

APTEFF, 41, 1-203 (2010)
DOI: 10.2298/APT1041105S

UDC: 665.3:66.061
BIBLID: 1450-7188 (2010) 41, 105-120
Review

CRITICAL REVIEW OF SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF SELECTED OIL SEEDS

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Supercritical carbon dioxide extraction, as a relatively new separation technique, can be used as a very efficient process in the production of essential oils and oleoresins from many of plant materials. The extracts from these materials are a good basis for the new pharmaceutical products and ingredients in the functional foods. This paper deals with supercritical carbon dioxide extraction of selected oil seeds which are of little interest in classical extraction in the food industry. In this article the process parameters in the supercritical carbon dioxide extraction, such as pressure, temperature, solvent flow rate, diameter of ground materials, and moisture of oil seed were presented for the following seeds: almond fruits, borage seed, corn germ, grape seed, evening primrose, hazelnut, linseed, pumpkin seed, walnut, and wheat germ. The values of investigated parameters in supercritical extraction were: pressure from 100 to 600 bar, temperature from 10 to 70°C, diameter of grinding material from 0.16 to 2.0 mm, solvent flow used from 0.06 to 30.0 kg/h, amount of oil in the feed from 10.0 to 74.0%, and moisture of oil seed from 1.1 to 7.5%. The yield and quality of the extracts of all the oil seeds as well as the possibility of their application in the pharmaceutical and food, industries were analyzed.

KEYWORDS: supercritical extraction, seed oils, process parameters, global extraction yield.

INTRODUCTION

Supercritical fluid extraction using carbon dioxide (SC-CO₂) is a particularly suitable isolation method for isolation of the valuable components from plant materials. A natural plant extract, free from chemical alterations brought about by heat and water, and without solvent residues and other artifacts can be obtained by this method. Carbon dioxide is non-toxic, non-explosive, readily available and easily removed from the extracted products. All products characterized by their internal content are claimed by the market's consumers to have a reproducible and stable quality. Technological solutions are sought that ensure these properties of the products and satisfy the standards in a more reproducible way. For extraction of oil seeds a variety of solvents, including alcohols, acetone and hexane can be used. However, these organic solvents leave adsorbed residues behind

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and elevated temperatures at the desolventization process can cause chemical transformation of the oleoresins. The solvent residues must be reduced to very small concentrations, generally in the range of 25-30 ppm or less (1).

Supercritical fluids are gases with properties between that of a „normal“ gas and a liquid. Variation of pressure changes properties of supercritical fluids continuously from more gas-like behavior to more liquid-like behavior. This behavior may lead to new technologies in processing natural materials (extraction and purification), in processes related to the environment (destruction of waste with supercritical water), and in reaction engineering (hydrogenation with mixtures of hydrogen and supercritical carbon dioxide). Supercritical fluids produce practically solvent-free products and avoid deteriorating reactions. Process temperatures are low to very moderate. Solvent handling is favorable because it can be easily and totally removed, it is readily available and cheap, and it is accepted and part of the environment (water, carbon dioxide). Even then, solvent losses are minimal when compared to ordinary men's activities for these substances, since the solvent is recycled. Situations where supercritical fluids may be useful emerge from the effects which supercritical fluids have on pure compounds and mixtures. Supercritical fluids change compound properties, phase equilibria, chemical equilibria, and related rate processes, as it has been discussed in many publications.

ANALYSIS OF EXPERIMENTAL DATA

Almond fruits (*Pinus dulcis*) are important products from trees found in European countries and elsewhere. They are valued for their reamakable nutrition properties leading to high economical value. They have high oil content, which contribute to their use as a source of energy in the Mediterranean diet and exhibit high levels of mono- and polyunsaturated fatty acids. Almond fruits have about 20% albumens, 50% lipid components, 17% glucose, and some of minerals, as well as potassium, phosphorus, calcium, iron, and vitamin B3. The amount of oil in the almond fruits is about 50%. The almond oil has oleic, linoleic, and palmitic acids. Almond fruits are rich a source of flavonic antioxidants, vitamin E, Mg, P, plant fiber, and very quality albumens. The almond fruit oil is light yellow color and gentle odor. It can be used as an oil for care and massage. The high production and competition among productive zones have hardened the commercialization of this product. On the other hand, almond oil is very appreciated for alternative medicine, cosmetics, etc. Thus, production of almond oil may be an option for expanding the almond market. In this way, using clean process such as supercritical fluid extraction for almond oil extraction may also lead to an almond revaloraziton as a „light“ product (partially defatted almonds).

Femenia et al. (2) presented the results of extraction of oil from crushed almond fruits by supercritical carbon dioxide (SC-CO₂) at 330 bar and 50°C, Table 1. The oil percentages in the feed ranged between 15.5 and 64.3% of extracted lipids. Moisture, oil fraction, protein, dietary fiber, ashes, and soluble sugars were determined for almond preparations before and after oil extraction. Samples of nontreated fresh almond seeds, fresh almond kernels, and toasted seeds were analyzed to allow comparison with further analyses performed on partially defatted samples. As expected, oil extraction by SC-CO₂

promoted an increase of the percentages of the remaining fractions. Thus, defatted almond samples contained large amounts of protein and dietary fiber. This latter fraction was particularly important in the case of whole almond seeds. From a nutritional point of view, the reduced fat content, together with the increased protein and dietary fiber fractions, might provide almond kernels as an interesting food source. In fact, dietary recommendations have called for a reduction of total calories received from fat with an equivalent increase in calories from complex carbohydrates (2). Marrone et al. (3) examined the extraction of oil from almond fruits with supercritical CO₂ at 350 bar and 40°C. Almond particles of three different mean sizes were tested (0.30, 0.70, and 1.90 mm). Extraction of the smaller particles was performed at two different solvent flow rates (0.72 and 1.43 kg/h), Table 1. The quantity of oil extracted flow divided by the weight of the initial charge (extraction yield), is usually given as a function of the ratio between the mass of CO₂ used and the mass of seeds charged in the extractor (specific mass of solvent). From the comparison between the experimental data in this system it is possible to observe that, during the first part of the extraction process, the mass of oil extracted is independent of both the particle size and the solvent flow rate. These considerations strongly suggest that the thermodynamic equilibrium of the oil between the solid and the fluid phases applies to this part of the extraction. For extraction yields larger than 10%, the extraction curves show a considerable spreading suggesting the onset of a mass transfer resistance. An increase of particle size increases the extraction time as it is expected from a corresponding decrease of the mass transfer rate. For very large extraction times, it can be identify an asymptotic value of the yield of about 50% by weight. This value is in good agreement with the oil content measured in a Soxhlet extraction with hexane, which gave about 54% of weight fraction of oil and was assumed to be the maximum possible yield, Y_{∞} . Leo et al. (4) gave the results of the SC-CO₂ extraction of almond fruits at pressure from 350 to 550 bar, temperature from 35 to 50°C, and solvent flow rates from 10 to 30 kg/h, Table 1. The results were compared with those obtained when hexane/methanol using as solvent. The almond oil obtained from the various fractions, extracted with either hexane/methanol or supercritical CO₂, was intensely yellow in colour having a variable content of tocopherols. After oil extraction, the residual defatted almond was a white flour with variable grain size, more or less fine, depending on the degree of grinding of the fresh matrix and on the effect of the operating conditions (pressure, flow rate, temperature) used to remove the oil. The almond seeds were homogenized and sieved to obtain four representative sub-samples: whole, broken (4-8 mm), milled (0.5-3.0 mm) and powered samples. The decreasing of the particle causes an increase in the oil content in the extract for the conventional extraction process of almond seeds. It was evident that the yield of SC-CO₂ extraction not only depended on the utilized pressure, temperature and flow rate of the solvent through the extraction bed, but also on the chemical and physical characteristics of the matrix such as oil composition, moisture content and particle size (4). The influence of pressure on the CO₂ supercritical oil extraction from almond seeds was determined at pressures of 350, 450 and 550 bar, respectively, and at a constant temperature of 50°C and a constant flow rate of 20 kg/h. The extraction curves obtained at 450 and 550 bar were characterized by an initial period in which the oil yield rose more steeply as the pressure was increased and a second period characterised by a higher value of the plateau

as the pressure increased. The extraction curve showed a linear period without a flex point at low pressure (350 bar).

Table 1. Amount of oil in the feed and process parameters in the Supercritical carbon dioxide extraction of the selected oil seeds

Oil seed	Amount of oil in the feed, %	Process parameter	Ref.
Almond fruits (<i>Prinus dulcis</i>)	15.5 - 64.3	P = 330 bar; T = 50°C; v = 20-40 kg/h; w = 1.1-5.5%	(2)
	54.0	P = 350 bar; T = 40°C; v = 0.72 - 1.43 kg/h; d _{av} = 0.30 - 1.9 mm	(3)
	45.0 - 60.0	P = 350 - 550 bar; T = 35 - 50°C; v = 10 - 30 kg/h; d _{av} = 0.5 - 8.0 mm	(4)
Borago seed (<i>Borago officinalis</i> L.)	31.0	P = 200 - 300 bar; T = 10 - 55°C; v = 7.5 - 12.0 kg/h; w = 6.6%	(5)
	31.0	P = 200 - 300 bar; T = 40 - 60°C; v = 0.20 kg/h; d _{av} = 1.125 mm; w = 7.99%	(6)
	30.0	P = 100 - 350 bar; T = 40°C; v = 30 L/h	(7)
Corn germ	45.0 - 55.0	P = 300 bar; T = 42°C	(8)
Grape seed (<i>Vitis vinifera</i> L.)	10.0	P = 280 - 550 bar; T = 40°C; d _{av} = 0.39 - 0.97 mm; v = 0.36 kg/h; w = 3.9%	(9)
	12.0	P = 100-500 bar; T = 40-60°C	(10)
Evening primrose seed (<i>Oenothera biennis</i> L.)	28.0	P = 200 - 300 bar; T = 40 - 60°C; v = 0.17 kg/h; d _{av} = 0.63 mm	(6)
Hazelnut (<i>Corylus avellana</i> L.)	56.0-60.0	P = 150 - 600 bar ; T = 40 - 60°C; v = 0.12 l/h; d _{av} = 1.0 - 2.0 mm	(11)
	60.0	P = 300 - 450 bar; T = 40 - 60°C; v = 0.06 - 0.30 kg/h; d _{av} = 0.85 - 1.00 mm	(12)
Linseed (<i>Linus usitatissimum</i> L.)	35.0-45.0	P = 300-500 bar ; T = 47-52°C; v = 8.8 kg/(kg h) ; d _{av} = 0.16 - 2.00 mm	(13)
Pumpkin seed (<i>Cucurbita peppe</i>)	42,0-54,0	P = 150 - 300 bar ; T = 40-60°C ; v = 0.20 kg/h ; d _{av} = 0.50 - 0.63 mm	(14)
Walnut (<i>Juglans regia</i> L.)	65.0	P = 180 - 234 bar; T = 35 - 48°C; v = 4.0 L/h; d _{av} = 0.01 - 0.50 mm	(15)
	74.0	P = 200 - 400 bar ; T = 25 - 70°C; v = 10.5 kg/h; d _{av} = 1.2 - 2.4 mm; w = 2.5 - 7.5%	(16)
Wheat germ	8.0-14.0	P = 200 - 350 bar; T = 40 - 60°C; v = 15-25 L/h	(17)
	11.0	P = 200 - 300 bar; T = 40°C	(18)

P – pressure, T – temperature, v – flow rate, d_{av} – average diameter of grinding material, w – moisture content

In view of these results it can be stated that at the beginning of the extraction process, when the physical phenomenon of quantitative extraction mass depends mainly on the available amount of oil extracted into the CO₂, an increment of the extraction pressure determines the yield increment of oil. On the other hand, at a constant flow rate an increase in pressure resulted in an increasing of extraction yield. As expected, using the same quantity of CO₂ per kg of matrix, as pressure increased the solubility of the almond oil in CO₂ increased. The results of the pressure effect on the oil yield suggest that the increased yield resulted from the increasing density of the solvent and consequently from increased solubility of almond oil in the CO₂. The results obtained clearly indicate that the extraction yield was promoted by an increase in temperature. At fixed conditions of pressure and flow rates, an increase in temperature produced an almost fourfold yield increment. An explanation of this phenomenon is that the oil solubility in the CO₂ is enhanced. However, the temperature effect on CO₂ extraction is more difficult to assess than the other parameters, pressure and solvent flow, because it influences both the solvent diffusion in the matrix and the oil dissolution in the solvent (4). From the experimental results it is evident that oil yield, at the initial stage of extraction, increased with increasing CO₂ flow rate from 10 to 30 kg/h, with constant pressure of 420 bar and constant temperature of 50°C. It appeared that flow the rate of supercritical fluid affects directly the extraction rate. In conclusion, these experiments demonstrated that when extracting a biological matrix at the described conditions, the oil yield increased with increasing pressure, temperature and flow rate (4).

Borago seed (*Borago officinalis* L) contains about 30% of oil, i.e. lipid components. The oil from borago seeds consists mainly of triglycerides consisting of C16 - C20 fatty acids. The fatty acid composition of the oil expressed in percentage by weight, is as follows: palmitic acid (9 - 12%), stearic acid (3 - 5%), oleic acid (15 - 20%), linoleic acid (30 - 40%) and γ -linolenic acid (18 - 25%). One of this acids, γ -linolenic acid (GLA), is the first intermediate formed during the conversion of linoleic acid 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 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, such as borago seed oil, evening primrose oil, etc.

The extraction of borago seed oil using compressed CO₂ was studied on a pilot plant apparatus with the aim to optimize plant performance and collect data for scale-up purposes, (5). Effects of extraction pressure (200 - 300 bar) and temperature (10 - 55°C), solvent flow rate (7.5 - 12.0 kg/h) and bed length (0.25 - 0.50 m) were examined, Table 1. It can be seen from experimental results that, in the early stages of the extraction, the curves present a steady state situation (near-constant extraction rate) and follow the solubility lines. After the extraction of around 70% of the oil (approximately 0.20 kg of oil/kg of seed) the extraction rate reduces progressively and the curve tends to the maximum oil yield as indicated by the horizontal line. This gradual decrease in the extraction rate is due to the exhaustion of the freely available oil and the mass transfer resistances turned to the intraparticle diffusion of the last fraction of oil. The form of the extraction curves is the same for all conditions despite of the considerable differences in solubility

arising from the different pressures and temperatures (5). The investigation of the effect of solvent flow rate on the extraction yield showed that the lower flow rate test resulted in a slightly higher oil load, but the higher flow rate leads to a shorter extraction time to reach the same yield as the lower flow rate test. Solvent flow rate affects both the residence time and the film coefficient. Within experimental ranges, the extraction rates were found to be mainly controlled by the solubility of the oil in compressed CO₂. The effects of flow rate on the extraction curves was smaller than those of pressure and temperature (5). Kotnik et al. (6) also investigated the high-pressure extraction of borage seed oil, containing the valuable GLA, Table 1. Extraction was performed with supercritical carbon dioxide on a semi-continuous flow apparatus at pressures of 200 and 300 bar, and at temperatures of 40 and 60°C. A constant flow rate of carbon dioxide in the range of 0.20 kg/h was maintained during extraction. Empirical results showed that the total yield and extraction rate increased with increasing pressure from 200 to 300 bar. At 200 bar, the temperature had a positive effect on the extraction yield and, oppositely, a negative effect on the extraction rate. At 40°C, a higher extraction rate was observed than at 60°C, which is a consequence of the higher density of CO₂ and the higher solvent power at lower temperature. At 300 bar, the temperature had no influence on the total extraction yield and extraction rate, and the highest yields were obtained. The extraction yields obtained using dense CO₂ were similar to those obtained by conventional extraction using hexane as solvent. The composition of extracted crude oil was determined by GC analysis. The best results were obtained at 300 bar and 40°C for borage seed oil, where the quality of oil was highest with regard to GLA content (6). Daukšas et al. (7) studied the influence of different pressures of CO₂ and the addition of caprylic acid methyl ester as an entrainer for the extraction process of borage seed, Table 1. The increase of CO₂ pressure from 100 to 350 bar resulted in the increase in extract yield from 0.14 to 24.29% (w/w) while the changes in the extract composition were not so considerable. The highest solubility of pure caprylic acid methyl ester in dense CO₂ was determined at 100 and 300 bar. The addition of this entrainer increased the yield of pure extract up to 47.8 times at 100 bar, 2.4 times at 200 and 300 bar. Due to the high solubility of caprylic acid methyl ester at the lower (100 bar) pressure it is easy to separate the entrainer, which constituted only 4.22% of the total borage seed extract. The highest extract yield and the fastest extraction rate were achieved after increasing the pressure above 250 bar. The extraction rate increased slightly with an increase of pressure from 250 to 300 and 350 bar; however, the final yields were 21.89, 21.59 and 24.29%, respectively, which were quite comparable for all these pressures. The appearance of the extracts obtained at different pressures was very similar and can be characterized as a yellow oil-like transparent liquid. However, some changes in the intensity of the color (darker at higher pressures) were observed (7).

Corn germ is a very quality product which contains a significant amount of oil. The light-yellow oil is rich in the tocoferols (vitamin E), amino acids, fatty acids, as well as in linoleic, oleic, palmitic acid, and contains small quantities of minerals: phosphorous, iron, magnesium, and sodium. Corn germ oil can be used in the production of margarine, and in some of culinary prescriptions. It is light-colored and has significantly lower refining loss and phosphorous content. The corn kernel contains only 5% of oil, so processing it for oil is uneconomical. Both wet and dry millers separate the lipid containing germ and recover the crude oils by expeller pressing and/or solvent extraction with hexane. The

literature reports that the dry-milled corn germ protein meal obtained by SC-CO₂ extraction showed better nutritive and physicochemical properties than when the protein defatted by hexane. The SC-CO₂ extraction dissolves only slightly polar lipids (phospholipids and glycolipids), so they essentially remain in the extracts. Thus increasing polar lipid content can influence the functional properties of germ proteins (8).

Ronay et al. (8) investigated the extraction rate of corn germ with carbon dioxide+ethyl alcohol mixtures. The effect of the amount of ethyl alcohol entrainer on the composition of oils as well as on the nutritive value and functional properties of defatted proteins was examined. The laboratory experiments were carried out at constant pressure (300 bars) and temperature (42°C) with amount of ethyl alcohol ranging from 0% to 10% by CO₂ weight, Table 1. Because of higher solubility of the oil with increasing alcohol concentrations the extraction time was decreased significantly. The global extraction yields were plotted against the specific CO₂ solvent mass passed through the extraction vessel. The extraction curves are characterized by a linear increase at the beginning and a region in which the curve approaches asymptotically the horizontal line determined by ultimate yield, and is controlled by diffusion in the solid phase at the end of the extraction. The former part represents the quasi-equilibrium conditions under which the extraction is conducted. The solvent is saturated with oil in this period. The alcohol content in CO₂ has a strong influence on the rate of the extraction. Increasing the amount of alcohol in the fluid decreases the extraction time and the amount of CO₂ consumption (8).

The grape seed oil (*Vitis vinifera* L.) is a product which is ever more appealing for its large availability, as a by-product of the wine-making industry, and for its properties which make it interesting for the food, cosmetic, and pharmaceutical industries. The industrial interest for grape seeds is demonstrated by their increasing cost and by some new technologies developed to preserve their properties while processed in the alcohol industry. Grape seeds are traditionally sold to the oil extraction industry and more recently they are asked for by cosmetic and pharmaceutical sectors for their use as a source of antioxidants. Grape seed is a well known oilseed crop containing typically 8 - 15% (w/w) of oil with recognized quality due to its high level of unsaturated fatty acids, namely oleic and linoleic and antioxidant-rich compounds. In terms of applications, it is becoming increasingly popular for culinary, pharmaceutical, cosmetics, and medical purposes. Grape seed oil contains a large percentage of free fatty acids (and high level of unsaturated fatty acids, such as linoleic and oleic acids), mono and diglycerides and large amounts of tannins. It is indicated for human consumption and in particular for infants and elderly people: its pharmaceutical activities concern the ability to contrast free-radicals, cardiovascular diseases, cholesterol. The same properties emphasized (accentuated in strength) are today commercially advertised in „new“ and costly products such as grape seed extract and grape extract (9).

Fiori (9) presented the results on supercritical CO₂ extraction of grape seed oil which has been analyzed both experimentally and theoretically. Extractions of crushed seeds at pressures varying in the range 280 - 550 bar and at a fixed temperature of 40°C were performed, Table 1. In the first part of extraction for all the pressure investigated the extraction yield increase (the slope of the curve is constant), then it lowers until the maximum yield is approached. The latter is about 8% for all the tests, except for the lowest pressure. Considering the various tests in their linear portion, increasing the pressure the

slope of the curve also increases. The initial linear portion of the experimental curves yielded oil solubility data in the supercritical solvent. It was found that the maximum oil yield appears to depend on the particle dimensions, the smaller the particle the greater the final yield. This results are in accordance with other papers dealing with the similar substrates. It is worth mentioning that small change in particle size (diameters of 0.49 and 0.51 mm) results in significant change in the extraction curve. This could be due to the physical nature of the substrate or to the intrinsic approximation of considering the particle dimensions as represented by an average value – the Sauter mean diameter (9). Murga et al. (10) have studied supercritical extraction with CO₂ extraction of grape seed at pressures from 100 to 500 bar, and temperatures from 40 to 60°C, Table 1. They determined the solubility of some natural, low-molecular weight phenolic compounds, 3,4-dihydroxy benzoic acid (protocatechuic acid), methyl 3,4,5-trihydroxybenzoate (gallic acid methyl ester or methyl gallate), and 3,4-dihydroxy benzaldehyde (protocatechualdehyde), in SC-CO₂. These phenolic compounds are contained in grape seeds and other natural substrates. The data presented in this work indicate the possibility are valuable to know the possibility of separation from their natural matrices by SC-CO₂.

Evening primrose seed (*Oenothera biennis* L.) contains about 28% of oil. Oil from evening primrose seeds is rich in GLA, cis-6,9,12-octadecatrienoic acid), n-6 essential fatty acid (FA), and free fatty acids (FFA) found in seeds oils, and thus have become a major commercial source of this essential polyunsaturated fatty acid (PUFA). This fatty acid is prone to oxidation and thermal rearrangement; therefore, the conventional recovery of the oil via mechanical expression and hexane extraction must be carried out under very mild and controlled conditions. Studies have shown that GLA can help people with breast cancer, skin problems, cardiovascular diseases, high blood pressure, and is useful in treating neurological problems related to diabetes.

Kotnik et al. (6) studied the supercritical carbon dioxide extraction of the evening primrose, Table 1. Extraction experiments were performed on a semi-continuous flow apparatus with CO₂ at pressures of 200 and 300 bar, and at temperatures of 40°C and 60°C. The flow rate of CO₂ was measured at atmospheric pressure and room temperature: approximately 0.20 and 0.17 kg/h for evening primrose seed extraction. The aim of this work was also to investigate the differences between the compositions of oils obtained by conventional extraction and SC-CO₂; therefore, extraction by hexane was performed using a Soxhlet apparatus. The median particle sizes of the extracted seeds of evening primrose was 0.63 mm, determined by sieve analysis. The composition of the oils extracted was determined by GC analysis and compared with those published in the literature. These authors (6) concluded that for evening primrose seeds, the total yield and extraction rate increased with increasing pressure from 200 to 300 bar. At 200 bar, the temperature had a positive effect on the extraction yield and, oppositely, a negative effect on the extraction rate. At 40°C, a higher extraction rate was observed than at 60°C, which is a consequence of the higher density of CO₂ and the higher solvent power at lower temperature. At 300 bar, the temperature investigated had no influence on the total extraction yield and extraction rate, and the highest yields were obtained. The highest contents of total FFA and GLA in evening primrose extract were obtained by CO₂ extraction at 200 bar and 60°C. The total yield obtained by conventional extraction of evening primrose seeds using the Soxhlet apparatus was similar to that obtained by CO₂ extraction,

with much lower contents of total FFA and GLA. Generally, it can be observed that the main difference is in the composition of FFA in the oil. The content of total and unsaturated FFA obtained by SC-CO₂ was higher compared to conventional extraction. Consequently, the content of saturated FFA in the oil obtained using Soxhlet extraction was higher.

Hazelnut (*Corylus avellana* L.) is the most produced in Mediterranean, especially in Turkey. The solubility of hazelnut particles (1-2 mm) in SC-CO₂ under each condition was determined from the of extraction curves, drawn as a g oil extracted vs. CO₂ at beginning of a each extraction. The solubility values flow rate slopes of linear part It has a high nutritional value, containing, generally, 65% oil, 14% protein, and 16% carbohydrates. More than 90% of its oil consists of unsaturated fatty acids, especially oleic (76 - 80%) and linoleic (6 - 14%) acids. It has been reported that high levels of mono- and polyunsaturated fatty acids and the sterol and tocopherol contents play a preventive role in many diseases, especially cardiovascular ones, because they contribute to lower the low density lipoprotein cholesterol (11).

Ozkál et al. (11) explored the SC-CO₂ extraction of hazelnut particle (1-2 mm) at 150-600 bar, and 40-60°C, Table 1. The solubility of hazelnut oil in SC-CO₂ under each condition was determined from the slopes of the linear parts of extraction curves drawn as g oil extracted vs. g CO₂ observed the beginning of each extraction. The solubility values calculated by using SC-CO₂ flow rate of 0.12 L/h were 10% lower than the ones calculated by using 4 times lower flow rate of 0.03 L/h. Therefore, the flow rate of 0.03 L/h was considered to be low enough to ensure saturation during the solubility measurements. The solubility values were constant for the entire extraction time. The solubility of an oil in SC-CO₂ changes during the extraction only if its composition changes upon fractionation. It was observed (11) that the solubility increased with pressure and temperature above 300 bar. However, at 150 bar the solubility showed a slight decrease with temperature. This is consistent with the crossover phenomena generally observed for some of the oils (12). The solubilities of oils in SC-CO₂ increase both with the density of SC-CO₂ and the volatility of fatty acids. The crossover phenomenon is due to the competing effects of reduction in density of SC-CO₂ and increase in the fatty acids volatility, which accompanis the temperature rise. Therefore, it could be concluded that the crossover pressure for hazelnut oil was in between 150 and 300 bar. The extraction occurred in two periods. The released oil on the surface of particles was extracted in the fast extraction period, and 39% of the initial oil was recovered under each conditions. However, the duration of the fast extraction period decreased with increased pressure and temperature. The unreleased oil in the intact cells was extracted in the slow extraction period. The maximum recovery was 59% at 600 bar and 60°C, for 180 min of extraction. The fluid phase and solid phase mass transfer coefficients increased with increased pressure and temperature. Ozkál et al. (12) used the Response Surface Methodology to determine the effects of solvent flow rate (0.06, 0.18 and 0.30 kg/h), pressure (300, 375 and 450 bar) and temperature (40, 50 and 60°C) on hazelnut oil yield in SC-CO₂ supercritical carbon dioxide extraction, Table 1. Oil yield was represented by a second-order response surface equation ($R^2=0.997$) using Box-Bhenken design of experiments. Oil yield increased with increasing SC-CO₂ flow rate, pressure and temperature. The maximum oil yield was predicted from the response surface equation as 0.19 g oil/g hazelnut (34% of initial

oil) when 4 g hazelnut particles (particle diameter < 0.85 mm) were extracted with 0.30 kg/h SC-CO₂ flow rate at 450 bar, and 60°C for 10 min. Total extraction time under these conditions was predicted to be 35 min (13).

Linseed (*Linum usitatissimum* L.) is very rich in oil which contains a high level of the essential fatty acids, quality proteins, soluble and insoluble fibers, flavonoids and phenolic acids. More than 70% of linseed oil consists of the polyunsaturated fatty acids. The most of acids constitute the α -linoleic acid (ALA), essential Ω -3 fatty acid, linoleic acid, and essential Ω -6 fatty acid.

Nikolovski et al. (13) investigated the SC-CO₂ extraction of linseed oil at the pressures of 300, 400 and 500 bar, temperatures of 47°C and 52°C, extraction times of 4 h, solvent flow rate 8.8 kg/(kg h), and fractions of grinding material of 0.16-0.315 mm, 0.315-0.80 mm and 0.80-2.00 mm, Table 1. The extraction yield increased with increasing pressure from 300 to 500 bar, if the temperature still constant, Figure 1. The extraction yield decreased with increasing temperature, but this effect was of smaller significance than the affect of the other process parameters. The maximum value of extraction yield was obtained when the smallest particle size of ground materials were used. This was caused by an increase the specific area for mass transfer and a decreasing of diffusion path in the solvent.

Pumpkin seed oil (*Cucurbita pepo* L.) can be usually obtained by cold pressing and it has a very good organoleptic properties, odor, flavor, and the color from dark green to brown red. The seed contains from 42 to 54% of oil. The oil has high nutritional value, containing, generally, 50% linoleic, 25% oleic, 10% palmitic, and 5% stearic acids. It is a rich source of micronutrients, as well as vitamin E, phytosterols and lignane (14). The higher dietary intake of phytosterols from the diet, the lower is cholesterol absorption and the lower is the serum cholesterol level.

Sovilj and Barjaktarović (14) studied SC-CO₂ extraction of pumpkin seed on a laboratory scale at pressures of 150, 225 and 300 bar, temperatures of 40 and 60°C, solvent flow rate of 0.20 kg/h, and diameter of ground materials of 0.56 mm, Table 1. The extraction yield obtained at 150 bar was rather low (18.4%) for an extraction time of 14 h. However, extractions at higher pressures yielded greater quantities of the oil; at 225 bar for 9 h, 36.3% of the oil and at 300 bar for 6 h, 41.0% of the oil, Figure 1. For comparison, hexane extraction of the same seed material yielded less than 40% of the oil. Temperature did not influence the extraction yield. At a pressure of 300 bar, the color of the fraction yielded during successive extraction time intervals varied greatly, from pale yellow (the first 2 h), through orange-yellow (from 2-4 h) to red (after 4 h).

Walnut (*Juglans regia* L.) is a crop of a high economic interest for the food industry. Walnut fruit contains high level of oil (52 - 70%) and it is consumed, fresh or roasted, alone or in other edible products. Among the by-products of the walnut industry the oil has not yet obtained popularity, although it has been demonstrated that its consumption has a lot of nutritional benefits. The major constituents in the walnut oil are fatty acids, such as linoleic acid (56.5%), followed by oleic acid (21.2%) and linolenic acid (13.2%). The presence of the other bioactive minor components, such as sterols, tocopherols and phytosterols, has been documented in the literature (15,16). The main component of sterols is β -sitosterol (85.16%), followed by campesterol (5.06%).

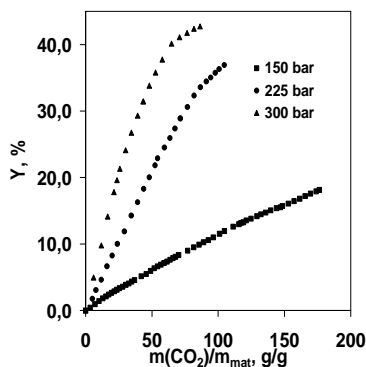


Figure 1. Dependence of the global extraction yield (Y) on the mass relation of carbon dioxide (m_{CD}) and ground particle bed (m_{mat}) (specific solvent flow) in the extraction of pumpkin seed oil (the mean particle size 0.56 mm, temperature 40°C, flow of CO_2 , 0.2 kg/h and pressures of 150, 225 and 300 bar); $Y = (m_{\text{E}}/m_{\text{F}}) 100$, m_{E} - extract yield (kg), m_{F} – feed (kg).

Oliviera et al. (15) examined the extraction of oil from walnut fruits with compressed carbon dioxide in the temperature range of 35 to 48°C and in the pressure range of 180 to 234 bar. The influence of particle size was also studied at a solvent flow rate of 4.0 L/h, Table 1. Fatty acids, sterol, and tocopherol compositions were not different from those of oil obtained with n-hexane. Extraction yield increased with the increase in pressure and under decrease in temperature. The particle size was also a very important parameter. At the conditions studied, the yields increased with the increase in the median particle diameter to an optimum value. At 150 min of extraction time, an optimal value of the yield occurred for the particle diameter range studied, recommending values of about 0.25 to 0.3 mm. At 150 min, the yield exhibited high dependence on the pressure and temperature, with high values for the yield at this extraction time at higher values of pressure and lower values of temperature (15). Martínez et al. (16) evaluated the oil extraction process from walnut seeds by pressing followed by extraction with supercritical CO_2 . The pre-pressed material resulting from the treatment at 7.5% moisture content and 50°C pressing temperature, was ground to 1.2 – 2.4 mm and then extracted with CO_2 in a high pressure pilot plant with single stage separation and solvent recycle. Extraction experiments were done at two pressures (200 and 400 bar) and temperatures (50 and 70°C) with a CO_2 mass flow rate of 10.5 kg/h, in an up-flow arrangement, Table 1. Pre-treatment of moisture conditioning did not affect the oil quality as measured by free fatty acid content. Increasing seed moisture content from 2.5% to 7.5% increased the extraction yield. The highest extraction yield (89.3%) was obtained at 7.5% moisture and 50°C. At this moisture content, an increase of temperature from 50 to 70°C produced a significant reduction in the extraction yield, due to the frequent choking problems during press operation. The drop in the extraction yield at 7.5% and 70°C was common at the end of the press operation (16). The oil quality data from walnut seeds pressed at different moisture and temperature conditions indicated significant variations for all parameters evaluated. The

most outstanding feature was the increase of tocopherol content and acid values with increasing pressing temperature. The CO₂ pressure had a pronounced effect on the extraction yield, the maximum recover being obtained at 400 bar. At this pressure, an increase of temperature had no significant effect on the extraction yield (16).

Wheat germ is a by-product of the wheat milling industry. Germ constitutes about 2 - 3% of the wheat grain and can be separated in a fairly pure form from the grain during the milling process. Wheat germ contains about 11% oil. Wheat germ oil is used in products such as foods, biological insect control agents, pharmaceuticals and cosmetic formulations. Wheat germ processing presents challenges due to its high content of polyunsaturated fatty acids and bioactive compounds. These compounds are prone to oxidation and degradation under the conditions used for conventional edible oil extraction and refining methods. Recent studies have demonstrated that wheat germ oil has several important physiological effects, which include the ability to lower plasma cholesterol, to reduce cholesterol absorption and to inhibit platelet aggregation (17).

Shao et al. (17) investigated the supercritical extraction of wheat germ with carbon dioxide as a solvent. Supercritical CO₂ extraction was carried out using a pilot plant extraction system. Thermostatic baths were switched on to reach the operating temperature required for extraction. Gaseous CO₂ was introduced into a compressor. The extraction vessel was 1000 ml volume capable of operating up to 500 bar and 75°C with the circulation of heated water. The independent variables were temperature (40, 50 and 60°C), pressure (200, 275 and 350 bar) and flow rate (15, 20 and 25 L/h), Table 1. After 200 g sample was placed in extraction vessel, the extraction temperature, pressure and flow rate were controlled automatically and maintained for 60 min. When the desired pressure, temperature and flow rate were reached, the extraction was started. The oil dissolved in the supercritical CO₂ was separated from the carbon dioxide and collected in the separator. Conventional extraction was carried out using hexane in a Soxhlet apparatus for 20 h (with a fraction wheat germ size of 0.75 mm and humidity less than 0.35%) to ensure maximum extraction efficiency. These values are considered very important to establish an indisputable basis for comparison to the high-pressure process. Many parameters can influence the separation performance of wheat germ oil extraction. It was shown that wheat germ oil yield has a complex relationship with independent variables that encompass both first and second-order polynomials and may have more than one maximum point. The best way of expressing the effect of any parameter on the yield within the experimental space under investigated was to generate response surface plots of the equation. The three-dimensional response surfaces were plotted as a function of the interactions of any two of the variables by holding the other one at a middle value. Contour plot and response surface curve showing predicted response surface of oil yield as a function of pressure and flow rate. It showed that at temperature 50°C the oil yield of wheat germ increased with increase in pressure. The oil yield increased from about 7.13% to 10.00% as the pressure was increased from 200 to 350 bar. The optimum pressure for the maximum yield of oil was around 350 bar. At lower pressure, the solubility of oil affected by vapor pressure of the oil, apparently at this stage CO₂ relatively act as an ideal gas that does not have any special characteristic of a solvent. However, at high pressures, the solubility of the oil increased due to the increase in density of CO₂. As the density increases, the distance between molecules decreases and the interaction between oil and

CO₂ increases, leading to greater oil solubility in CO₂. Piras et al. (18) explored the SC-CO₂ of wheat germ oil. The effects of pressure (200-300 bar at 40°C) and extraction time on the oil quality/quantity of feed were studied, Table 1. A comparison was also made between the relative qualities of material obtained by SC-CO₂ extraction and by organic solvent extraction. The extracts were analyzed for α-tocopherol and polyunsaturated fatty acid content. The maximum wheat germ oil yield at about 9% was obtained with SC-CO₂ carbon dioxide extraction at 300 bar, while fatty acid and α-tocopherol composition of the extracts was not remarkable affected by either pressure or the extraction method. The SC-CO₂ gives an CO₂ extraction yield not significantly that of different from organic solvent extraction. A comparison is made between the relative qualities of the oils produced by SC-CO₂ and by organic solvent extraction (hexane, methanol, chloroform-methanol 2:1 mixture) in terms of a much lower selectivity. CO₂ yielded oil contained as undesirable compounds. The effect of the specific mass of solvent, q (kg CO₂/kg feed) on the global extraction yield Y (%) for all the seed oils investigated is presented in Figure 2.

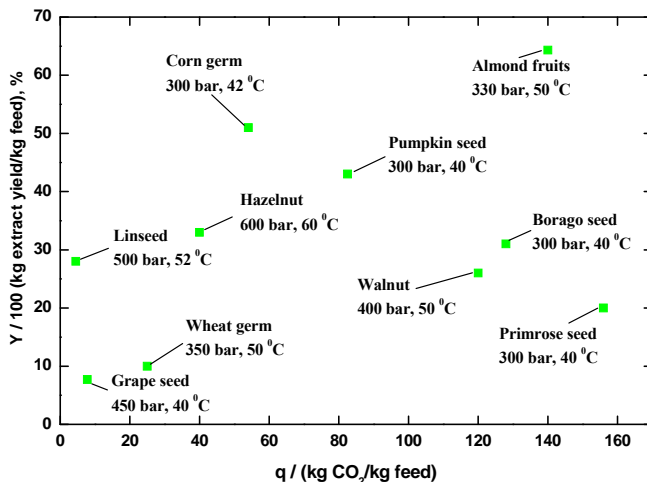


Figure 2. Effect of the specific solvent flow (q) on the global extraction yield (Y) in supercritical extraction of the selected oil seeds; q = kg CO₂/kg feed, Y = (m_E/m_F) 100, m_E - extract yield (kg), m_F - feed (kg).

RESULT AND DISCUSSION

The survey all the above papers related to the effect of extraction pressure on the global extract yielded the SC-CO₂ extraction showed that the extract yield increases with increasing pressure due to the increase of the solvents density, if the temperature, the solvent flow rate, and the diameter of the ground material were constant.

It was observed that the solubility increased with pressure and temperature above 300 bar. However, at 150 bar, the solubility showed a slight decrease with temperature (11). This is consistent with the crossover phenomena generally observed for some of the oils, which results presented in the literature.

In general, the increase of the temperature resulted in the decrease of the extraction yield, due to the decrease of the solvents density, whose effect seems to have dominated over the increase of the solute vapor pressure. In some of the cases the temperature had a positive effect on the extraction yield and, oppositely, a negative effect on the extraction rate (6). At higher pressure (300 bar) it was shown that the temperature had no influence on the total extraction yield and extraction rate, and the highest yields were obtained (6).

In the most of systems investigated solvent flow rate did not influence the extraction yield. From the experimental results for the system almond fruits oil - CO₂ (4) it is evident that oil yield, at the initial stage of extraction, increased increasing CO₂ flow rate, with constant pressure and constant temperature. On the other hand, in the system borage seed - CO₂, the investigation of the effect of solvent flow rate on the extraction yield showed that the lower flow rate test resulted in a slightly higher oil load, but the higher flow rate lead to a shorter extraction time to reach the same yield as the lower flow rate test (3).

The extraction yield increases by decreasing the particle size of the ground materials. It is due to the higher amount of oil released as the substrate cells are destroyed by milling, and this because of the amount of oil is easily extracted for direct exposure to the supercritical CO₂. Moreover, shorter diffusion paths in the milled solid matrix result in a smaller intraparticle resistance to diffusion.

The moisture containing pre-treatment conditionig did not affect the oil quality as measured by free fatty acid content. Increasing seed moisture content from 2.5% to 7.5% increased the extraction yield (16).

In two of the systems, the addition of entrainers was achieved in the aim of enhancement the extract, efficiency were added with aim to achive enchacement SC-CO₂ extraction process (7,8). Caprylic acid methyl ester as an entrainer in the extraction process of borage seed was added (7). The highest solubility of pure caprylic acid methyl ester in dense CO₂ was determined at 100 and 300 bar. The addition of this entrainer increased the yield of pure extract at the pressures investigated. Due to the high solubility of caprylic acid methyl ester at the lower pressure it was easy to separate entrainer, which constituted only 4.22% of the total borage seed extract (7). In the ectraction extraction of corn germ laboratory experiments were carried out at constant pressure (300 bar) and temperature (42°C) with amount of ethyl alcohol (entrainer) ranging from 0% to 10% by weight in CO₂ (8). The alcohol content in the solvent had a strong influence on the rate of the extraction. Increasing the amount of alcohol in the fluid decrease the extraction time and the amount of CO₂ consumption (8).

CONCLUSIONS

In this study, supercritical carbon dioxide extraction of some selected oil seeds are reviewed. The influence of process parameters, such as pressure, temperature, solvent flow rate, diameter of ground materials, and moisture of oil seed on the SC-CO₂ extraction for the selected seed oils: almond fruits, borage seed, corn germ, grape seed, evening primrose, hazelnut, linseed, pumpkin seed, walnut, and wheat germ was presented. The yield and quality of the extracts all of the oil seeds investigated as well as the possibility of

their application in the pharmaceutical and food industries were analyzed. The global extraction yield varied between 7.5 (grape seed) and 65.0% (almond fruits).

Acknowledgements

This research was supported by the Ministry for Science and Technological Development of the Republic of Serbia (Project No. 20022).

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КРИТИЧКИ ПРЕГЛЕД ЕКСТРАКЦИЈЕ ОДАБРАНИХ УЉАРИЦА НАТКРИТИЧНИМ УГЉЕНДИОКСИДОМ

Милан Н. Совиљ

Наткритична екстракција угљендиоксидом, као новија сепарациона метода, користи се као ефикасан поступак за добијање етарских и масних уља из великог броја биљних и лековитих сировина, при чему се добијају екстракти који служе као основа за производњу низа лековитих препарата и додатака прехранбеним производима. У овом раду дат је преглед добијања уља помоћу наткритичног угљендиоксида из мање познатих уљарица, чији се производи такође могу користити као лековити препарати или служе као додаци у функционалној храни. Приказани су процесни параметри коришћени у наткритичној екстракцији угљендиоксидом за следеће уљарице: бадем, коштице грожђа, кукурузна клица, лешник, орах, пшенична клица, семе боражине, лана, ноћурка и уљане тикве. Опсег вредности појединих процесних параметара је био: притисак (150 - 600 bar), температура (10 - 70°C), средњи пречник чврстих честица уситњеног материјала (0,16 - 2,00 mm), проток коришћеног растварача (0,06 - 30,0 kg/h), садржај уља у полазном материјалу (10,0 - 74,0%) и садржај влаге у полазном материјалу (1,1 - 7,5%). Анализирани су принос и квалитет добијених екстраката и могућност примене у фармацеутској и прехранбеној индустрији за сваку од испитиваних уљарица.

Received 8 February 2010
Accepted 21 May 2010