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Quality of borage seed oil extracted by liquid and supercritical carbon dioxide

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Abstract

This paper describes the extraction of borage seed oil by liquid and supercritical carbon dioxide, from the point of view of both operative method and pre-treatment of raw material. The best conditions for borage seed oil extraction are obtained at 300 bar, 40 °C and a solvent flow rate of 1.5 l/min at STP. The yields obtained are very similar to those resulting from the conventional extraction process using hexane as solvent (26.0 wt.%); however, the quality of oil extracted by supercritical fluid is higher (acidity, 11.0; unsaponifiables, 1.8). The fatty acid composition of the borage seed oil extracted by SFE is particularly rich in unsaturated fatty acids, especially the γ-linolenic acid (21.7%). For these reasons, the borage seed oil extracted using carbon dioxide could be competitive with the conventional process, since it simplifies considerably the oil refinement stages and completely eliminates the solvent distillation stage, which are the most costly processing stages in terms of energy consumption.

Keywords: Supercritical fluid; Supercritical fluid extraction; Carbon dioxide; Borage seed oil; Linolenic acid; GLA

1. Introduction

γ-Linolenic acid (18:3 Δ 6, 9, 12) is the first intermediate formed during the conversion of linoleic acid (18:2 n-6) to prostaglandins by means of the dehydrogenation of the C-6 position by the enzyme A-6 dehydrogenase. However, factors like stress or diabetes, can reduce, or even inhibit, the formation of γ-linolenic acid (GLA), causing an alteration of the metabolism and series of dysfunctions [1]. Consequently, the possibility to restore the levels of GLA appropriately through a supplementary diet has motivated a growing interest in the search of vegetables oils rich in this essential fatty acid [1–4].

The main commercial sources of GLA are the oils of primrose seed (*Oenothera biennis* L.), borage (*Borago officinalis* L.) and currant (*Ribes nigrum* L.). Of these, the one that presents the highest levels in GLA is the borage seed oil [5,6].

Traditionally, these vegetable oils have been obtained by means of conventional extraction with organic solvent. However, possible changes in the physicochemical properties of the GLA can alter their functionality; thus, these extraction processes should be carried out at moderate conditions, paying special attention to temperature [5].

Supercritical fluid extraction (SFE) is an alternative to supplement or to substitute the conventional separation systems (distillation or liquids solvents extraction), because energy yields and separation effectiveness is good [7].

The SFE is a unitary mass transfer operation based on the use of fluids at temperatures and pressures above the critical values [8]. These conditions make the supercritical fluid (SF) present peculiar physicochemical properties between the gas and liquid states, which confers them with exceptional characteristics solvent [8,9].

This separation technique offers extraction yields very similar to those obtained by conventional extraction processes using liquid solvents, but it requires a certain combination of operating temperature and pressure. Its advantages, compared to organic solvents, are that carbon dioxide is non-toxic, non-flammable, non-corrosive; it is cheap and readily available in bulk quantity, with a high degree of purity [10]. In processing terms, carbon dioxide has a low critical temperature and pressure (31.1 °C and 73.8 atm, respectively) which makes it the ideal solvent for natural products, since they do not suffer thermal degradation reactions during the process [11].

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In this work, the extraction of borage seed oil is studied using liquid and supercritical carbon dioxide. Working conditions of the process (operating pressure, temperature and solvent flow rate) and the pre-treatment of the raw material (grain size and humidity) have been optimised. In a second study, a comparison between the relative qualities of the oils obtaining by SFE and by organic solvent extraction using hexane has been made. The composition of fatty acids under the best conditions of extraction is also determined.

2. Materials and methods

The raw material used for the processes was borage seed (*B. officinalis* L.) supplied by Intituto de Agricultura Sostenible (Córdoba, Spain).

If necessary, the borage seed was crushed in a coffee mill "Futurmat" (Barcelona, Spain) model FP, of 2 kg capacity and capable of milling to 18 grades of particle size. Dehydration of the raw material was carried out by heating to 65 °C until constant weight.

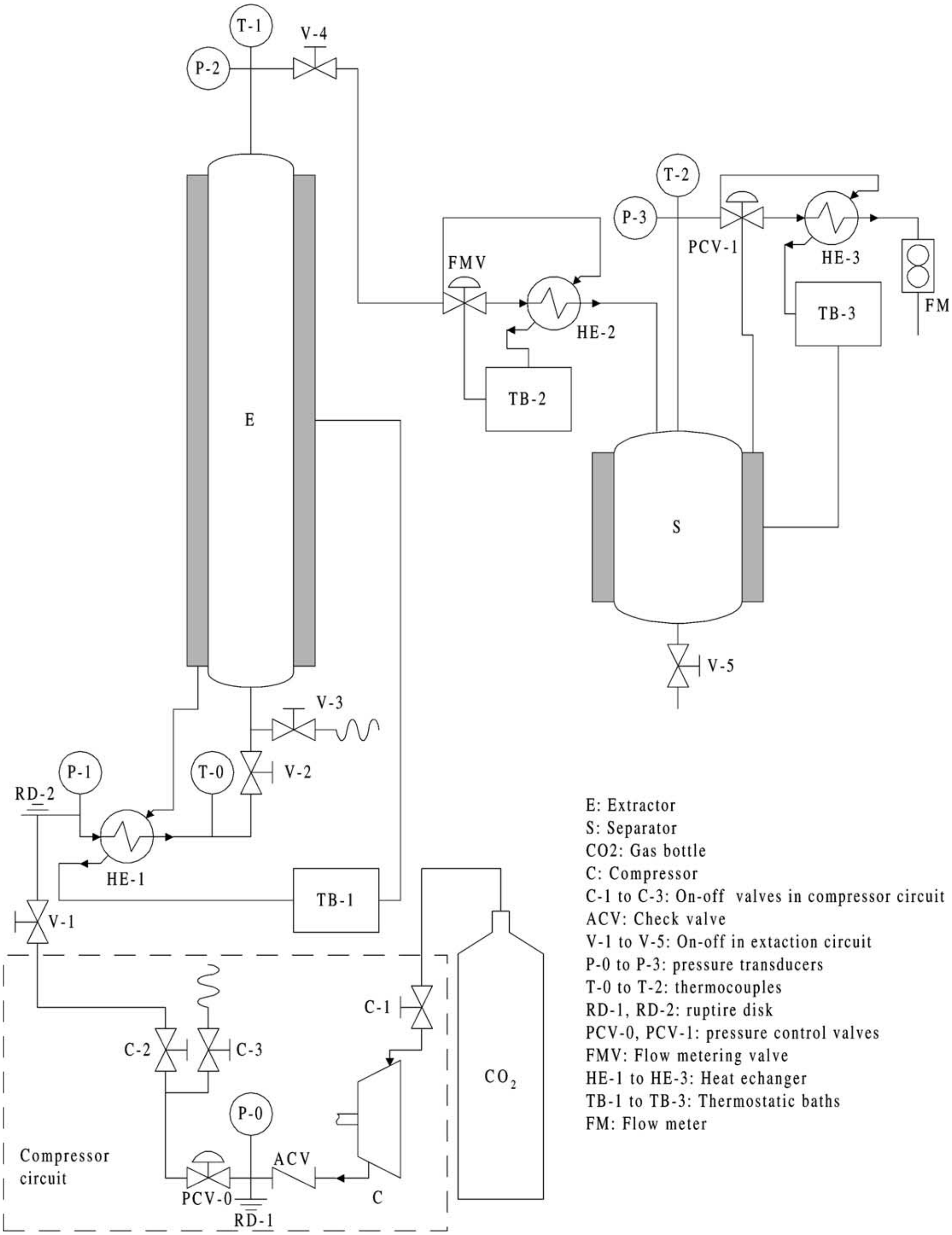

Fig. 1. Schematic diagram of the equipment used for the SFE.

In order to compare the conventional extraction process with liquid solvents, a “Soxhlet” type apparatus with hexane as solvent (250 ml of hexane and 35 g of borage seed) was used. Extraction time was 16 h, which guaranteed the full depletion of the grain and allowed the maximum possible extractive yield to be determined. These values are considered very important to establish an indisputable basis for comparison to the high-pressure process.

The equipment used for the SFE processes with carbon dioxide was the “high-pressure system” model manufactured by “Nova Swiss” (Effretikon, Switzerland) and it is shown (as a schematic diagram) in Fig. 1.

Thermostatic baths were switched on to reach the operating temperature required for extraction. Gas CO_2 was introduced into a compressor capable of delivering a maximum outlet pressure of 1600 bar. The extraction vessel was a 75 ml volume, 316 SS high-pressure cylinder, capable of operating up to 700 bar and 80 °C, with brass tube jacket for the circulation of water. A sample of about 40 g was placed in the extractor vessel. The pressure of the CO_2 cylinder was maintained throughout the system by opening valves V-1, V-2 and V-4 and closing the other valves (V-3, V-5 and PCV-1). The compressor was switched on and its output pressure increased by the pressure-regulating valve to reach the required processing pressure. When both the desired pressure and temperature were reached, the extraction was started and the flow rate of carbon dioxide through the extractor vessel was regulated by the flow micrometer valve. The oil dissolved in the supercritical CO_2 was separated from the carbon dioxide and collected in the separator of 200 ml capacity capable of operating up to 700 bar and 80 °C. The pressure in the separator was regulated using the PCV-1 valve; in this work, the separator was maintained at ambient temperature and pressure. The uncompressed CO_2 was passed through an inline volumetric flow meter, which controlled the quantity and flow rate of CO_2 used. The flow meter used was a model FC-70 supplied by “EG&G Flow Technology” (Phoenix, AZ).

The optimisation of the extraction process was carried out along the following ranges of operating conditions: pressure, from 50 to 350 bar; temperature, 10, 40 and 60 °C; solvent flow rate, from 0.5 to 2.0 l/min.

The physicochemical characterisation of the oils extracted was performed according to the legislation for the analysis of oils for human consumption [12]. The parameters analysed were: density, viscosity, and absorbance in the UV region. The indexes determined were: refraction, acidity (expressed as the % of oleic acid), iodine (by the Hanus method), saponification and peroxides. The percentage of unsaponifiables was also determined.

The fatty acid composition of the oil was determined, after methylation, by gas chromatography using a model 5890 Hewlett Packard (Pittsburgh, PA) fitted with a “Carbowax 20M” (Supelco, Bellefonte, PA) capillary column and an FID detector.

All the products and chemical reagents used were of analytical quality. The carbon dioxide used was of 99.95% purity from Carburos Metálicos, S.A. (Sevilla, Spain).

3. Results and discussion

3.1. Extraction of borage seed oil by liquid and supercritical carbon dioxide

Fig. 2 shows the influence of the operating pressure for the operating temperatures tested. As can be seen from this figure, for all temperatures, the yield of borage seed oil obtained with supercritical carbon dioxide increases with the operating pressure. The maximum extraction yield is

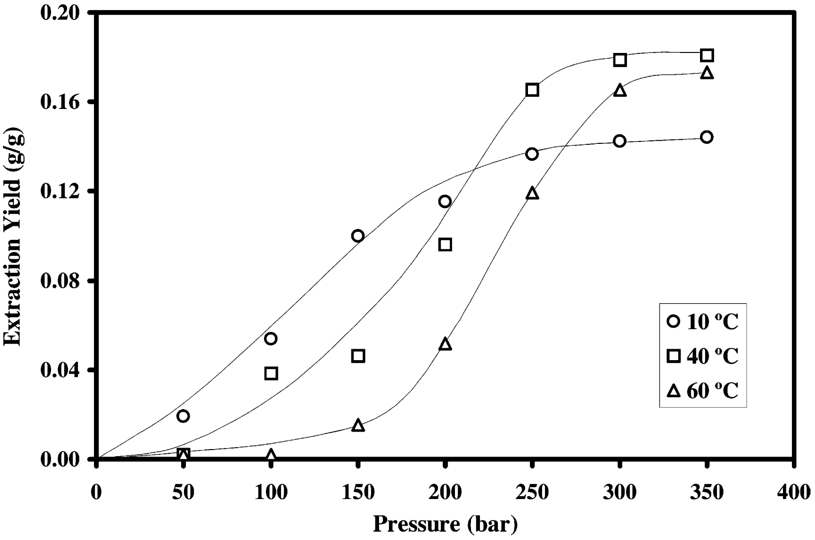

Fig. 2. Influence of the pressure in the extraction of borage seed oil by liquid and supercritical carbon dioxide. Operating conditions: extraction time, 3 h; solvent flow rate, 1.5 l/min (STP); borage seed humidity, 7.40%; grain size, 0.75 mm.

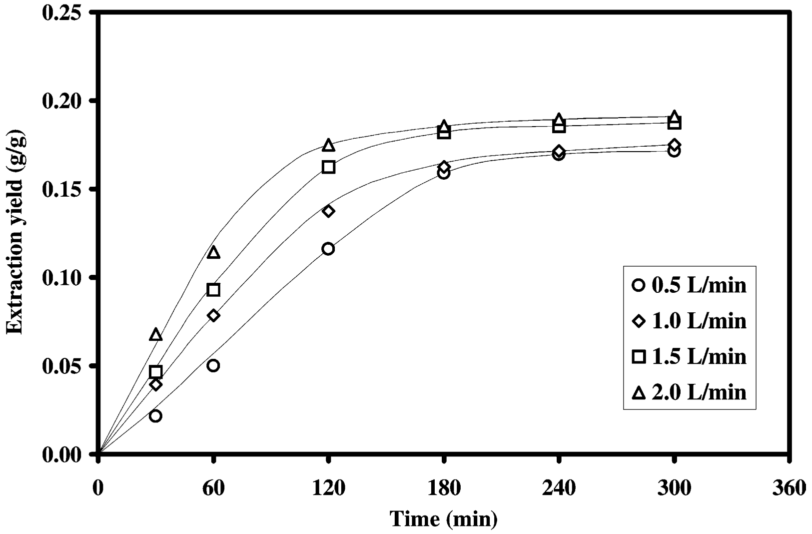

Fig. 3. Effect of supercritical carbon dioxide flow rate on the extraction yield of borage seed oil. Operating conditions: pressure, 300 bar; temperature, 40 °C; borage seed humidity, 7.40%; grain size, 0.75 mm.

achieved using supercritical carbon dioxide at 300 bar (40 and 60 °C). However, the extraction carried out using liquid carbon dioxide did not reach that maximum yield. Nevertheless, for operating pressures below 200 bar, the amount of borage seed oil dissolved in liquid carbon dioxide is greater than in supercritical CO_2. These results are consistent with those presented in other studies [10,13,14].

The differences observed when liquid and supercritical carbon dioxide was used are coincident with previous studies on the extraction of grape seed oil [11]. In the same way, this divergence resides in the physical nature of the seed, since the oil is located in the most internal parts of the seed, the liquid CO_2 has a lower solvent capacity.

Fig. 3 shows the effect of CO_2 flow rate on the extraction yield, at operating conditions of 250 bar and 40 °C. It can be seen that the maximum oil extraction yield together with the minimum consumption of solvent is achieved at about 1.5 l/min. A higher flow rate will give a somewhat higher yield but with a much higher solvent consumption; a lower flow rate will reduce solvent consumption but produce notably lower yields (and even for moderate yields requires processing times longer than 5 h, thus using considerably more solvent).

The flow rates of 1.5 and 2.0 l/min reach maximum extraction yields for a operating time of about 3 h. Lower flow rates maintains better the solubility equilibrium in

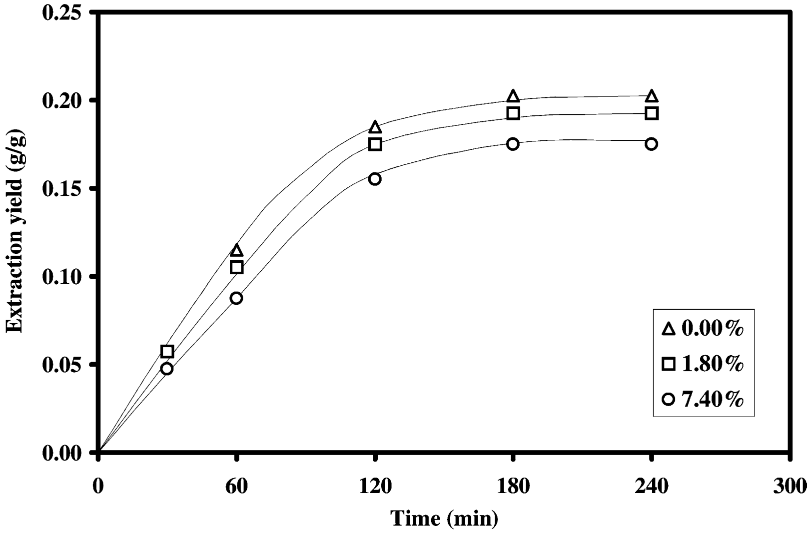

Fig. 4. Influence of seed humidity on the supercritical extraction of borage seed oil by supercritical carbon dioxide. Operating conditions: pressure, 300 bar; temperature, 40 °C; solvent flow rate, 1.5 l/min (STP); grain size, 0.75 mm.

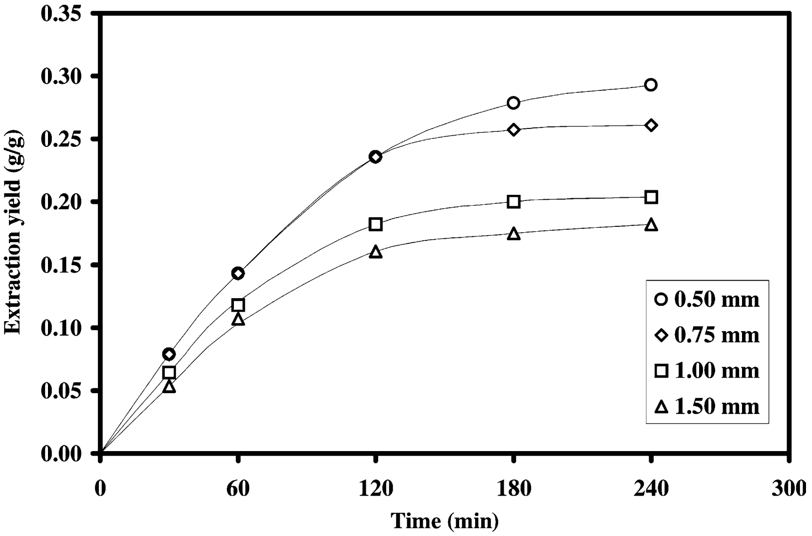

Fig. 5. Influence of particle size in the supercritical extraction of borage seed oil by supercritical carbon dioxide. Operating conditions: pressure, 300 bar; temperature, 40 °C; solvent flow rate, 1.5 l/min (STP); borage seed humidity, 7.40%.

the later stages of the extraction process (when the remaining quantity of oil is very small, needed larger processing time for a maximum yield). Therefore, the flow rate of 1.5 l/min gives a maximum yield with a minimum solvent consumption, in comparison to the 2.0 l/min rate [15].

No significant differences were detected in the extraction yields obtained when the extraction processes were carried out with partially hydrated borage seed samples and almost fully dehydrated samples. As can be seen in Fig. 4, the humidity of borage seed oil influences negatively the extraction process due to the variation of the solvent capacity of carbon dioxide, decreasing the extraction yield for the hydrated samples.

Fig. 5 shows the influence of grain size on extraction yield of borage seed oil with supercritical carbon dioxide. An increase of the size is accompanied by a decrease in the extraction yield; this variation is due to the increase of the mass transfer resistance between the surface of the seed and the supercritical carbon dioxide. This effect is observed for other vegetable oils [11]; however, other raw materials, like wheat germ oil, do not need to be milled due to their excellent mass transfer properties [16].

Table 1 gives the experimental yield values for the extraction of borage seed oil using supercritical carbon dioxide, at the optimised operating conditions, together with the corresponding data for conventional extraction using hexane. As can be seen, the apparent yield from the high-pressure extraction of borage seed oil is slightly lower than extraction with hexane. This difference has already been widely discussed in the bibliography [10,15,17], where it has been interpreted that hexane is much less selective than CO_2 in the extracted oil and produces oil containing some undesirable compounds.

Table 1
Comparison of oil extraction yield obtained using hexane and supercritical carbon dioxide for various vegetable seeds

Seed	Yield (wt.%)	
	SFE using CO_2	Extraction using hexane
Borage seed (this work)	29.0	30.1
Grape seed [11]	6.9	7.5
Soybean [13]	16.4	19.9
Sunflower seed [13]	36.0	38.4
Rape seed [13]	39.3	40.1
Wheat germ [16]	8.0	8.6

3.2. Characterisation of extracted oils

Table 2 presents a comparison of the physicochemical characterisation of borage seed oil extracted by the SFE and by the "Soxhlet" equipment using hexane as solvent. There are appreciable variations between the oils obtained in terms

Table 2
Comparison of properties of borage seed oils extracted using hexane and supercritical carbon dioxide

Physicochemical parameter	Extraction method	
	CO_2 (300 bar, 40 °C, 3 h)	Hexane (Soxhlet, 16 h)
Refractive index	1.4735	1.4702
Density ($g\,ml^{-1}$)	0.9197	0.9052
Viscosity (cP)	58.5	48.5
Absorbance at 270 nm (Igε)	0.72	0.00
Free fatty acid (%)	11.0	20.2
Iodine index (g per 100 g)	198	240
Saponification index	163	180
Unsaponifiable fraction (%)	1.8	3.0
Peroxide index (meq per kg)	33.5	7.8

of the analysed indexes, except for refractive index, density and viscosity.

The degradation of the extracted oils is analysed through the acidity and peroxides indexes, as well as the absorbance in the UV region. The first of these provides information about the hydrolytic deterioration that may have occurred in the oil. On the other hand, the peroxides index tries to evaluate the initial oxidation state of an oil, as well as the deterioration that certain components have suffered, such as the tocopherols (with nutritional and antioxidant values) and the polyphenols (antioxidants value). Due to its lower representation of the global oxidation state of an oil, this index is supplemented with the absorbance at 270 nm.

The acidity index of the oil extracted with supercritical carbon dioxide is lower than obtained using hexane, because the supercritical extraction using carbon dioxide extracted a lower amount of free fatty acid. These results confirm the greater selectivity of supercritical carbon dioxide.

The low values of peroxide index show that both oils present a minimum degree of oxidation, being very inferior in comparison with other vegetable oils extracted at the same conditions [11,16]. However, the oil obtained with supercritical carbon dioxide presents a higher value. Preliminary studies [18] indicate that the reason for this increase may be due to the reaction of the CO_2 with some compounds of the oil, facilitating the later degradation of these compounds with oxygen.

In relation to the absorption in the UV region, the UNE normative establish the analysis of the specific extinction coefficients at 232 and 270 nm. The first of these, corresponds to a measure of the formation of hydroperoxides due to the linoleic acid oxidation, as long as the second are indicative of the content in α-diacetones and cetones α,β-unsaturated [19]. Due to the higher stability of these compounds, the measure at 270 nm is considerate more reliable. The UV spectra shows a small peak at 270 nm for the oil extracted with supercritical carbon dioxide, confirming that the degradation state of oils is minimum.

The iodine index is a quantitative expression of the unsaturation level of a oil. Discounting the amount of free fatty acids present in both oils, the values of this index gives the quantity of triglycerides present in the extracted oil. Thus, the amount and composition of triglycerides presents in both oils is very similar, and independent of the extraction technique.

The results obtained for saponifiables matter are similar for both oils, with these higher values mainly due to the high content in triglycerides.

The unsaponifiable fraction is formed basically by sterols, hydrocarbons, tocopherols and pigments [20]. From this parameter, significant differences are observed for the oil extracted by supercritical carbon dioxide in comparison to the oil extracted with hexane. These results are in concordance which obtained by Molero Gómez et al. [11] and it is due to the small selectivity of hexane.

Table 3
Fatty acid composition of borage seed oil extracted using hexane and supercritical carbon dioxide

Fatty acid composition (%)	Extraction method	
	CO_2 (300 bar, 40 °C, 3 h)	Hexane (Soxhlet, 16 h)
Palmitic	13.32	13.28
Palmitoleic	0.19	0.18
Stearic	4.58	5.06
Oleic	19.78	20.77
Linoleic	39.57	39.02
γ-Linolenic	22.29	21.04
α-Linolenic	0.27	0.65

3.3. Fatty acids composition

Table 3 shows the free fatty acid of the borage oil obtained using hexane as solvent in “Soxhlet” apparatus, as well as the extracted by supercritical carbon dioxide. As can be observed, the content in fatty acids does not depend on the extraction technique. These results are coincident with those obtained by other authors that studied this oil [5,6].

The high content in unsaturated fatty acids (82.1%) and the lower proportion of saturated fatty acids (17.9%) makes it very appropriate to use this oil in the alimentary industry. Additionally, the great amount of γ-linolenic acid in this oil even reinforces its nutritional value.

4. Conclusions

As detailed in the previous discussion, the following conclusions have been reached.

1. The best yields of extraction of borage seed oil by supercritical carbon dioxide are obtained at 300 bar of pressure, 40 °C of temperature, solvent flow rate of 1.5 l/min (at STP) and a milled seed size of 0.75 mm. At these conditions, the extraction time is 3 h.
2. The oil extracted by SFE using carbon dioxide is solvent-free. For this reason, the distillation process to recover the solvent is not needed (a difference of the conventional extraction with organic solvent).
3. The quality of borage seed oil extracted by SFE is similar to that of oil refined and extracted by organic solvent. Consequently, the refining process may be used in minor scale.
4. Despite the high costs of process plant, the SFE could be more economical than conventional organic extraction. However, further economical studies are needed to confirm these last conclusions.

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