applied, attributing it to a higher concentration of canolol while, as in the present work, did not detect significant differences in the content of tocopherols. For all the previously discussed, the canolol seems to be better to handle the canola oil oxidation. Matthaüs et al.[42] obtained a canolol enriched extract from rapeseed meal wich was added to frying medium improving thermal stability. In Adition, More than 90% of the canolol is eliminated during the refining step of the crude oil, being necessary to optimize the refining processes in order to increase the canolol concentration in edible canola oils;[3,43] thus the proposed P2 would allow to recover this valuable component prior to the refining step in an industrial process.

The canolol content of the OF obtained by P1 from the untreated sample was significantly higher than that of the oil obtained with hexane, indicating a greater extraction capacity of this compound with ethanol than hexane.

Although significant differences in AV were detected, all the values were below the maximum limit established for virgin oils (4.0 mg KOH/g oil, CODEX STAN 192–1995[44]).

Significantly lower peroxide indices (PI) were observed for the pretreated samples compared to the unpretreated ones. However the PI of the P2 OF was higher than that of the P1, and it was not detected in the sample extracted with hexane compared to the unpretreated sample, possibly due to the fact that the hexane

Table 2. Fatty acid composition of the oil fractions (OF) obtained by process P2 and P1 (microwave-pretreated and unpretreated samples, respectively), and with hexane.

	Ethanol				
	Untreated sample		Microwave pretreated sample		Hexane
Solvent	P1	P2	P1	P2	Untreated sample
C16:0 (%)	$4.53^{a} \pm 0.41$	$4.91^{a} \pm 0.44$	$4.95^{a} \pm 0.44$	$5.22^{a} \pm 0.46$	$5.60^{a} \pm 0.5$
C18:0 (%)	$1.96^{a} \pm 0.14$	$1.78^{a} \pm 0.13$	$1.98^{a} \pm 0.14$	$1.85^{a} \pm 0.13$	$1.72^{a} \pm 0.12$
C18:1 (%)	$66.59^{a} \pm 6.09$	$66.72^{a} \pm 6.10$	$66.79^{a} \pm 6.11$	$66.64^{a} \pm 6.10$	$65.71^{a} \pm 6.01$
C18:2 (%)	$18.83^{a} \pm 0.36$	$18.58^{a} \pm 0.35$	$18.42^{a} \pm 0.35$	$18.43^{a} \pm 0.35$	$19.03^{a} \pm 0.36$
C18:3 (%)	$8.08^{a} \pm 0.57$	$8.00^{a} \pm 0.56$	$7.86^{a} \pm 0.51$	$7.86^{a} \pm 0.55$	$7.94^{a} \pm 0.55$

P1, method 1; P2, method 2. Different letters in the same line indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 3. Tocopherols and canolol of the oil fractions (OF) obtained by process P2 and P1 (microwave-pretreated and unpretreated samples, respectively), and with hexane.

	Ethanol				
	Untreated sample		Microwave pretreated sample		Hexane
Solvent	P1	P2	P1	P2	Untreated sample
TTC ($\mu g\,g\,e^{-1}$)	$650.3^{b} \pm 6.4$	$442.9^{a} \pm 29.6$	$708.9^{b} \pm 8.4$	$403.2^{a} \pm 25.1$	$780.2^{b} \pm 9.4$
α-TC($\mu g\,g\,e^{-1}$)	$221.3^{b} \pm 3.7$	$152.1^{a} \pm 13.3$	$226.3^{b} \pm 5.7$	$137.4^{a} \pm 5.0$	$301.4^{c} \pm 5.5$
β-TC ($\mu g\,g\,e^{-1}$)	$44.0^{b} \pm 0.1$	$55.7^{c} \pm 3.7$	$33.7^{a} \pm 2.5$	$50.0^{b,c} \pm 3.1$	$31.2^{a} \pm 0.6$
γ-TC ($\mu g\,g\,e^{-1}$)	$371.8^{b} \pm 0.9$	$227.4^{a} \pm 11.5$	$424.7^{c} \pm 6.5$	$208.3^{a} \pm 10.7$	$445.6^{c} \pm 4.7$
δ-TC ($\mu g\,g\,e^{-1}$)	$13.1^{a,b} \pm 1.8$	$7.8^{a} \pm 0.4$	$24.3^{b} \pm 6.3$	$7.6^{a} \pm 6.3$	$1.9^{a} \pm 0.2$
Canolol ($\mu g\,g\,e^{-1}$)	$15.8^{c} \pm 0.1$	$3.7^{a} \pm 0.2$	$65.9^{d} \pm 0.4$	$11.0^{b} \pm 0.4$	$10.8^{b} \pm 0.1$

P1, method 1; P2, method 2; TTC, total tocopherol; α-TC, alpha tocopherol; β-TC, Beta tocopherol; γ-TC, Gamma tocopherol; δ-TC, Delta tocopherol. All tocopherol contents are expressed as μg tocopherol g^{-1}. e: HSE or oil, as appropriate. Different letters in the same line indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 4. Content of tocopherols and canolol of the hexane-solubles in the ethanol-rich phases (H-EthF) obtained by P2.

	H-EthF	
	Untreated sample	Microwave-pretreated sample
TTC ($\mu g\,g^{-1}$)	$2165.6^{a} \pm 36.7$	$2285.0^{a} \pm 20.7$
α-TC($\mu g\,g^{-1}$)	$739.5^{a} \pm 7.1$	$747.4^{a} \pm 14.8$
β-TC ($\mu g\,g^{-1}$)	$87.3^{a} \pm 1.6$	$89.9^{a} \pm 1.8$
γ-TC ($\mu g\,g^{-1}$)	$1317.8^{a} \pm 7.9$	$1384.6^{b} \pm 20.3$
δ-TC ($\mu g\,g^{-1}$)	$20.9^{a} \pm 20.0$	$63.1^{a} \pm 17.0$
Canolol ($\mu g\,g^{-1}$)	$95.5^{a} \pm 5.6$	$293.1^{b} \pm 7.6$

TTC, Total tocopherols; α-TC, Alpha tocopherol; β-TC, Beta tocopherol; γ-TC, Gamma tocopherol; δ-TC, Delta tocopherol. All tocopherol contents are expressed as μg tocopherols g^{-1}. e: Hexane-solubles of the ethanol-rich phase. Different letters in the same line indicate significant differences (Tukey's test, $p \leq 0.05$).

extraction presents a smaller number of stages. All the PI values were lower than the maximum limit established for virgin oils (15 meq O2/kg oil, CODEX STAN 192-1995[44]).

The values of p-anisidine (pAV) exhibited the same behavior as PI with respect to the microwave effect (which was lower in the case of the microwave pretreated samples); however, when comparing OF, pAV presented the opposite behavior to PI. The pAV of the sample extracted with hexane was not significantly different from that obtained by P2 for microwave-pretreated seeds. Since PI is an indicator of the primary oxidation products while pAV analyzes the secondary oxidation products, the total oxidation products were determined by calculating the Totox index in order to analyze the effects of the microwave pretreatment and the extraction process. The same behavior as the quality indices analyzed above was observed with respect to microwave treatment, while differences in the values of OF for P2 with respect to P1 were smaller, following the trend of PI. The sample extracted with hexane presented the lowest Totox value, which is consistent with the behavior of pAV.

The different behaviors of the indices PI, pAV, and Totox between the fractions obtained by P1 and P2 may be due to a heterogeneity in the distribution of oxidation compounds between the phases obtained during phase separation in P2 (OF and EthF), which results in different PI, pAV, and Totox values for P2 OF compared to OF values for P1. In turn, the differences between PI, pAV, and Totox values for the microwave-pretreated samples compared to the unpretreated samples could be attributed to an antioxidant protection effect of canolol, which is described as a potent and effective antioxidant in the literature[2,15,45] which increases oxidative stability.[46]

Table 5. Quality analysis of the oil fractions (OF) obtained by process P2 and P1 (microwave-pretreated and unpretreated samples, respectively), and with hexane.

	Ethanol				
	Untreated sample		Microwave pretreated sample		Hexane
Solvent	P1	P2	P1	P2	Untreated sample
AV ($mgKOH/g_{oil}$)	$1.76^{d} \pm 0.01$	$0.71^{a} \pm 0.02$	$1.61^{c} \pm 0.05$	$0.72^{a} \pm 0.01$	$1.09^{b} \pm 0.03$
PI ($meq\,kg^{-1}$)	$7.03^{c} \pm 0.01$	$10.90^{d} \pm 0.07$	$3.16^{a} \pm 0.02$	$6.14^{b} \pm 0.02$	ND
pAV	$9.36^{d} \pm 0.57$	$4.39^{b} \pm 0.08$	$6.59^{c} \pm 0.12$	$3.13^{a} \pm 0.12$	$2.05^{a} \pm 0.07$
Totox	$23.42^{d} \pm 0.59$	$26.19^{e} \pm 0.06$	$12.90^{b} \pm 0.15$	$15.41^{c} \pm 0.08$	$2.05^{a} \pm 0.07$

P1, method 1; P2, method 2; AV, acid value; PI, peroxide index; pAV, p-anisidine index; Totox, Total oxidation index; ND, not detected. Different letters in the same line indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 6. Carbohydrate content of hexane-insoluble fractions.

Fraction	Carbohydrate content (%db)
Untreated sample	
HIE	$3.1^{c,d} \pm 0.2$
SF	$0.7^{a} \pm 0.1$
I-EthF	$2.3^{b,c} \pm 0.3$
Microwave-treated samples	
HIE	$3.9^{d} \pm 0.2$
SF	$0.6^{a} \pm 0.1$
I-EthF	$1.8^{b} \pm 0.2$

%db, Percentage on dry basis relative to the original sample of ground seeds; HIE, Fraction of hexane-insoluble extract for P1; I-EthF, Hexane-insolubles of the ethanol-rich fraction for P2; SF, Solid phase obtained by P2; H-EthF, Hexane-solubles of the ethanol-rich phase obtained by P2. Different letters indicate significant differences (Tukey's test, $p \leq 0.05$).

3.3.2. Hexane-Insoluble Fractions

Table 6 shows the characterization of the hexane-insoluble fractions as a function of total carbohydrate content.

The presence of carbohydrates was observed in all the analyzed fractions, which is consistent with the comments in section 3.2.

No significant effect of the microwave treatment was detected on the total carbohydrate content for any fraction. A larger content of carbohydrates was observed in the I-EthF fractions than in the SF fractions, showing that most of the extracted carbohydrates have a high solubility in ethanol. The precipitated fractions (SF) contained a low carbohydrate content, suggesting the difference observed between the total hexane-solubles obtained by P2 and by P1 (4.2%db), with a significant oil retention in these fractions.

4. Conclusions

The process of solid-liquid extraction with ethanol applied to canola seeds allowed to obtain a solvent-free total extract of approximately 45%db containing hexane-insoluble compounds. The three generated fractions were oil-rich phase (OF + Ethanol), a solid phase (SF), and a solvent-rich phase (EthF + Ethanol). The latter phase, in turn, contained hexane-soluble compounds (H-EthF) and hexane-insoluble compounds (I-EthF). A 41% of the hexane-insoluble compounds could be separated as SF.

Despite that the OF yield found for the free-hexane process (P2) was 78% of OF yield obtained with a process using ethanol and hexanes (P1), by applying P2 it was possible to obtain an OF free of hexane insolubles without using hexane as solvent. It is worth mentioning that oil can be extracted from the solvent-rich phase using hexane, being the use of EthF fraction another promising alternative, due to its rich in antioxidants composition. The use of microwaves as pretreatment allowed to improve the protection against oxidation of OF. In addition, H-EthF fractions obtained by P2 showed a high antioxidants content, with a high tocopherol concentration and also a higher canolol content; which indicates an important contribution of this process to the quality of the products, being promising its incorporation to an industrial process. The results show the viability of the microwave pretreatment and the extraction and separation of oil with ethanol without using hexane, without loss of quality of the obtained oil, and obtaining byproducts with high antioxidant properties. Besides, the results obtained in this study may represent a starting point for future research where scaling up and industrial implementation are raised, with the consequent energetic costs reduction and the deepening of the applications of the obtained subproduct.

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Nomenclature

Et	total extract
E	total solvent-free extract
P1	process 1: Hexane washing
P2	process 2: Phase separation
HIE	fraction of hexane-insoluble extract for P1
OF	Oil fraction, hexane-soluble extract
OF+ethanol	oil-rich phase
EthF+ethanol	ethanol-rich phase
SF	solid phase
EthF	solubles in the ethanol-rich phase
I-EthF	hexane-insolubles of the ethanol-rich phase
H-EthF	hexane-solubles of the ethanol-rich phase
AV	acidity value
PI	peroxide index
pAV	p-anisidine value
Totox	total oxidation index

Conflict of Interest

The authors declare no conflict of interest.

Keywords

canola oil, canolol, ethanol, extraction, microwaves

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