SUPERCRITICAL EXTRACTION OF BORAGE SEED OIL COUPLED TO CONVENTIONAL SOLVENT EXTRACTION OF ANTIOXIDANTS

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Introduction

Borage (*Borago officinalis*) is a plant native from Asia; nowadays the main producers are UK, Holland, New Zealand and Canada. Borage seed contains 30% of oil, with 34-38% linolenic acid (LA), and 24% of γ-linolenic acid (GLA) (omega-6)[1]. GLA is the first intermediate formed during the conversion of LA to prostaglandins. These molecules have antiinflammatory and anti-thrombotic properties[2], also borage seed oil has been used for treating arthritis, certain skin problems, cardiovascular diseases and neurological problems related to diabetes[1, 3]. The traditional industrial extraction process is carried out with solvents or by pressing coupled to conventional solvent extraction. The latter of these processes consists of cleaning, drying, crushing, and pressing; the oil remaining in the pressing cake is further extracted with hexane and has to be refined. These processes can induce thermal degradation and a reduction of the oil quality, the GLA physicochemical properties and their functionality can be altered during pressing, solvent extraction and subsequent refining or degumming stages. Supercritical fluid extraction (SFE) is an alternative to the conventional separation systems because energy yields and separation efficiency is good[4]. The oil obtained by SC-CO_2 is free from chemical and thermal degradation compounds and from solvent residue[5]. The supercritical CO_2 extraction could replace both pressing and solvent extraction, with the advantage of avoiding refining because phospholipids are practically insoluble in this solvent.

To avoid the oxidation of this oil, with high unsaturated fatty acid content, the seed probably contains an efficient antioxidant system. The present work aims at assessing the extraction of seed oil by supercritical CO_2 (SC-CO_2) and the further conventional solvent extraction of antioxidants from the SC-CO_2-extracted borage meal.

Materials and Methods

Materials. Borage seeds were supplied by the Chilean industry LONCOPAN S. A. (Santiago, Chile). All the products and chemical reagents used were of analytical quality and purchase by Sigma-Aldrich (Madrid, Spain).

Soxhlet extraction. In order to compare the conventional solvent with SFE extraction yields, a Soxhlet type apparatus with hexane as solvent was used.

Supercritical CO_2 extraction. All extractions were carried out in an automated and computerized laboratory-pilot extraction plant, SFF model (Iberfluid, Spain). The extraction pressure was controlled by micrometering valves and the carbon dioxide used was Premier-X50S from Carburos Metálicos, S. A. (Ourense, Spain). For each experiment, the extraction cell was filled with 20 g of borage seeds. The range of extraction pressures tested was 150 to 250 bar.

Conventional solvent extraction of the SC-CO_2- extracted meal. Antioxidant extractions from SC-CO_2-extracted meal were carried out with water, methanol, ethanol and ethyl acetate as solvent at a solid-liquid ratio of 20:1 (w:w) solvent:meal ratio at 323 K during 13 h in a rotatory shaker. The extracts were obtained after vacuum filtration to remove the solvent, then they were weighed to determine the extraction yield and stored at 4ºC until use.

Analytical methods. The total phenolic content was determined by the Folin-Ciocalteu colorimetric method, and expressed as Catechin Equivalents (CE).

Antioxidant methodology. The antioxidant activity of the solvent extracts was evaluated using *in vitro* tests for radical scavenging (DPPH and ABTS) and ferric reducing antioxidant power (FRAP).

Results

Extraction of borage seed oil by supercritical carbon dioxide. Figure 1 shows the effect of the extraction conditions on the oil extraction yield from borage seeds. The higher oil yield was obtained at 303 K and 200 bar. At 303 K, the increase in pressure does not improve the oil extraction yields (57%), whereas at 323 K, a linear increase in the extraction yield was observed in the range 150 to 250 bar.

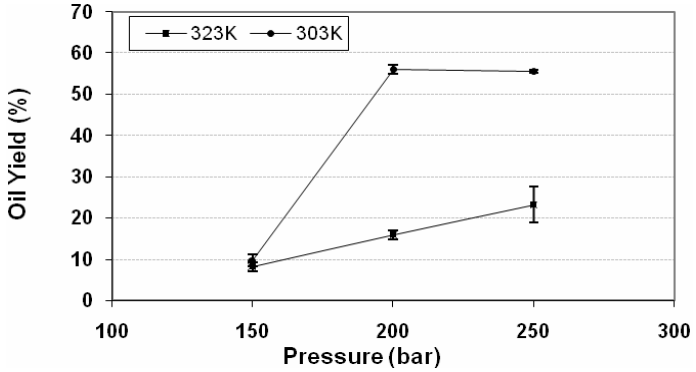

Figure 1. Influence of the pressure and the temperature in the extraction of borage seed by SC-CO_2 with a solvent flow of 1.5 L/h during 2 h.

Molero-Gómez & Martínez de la Ossa (2002)[2] reported a similar effect on the oil extraction yields from borage seeds. These authors found that in operation at pressures under 200 bar, the extraction yield was higher at 283 K than at 313 and 333 K. Kotnik *et al* (2006)[1] found the same behavior from borage seeds when the extraction was carried out at 313 K and 200 bar. On the other hand, when the operation pressure was 300 bar, the extraction yields at 313 and 333 K did not differ. Rao *et al* (2007)[6] did not report significant differences in oil extraction yields from black seeds at 313 and 323 K in the pressure range of 200-400 bar, and at higher temperatures lower yields were observed.

Table 1 summarizes the fatty acid composition the oil extracted by SC-CO_2 operation with 1.5 L/h of solvent flow rate during 2.5 h extraction time. The relative proportion of unsaturated fatty acid (UFA) to saturated fatty acid (SFA) decreased with the extraction temperature for a fixed extraction pressure. Similarly, at a fixed temperature an increase in the extraction pressure involved a lower UFA:SFA ratio. The UFA:SFA of three found in this work was lower than the value (4.5) reported by Molero-Gómez & Martínez de la Ossa (2002)[2] at 300 bar and 313 K.

Conventional solvent extraction. Yield and total phenolic content. In order to evaluate the potential of SC-CO_2 extracted borage meal as a source of antioxidants and the effect of the supercritical fluid extraction in the potency of the antioxidants recovered, water and methanol were used as solvents. Table 3 summarizes the antioxidant extraction yield, the phenolic content in the extract processed and their free radical scavenging activity. The highest recovery was observed when the meal obtained after supercritical extraction at 200 bar and 303 K, was processed using water as solvent (8.86 g extract/100 g SC-CO_2 extracted meal), this value was similar to those reported by De Leonardis *et al.* (2005)[7] and Peschel et al. (2007)[8] for the water extraction of sunflower seeds and evening primrose, respectively. Using water as solvent for burdock[9], extraction yields twice higher were obtained. Matthäus (2002)[9] reported yields of water extractable compounds from residues of different oilseeds up to five times higher than those obtained in the present work.

Table 1: *Fatty acid composition of borage seed oil obtained with SFE operating with a solvent flow rate of 1.5 L/h during 2.5 h.*

Fatty acid	Relative Fatty acid content (%)				
	323K		303K		
	150 bar	200 bar	150 bar	200 bar	250 bar
Palmitic acid	15.81	17.10	16.60	17.63	18.34
Stearic acid	3.80	4.64	3.60	4.28	4.28
Oleic acid	24.32	21.27	22.12	21.54	21.56
Linoleic acid	35.46	35.96	36.72	35.99	34.92
Linolenic acid	15.00	15.42	16.06	15.64	15.61
Arachidic acid	3.13	3.13	3.00	2.91	3.05
Erucic acid	1.46	1.49	1.10	1.31	1.23

The highest phenolics extraction yield was observed when the meal obtained after supercritical extraction at 150 bar and 303 K was processed using methanol as solvent (13.46 g catechin/100 g extract). Phenolic extraction yields in the range of 7.5 – 8.5 were achieved from meal processed with water as solvent. Peschel *et al* (2007)[8] reported a similar behaviour for burdock and sesame extracts obtained with water. The phenolic content in the water extract was superior than that reported from Mexican chia by Reyes-Caudillo *et al* (2008)[10] and from rapeseed by Vuorela *et al* (2004)[11] .

Table 2: *Antioxidant extraction yield, phenolic content and antioxidant activity in extracts obtained from the supercritical spent borage seeds (SPS).*

	P (bar)	Extraction Yield (g extract/ 100 g SPS)		Phenolic content (g CE/100 g extract)		TEAC (mmol Trolox/ 100 g extract)		DPPH EC_{50} (g/L)	
		Water	*Methanol*	Water	Ethanol	Water	Methanol	Water	Methanol
303 K	150	8.04^{a}	8.45^{a}	8.27^{a}	4.75^{b}	111.92^{a}	167.90^{a}	$1.74^{c,d}$	1.41^{a}
	200	8.86^{a}	6.78^{b}	3.97^{c}	7.58^{a}	79.72^{c}	$55.81^{c,d}$	$1.69^{b,c}$	3.57^{c}
	250	7.45^{a}	5.88^{c}	7.62^{b}	2.84^{c}	93.60^{b}	50.90^{d}	1.58^{a}	ND
323 K	150	8.32^{a}	3.64^{c}	8.48^{a}	6.53^{a}	88.53^{b}	90.51^{b}	2.03^{d}	4.76^{c}
	200	5.64^{c}	7.55^{a}	7.96^{a}	3.58^{b}	110.9^{a}	58.29^{c}	$1.62^{a,b}$	4.54^{c}
	250	6.85^{b}	6.30^{b}	8.06^{a}	2.61^{c}	93.25^{b}	40.12^{e}	$1.73^{c,d}$	ND

Antioxidant properties of the extracts. Three different methods have been used for the determination of the antioxidant properties of the extracts obtained from the different supercritical residues: DPPH radical scavenging, ABTS radical scavenging and ferric reducing antioxidant power. Table 2 shows the activity of the different extracts on the DPPH and ABTS free radicals. Methanol and water extracts from meals processed at 150 bar and 323 K had the highest ABTS radical scavenging activity. As a general trend, water extracts achieved the higher values on the residual meals from all operational conditions. All these TEAC values were better than those reported from several vegetable oils (flaxseed, grape, peanut, rapeseed, sunflower) [12]. A good correlation between TEAC values and the phenolic content on the extracts for water and ethyl acetate extracts obtained for residues processed at 303 K can be established.

The highest DPPH radical scavenging was achieved using methanol to extract the supercritical residue produced at 150 bar and 303 K. The EC_{50} values of the water extracts were similar to those reported for the water extracts of different oilseeds[10]. Peschel *et al.* (2007)[8] reported better DPPH values from extracts processed from evening primrose, burdock, sesame and woad (*Isatis tinctora*). Figure 4 shows the FRAP values of the extracts from residual borage meal. Water (W-50, W-30) and methanol (M-50, M-30) extracts achieved the highest FRAP values. The FRAP values were directly related to the phenolic content of the extract, a higher phenolic content involves a higher FRAP values.

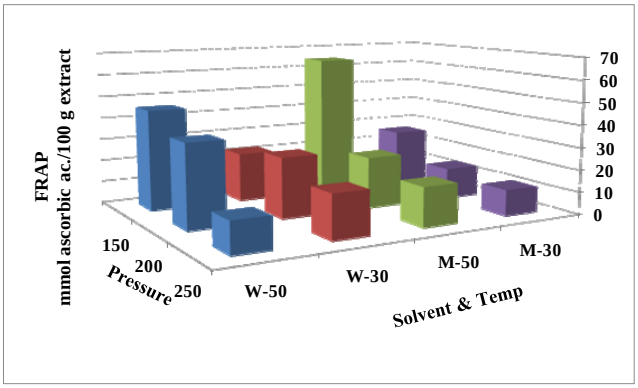

Figure 4. FRAP values of the extracts obtained from supercritical CO_2 spent borage meal residues.

Conclusions

The best yield oil extraction of borage seed oil by supercritical carbon dioxide was obtained at 200 bar, 30ºC, solvent flow rate 1.5 l/h and extraction time of 2 h. The highest oil quality was obtained at 150 bar, 50 ºC, solvent flow rate 1.5 L/h during 2 h. Water attained the best extraction yields and phenolic content on the extracts, which showed high ABTS radical scavenging capacity.

Acknowledgments

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