

EXTRACTION OF CAFFEINE, CHLOROGENIC ACIDS AND LIPIDS FROM GREEN COFFEE BEANS USING SUPERCRITICAL CARBON DIOXIDE AND CO-SOLVENTS

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Abstract - The paper reports on experimental data on the extraction of caffeine, coffee oil and chlorogenic acids from green coffee beans using pure supercritical CO₂ and supercritical CO₂ modified with ethanol (5% w/w) and isopropyl alcohol (5% w/w) at 50 and 60°C and 15.2, 24.8 e 35.2 MPa. In this study extraction kinetics were obtained for all assays i.e. samples were collected at several time intervals for each solvent and mixed solvent. When pure CO₂ and CO₂-ethanol mixed solvent were used, an increase in pressure resulted in an increase in the amount of oil extracted. When CO₂ was modified with isopropyl alcohol, the amount of coffee oil extracted also increased with pressure. Caffeine extraction initially increased and subsequently decreased with pressure. Chlorogenic acids were only extracted when isopropyl alcohol was used as a co-solvent. An increase in extraction temperature resulted in a decrease of caffeine and oil extraction (retrograde condensation) when only CO₂ was used as solvent. With the use of co-solvent this retrograde behavior was no longer observed and the increase in temperature resulted in the increase in the extracted amounts of caffeine, coffee oil and chlorogenic acids.

Keywords: Supercritical extraction; Coffee; Coffee oil; Caffeine; Chlorogenic acid; Co-solvent.

INTRODUCTION

Active principles obtained from natural products are widely used by the pharmaceutical, cosmetic and food industries as raw materials for a large number of industrialized products (Cordel, 2000). Coffee beans are an important source of some active principles. Caffeine, the most widely consumed alkaloid in the world, is found in coffee beans (approximately 1-2 wt.%), together with others valuable active principles in still higher

concentration than caffeine. These active components include coffee oil, which is of special interest to the cosmetic and pharmaceutical industries, and chlorogenic acids to which several therapeutic properties have been attributed and are typically found in concentrations of 7-13 wt.% and 6-9 wt.%, respectively (Folstar, 1985, Clifford, 1985; Mazzafera et al. 1998; Lima et al., 2000)

Alkaloids, vegetable oils and chlorogenic acids are commonly extracted by conventional methods using organic solvents (chloroform, dichloromethane,

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etc), which are dangerous to handle and harmful to human health and environment (Mohamed, 1997), and under severe process conditions which could result in product thermal degradation (particularly when steam distillation is involved). Despite the high extraction yield of these conventional processes, the selectivity is often low and the purification of the extracted products is very costly (Reverchon et al., 2000).

Supercritical CO₂ extraction is among the new emerging clean and environmental friendly technologies for the processing of food and pharmaceutical products (Subramanian et al., 1997; Perrut, 2000). Alkaloids (Santana et al., 2006) and phenolics (Okuno et al., 2002) have been extracted from plants using supercritical CO₂. However, this technique strongly depends on the solubility of low-volatile substances in supercritical fluids, usually CO₂, a non-polar solvent, with low affinity for polar substances. So, the solubility of substances in supercritical CO₂ decreases with the increase in the number of polar functional groups (e.g. hydroxyl, carboxyl, amino and nitro). Thus the solubility of chlorogenic acid molecules is expected to be low, particularly as the molecular weight increases (Clifford, 1985; Brunner, 1994; Taylor, 1996).

Small additions of polar co-solvents are usually employed to increase the solubility of polar and high molecular weight substances, despite a possible decrease in selectivity (Brunner, 1994). Two major effects are associated with the addition of a co-solvent: I)- its contribution to the enhancement of physical interactions between solute and solvent molecules which, depending on the nature of the solute, can lead to chemical interactions such as hydrogen bonding, and a consequent increase of the overall solubility (Ting et al., 1993; Brunner, 1994), and II)- the higher critical temperature of the mixed solvent when compared to pure solvent (Kim and Johnston, 1987; Brunner, 1994). In the vicinity of the critical point the isothermal compressibility assumes high values, which leads to the clustering of solvent molecules around the solute molecule and thereby enhancing the solubility (Debenedetti et al., 1989; Brunner, 1994). A good example of the co-solvent effect can be seen in effective extraction of caffeine from coffee beans using moistened green coffee beans and water saturated supercritical CO₂ as a solvent (Peker et al., 1992; Lack and Seidlitz, 1993).

However, depending on the compound to be extracted the presence of moisture can have negative influence on the extraction process. Snyder et al. (1984) investigated the effect of moisture content on the extraction of soy oil from seeds using supercritical CO₂. The authors reported lower

extraction rates for moisture contents higher than 12 wt%. Eggers (1996) also reported a similar result.

Several studies on the extraction of lipids from oleaginous seeds and alkaloids from natural products with supercritical CO₂ and supercritical CO₂ modified with aliphatic alcohols as co-solvents can be found in the literature. Azevedo and Mohamed (2001) reported that the addition of ethanol to supercritical CO₂ decreased the extraction time and the amount of solvent necessary for the extraction of lipids from cupuaçu. Saldaña et al. (2002a,b) used supercritical CO₂ and ethanol as a co-solvent in the extraction of methylxantines from guaraná seeds, mate leaves and cocoa beans.

The main objective of this work is to explore and compare the capacity and selectivity of CO₂ and CO₂ modified with ethanol or with isopropyl alcohol (both acceptable solvents for cosmetics, pharmaceuticals and food processing) in the extraction of caffeine, chlorogenic acids and coffee oil from green coffee beans.

MATERIALS AND METHODS

Materials

Green coffee beans (*Coffea arabica* variety Mundo Novo) were supplied by the Instituto Agronômico de Campinas (Campinas, Brazil). The beans were frozen using liquid nitrogen and ground in a manual mill to avoid oil losses. Ground beans were immediately classified by particle size using a sieve series, and then stored in sealed plastic bags and kept in a freezer until use. The total oil content in this ground material was obtained by gravimetry after hexane extraction in a Soxhlet apparatus. Before each experiment the ground coffee beans were dried in an oven at 65°C during 24h to reduce sample moisture. This limit of temperature was adopted to avoid the oxidation of the coffee oil. Carbon dioxide (99.9%) and carbon dioxide, ethanol or isopropyl alcohol mixtures (5% wt/wt) were purchased from White Martins Co. (Campinas, Brazil). Methanol and ethanol, acetone and acetonitrile in HPLC grade (Merck, Rio de Janeiro, Brazil) were purchased from a local supplier.

Experimental Apparatus

A semi-continuous flow experimental apparatus (Fig. 1) that allows an independent control of temperature and pressure was used for the extraction experiments. The apparatus was designed and

assembled by LEPPBIO/FEQ/UNICAMP supercritical fluid process research group for pressures up to 41.3 MPa at 200°C. The major components of this apparatus included positive liquid displacement pumps (P-1, P-2) (Thermal Separation Products, Riveira Beach, FL, USA) for solvent delivery (46-460 mL/hr), one 300 mL high-pressure extraction vessel (E-1) (Autoclave Engineers, Erie, PA, USA) and two high-pressure columns (C-1, C-2) (300 mm X 12,7 mm I.D.) and separator flasks. The extraction vessel and the columns were supplied with heating jackets and temperature controllers and could be operated in series with three different arrangements. Heating tapes were used throughout the apparatus to maintain constant temperature in the entire equipment. In order to ensure constant and steady solvent delivery, pump heads were cooled circulating fluid through a chiller, CFT R134a (NESLAB Instruments, Newington, NH, USA). Flow rates and accumulated gas volumes passing through the apparatus were measured using a flow computer measuring device (FM-1) (EG&G Instr. Flow Technology, Farmington Hills, Michigan, USA). Micrometering valves VM-1 (Autoclave Engineers, Erie-Pennsylvania, USA) were used for flow control throughout the apparatus. Heating tapes were also used around these valves to prevent freezing of solvents or solid solute

precipitation following depressurization. Behind those valves three flasks were positioned in series to collect the solute after depressurization. The pressure in the extractor and columns was indicated by a digital transducer system (G1, G2, G3), Heise Series 901A RTS, acquired from Dresser Industries (Stratford, CT, USA) with ± 0.03 MPa of precision. Extractor and column temperatures were controlled within $\pm 0.5^\circ\text{C}$ of precision.

The compositions of the coffee extract fractions were determined by HPLC using a LC-10AD chromatograph (Shimadzu, Kyoto, Japan) and a C_{18} column (25 cm x 4.6 mm, 5 μm , Supelco, USA). For the determination of the oil content in the extracts the mobile phase used was acetone/acetonitrile (62:38% v/v) and the flow rate was 1.0 ml/min (González et al., 2001). A refractive index detector model RID 10A (Shimadzu, Kyoto, Japan) was used and the peaks were identified by comparison with pure standards (GC grade, $\geq 99\%$, Sigma, St Louis, USA) and chromatographic profiles presented in the literature (González et al., 2001). The amounts of caffeine and chlorogenic acids in the extracts were determined using a detector SPD 10 AV (Shimadzu, Kyoto, Japan) operating at 280 and 313 nm respectively. Methanol (50% v/v) and (40% v/v) in aqueous sodium acetate (0.5%) at a flow rate of 0.8 ml/min was used as the mobile phase.

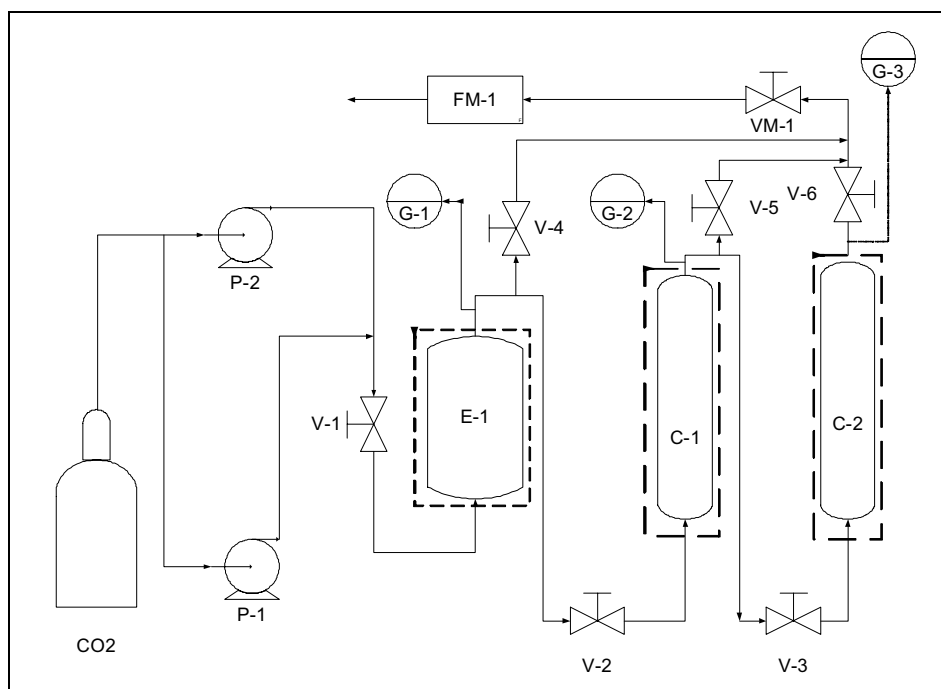


Figure 1: Experimental apparatus.

Experimental Procedures

In order to explore the capacity of CO₂ and CO₂-aliphatic alcohol mixed solvents in the extraction of caffeine, coffee oil and chlorogenic acids from green coffee beans, experiments were carried out at 50°C and 60°C and pressures of 15.2, 24.8 and 35.2 MPa. Solvent mass flow rate was kept at 1.8 g/min. At this flow rate it can be assumed that equilibrium concentration for the solvent and solute is achieved (Filippi, 1982; Mohamed et al. 2000; Azevedo et al., 2003). Samples of 15 g of dried ground green coffee beans (average diameter of 0.725 mm) were placed in the extractor. In a typical extraction experiment, solvent (CO₂ or CO₂-alcohol mixed solvents) was delivered by the pumps as a liquid and slowly allowed into the extractor until the desired extraction pressure was reached. The extractor was heated until the extraction temperature and the micrometering valve positioned downstream of the extractor was slowly opened while maintaining the pressure constant in the extractor. The effluents of the extractor (supercritical fluid and the extracted components) were depressurized through the micrometering valve. The precipitated extract following depressurization was recovered in the separator flasks immersed in a cooling bath. Ethanol was placed in the last separator flask to assure complete recovery of precipitated coffee extract. In these particular experiments, fractions were collected at time intervals characterized by the passage through the extractor of 183 and 91.5 g of CO₂ and, CO₂-alcohol mixed solvents, respectively. The amount of solvent was determined through the gas flowmeter.

In order to estimate the oil extraction yield, the total oil content of the coffee beans was obtained by gravimetry after benzene extraction in a Soxhlet apparatus.

RESULTS AND DISCUSSION

Coffee Oil

The results obtained for the extractions carried out at both isothermal and isobaric conditions for supercritical CO₂, CO₂-isopropyl alcohol and CO₂-ethanol are shown in Figs. 2a, b and c, respectively. Each experimental point on the extraction curve represents the average value of two independent experiments with reproducibility within $\pm 6\%$ for all extractions using supercritical CO₂, CO₂-isopropyl alcohol and CO₂-ethanol. The data reveal that the amounts of coffee oil extracted using CO₂-alcohol mixed solvents were higher than those obtained with supercritical CO₂ at the same process conditions. The addition of co-solvents (5 wt.%) in the formulation

of the mixed solvent resulted in a reduction of 60% on the extraction time and the amount of solvent required to achieve an oil extraction yield of 70%. The oil extraction yield was calculated comparing the extracted oil with the total oil content of the ground beans obtained by Soxhlet extraction. The results revealed that it is possible to obtain coffee oil extraction yields of 70%, 93% and 99% by weight when pure supercritical CO₂, CO₂-isopropyl alcohol and CO₂-ethanol were used as solvents, respectively, at 60°C and 35,2 MPa.

The increase in the extraction yields obtained with the addition of alcohols as co-solvents can be attributed to the enhancement of solvent density and to modifications in both physical and chemical intermolecular interaction forces in the system (Ke et al., 1996). Experimental studies reported by Yonker and Smith (1988) and by Bulgarevich et al. (2002) showed that the addition of a co-solvent increases the local density around the solute molecule increasing the physical interaction, which are short range forces. Depending on the molecules characteristics it leads to the formation of specific interactions such as hydrogen bonding. The combination of an increase in density with the development of physical and chemical interactions has an important role on the formation of the solvation complex and consequently on the solubility. The improvement in the oil extraction process obtained with the addition of co-solvents is in agreement with extraction results of other oils from plant seeds as reported by Cocero and Calvo (1996), Azevedo and Mohamed (2001), among others.

At the same temperature and pressure higher yields were obtained when ethanol was used as a co-solvent instead of isopropyl alcohol. The mole fraction of ethanol and isopropyl alcohol in the mixtures were 0.048 and 0.038, respectively, and at these compositions the mixed solvents presents approximately the same density as can be seen from the equilibrium data reported by Pöhler and Kiran (1997) and Zuniga-Moreno et al. (2002). The higher yields can therefore be attributed to the higher molecular ethanol content in the system. Steric effects can also negatively influence the efficiency of isopropyl alcohol as a co-solvent, due to the relative position of the hydroxyl group in the molecule (Ting et al., 1993).

The slope of the extraction curves in Fig. 2 gives the extraction rate, in g extracted oil /g of solvent. At the beginning of the extraction, the process is phase equilibrium controlled and the diffusion of coffee oil from the seed surface to the supercritical fluid bulk phase is the unique mass transfer resistance considered here. The initial portion of the extraction curves in Fig. 2 describes the extraction rate due to the solubilization of surface available oil by the supercritical solvent (Hedrick et al., 1992). As

expected, increasing pressure at constant temperature, increases solvent density and thus results in higher coffee oil extraction ratios.

The effect of pressure and temperature on the coffee oil extraction ratio at this solubility-controlled region is shown in Fig. 3. From these curves it can be seen that the extraction ratio of coffee oil varies from 0.2 to 3.5 g/kg solvent as the CO₂ density varies from 550 to 890 kg/m³ (at 60°C) for the system CO₂-triacylglyceride. Based on the characteristics of the solute and solvent molecules, the only intermolecular interaction forces present in the system are of physical nature (dispersion and induction ones). These are short range forces and their intensity increase as the distance of the molecules decreases, leading to higher solvation complex formation (Morita and Kajimoto, 1990).

The influence of co-solvent on intermolecular interactions forces becomes evident when results of the effect of pressure on coffee oil extraction with CO₂-alcohol mixtures are compared with those when pure CO₂ was used. Densities of the mixture 90/10% CO₂-ethanol at 50°C and pressures ranging from 10 to 57 MPa are slightly higher than densities of only CO₂ at the same temperature and pressures (Pohler and Kiran, 1997). So, the reduction of the extraction

ratio for CO₂ in comparison with CO₂-isopropyl alcohol and CO₂-ethanol mixtures, at the same pressure and temperature, as shown in Fig. 3, can be attributed to the presence of the induced dipole and the change on the dispersion interaction forces. The occurrence of specific interactions between the oxygen of the esterified carboxyl group of the triacylglyceride molecule and the hydroxyl group of the alcohol will also enhance the extraction rate. The co-solvent effect on CO₂ mixtures diminishes as the pressure increases due to the saturation of the close region around the solute molecule and the self-association of co-solvent molecules (Bulgarevich et al., 2002; Santos et al., 2004).

Experimental data of oil extraction from seeds indicate the occurrence of a retrograde behavior, as reported by Friedrich et al. (1982) and Hadolin et al. (2001), and also by Reverchon et al. (2000) for the supercritical extraction of hiprose seed oil using CO₂ as a solvent at temperatures of 40 and 70°C. The small temperature interval investigated in this study with only two temperature levels suggests the existence of a retrograde behavior when using pure supercritical carbon dioxide and CO₂-ethanol- mixed solvent with negligible influence on oil extraction rate.

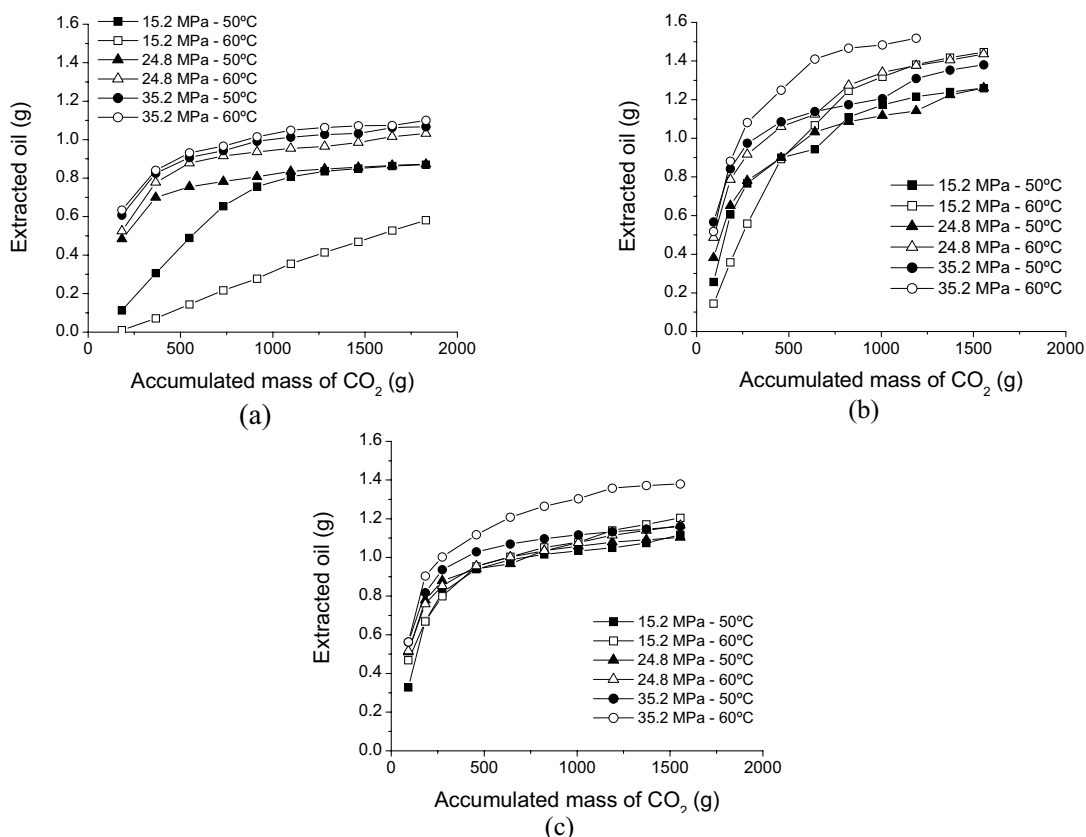


Figure 2: Coffee oil extraction curves. (a) pure CO₂, (b) CO₂/ethanol and (c) CO₂ isopropyl alcohol.

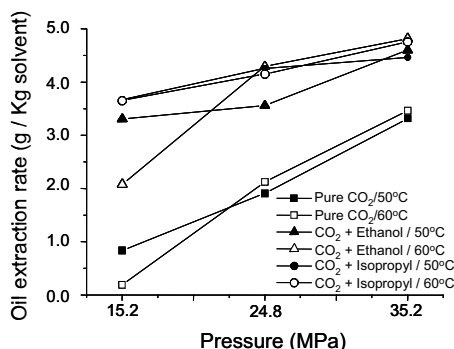


Figure 3: Coffee oil extraction rate as a function of pressure.

Caffeine

Figs. 4a, b and c present the extraction curves obtained for caffeine. The yields, calculated as g caffeine / g of solvent, were 1.7%, 17% and 2% when supercritical CO₂, CO₂-ethanol and CO₂-isopropyl alcohol were used as solvents, respectively. These yields are smaller than those reported by Peker et al. (1992) and Lack and Seidlitz (1993) for caffeine extraction from moistened coffee beans and using supercritical CO₂ saturated with water. The low yield values can be attributed to the fact that caffeine molecules in coffee beans are complexed with chlorogenic acids (Horman and Viani, 1972), and the hydrogen bonds between caffeine and chlorogenic acid molecules have to be broken. The low co-solvent concentration could not be sufficient to break the complex and solvate the caffeine molecules. Kopcak et al. (2004) obtained yields of approximately one order of magnitude higher when the ethanol concentration in carbon dioxide was increased from 5 to 10% in mass for the extraction of caffeine from guaraná (*Paullinia cupana*) seeds.

The influence of the solute-matrix interactions was previously reported by Bjorklund et al. (1998) when extracting clevidipine from different matrices. As discussed above, CO₂ has a high affinity to non polar species (Taylor, 1996), which is the case of triglyceride molecules, and the addition of a polar modifier can improve the extraction of polar species due to changes in intermolecular interactions. This explains the increase in caffeine extraction yields using ethanol and isopropyl alcohol as modifiers. The low caffeine yields obtained throughout this work can partially be attributed to additional resistance to mass transfer. Caffeine is found

homogeneously distributed over the vegetable matrix and migrates by diffusion to the surface (Brunner, 1994).

The lower caffeine extraction yields observed when CO₂-isopropyl alcohol was used as a solvent instead of ethanol can be attributed to the lower number of hydroxyl groups available to chemically interact (hydrogen bonding) with the alkaloid, and also to the loss of selectivity since chlorogenic acids were detected in the extracts only when isopropyl alcohol was used as a co-solvent, as discussed in the next section.

The effect of pressure and temperature on the initial caffeine extraction ratio is shown in Fig. 5. There was an increase in the amount of caffeine extraction with the increase in pressure when supercritical CO₂ and supercritical CO₂-ethanol were used as the solvents due to the increase in density and, thereby, an increase in extraction capacity. With supercritical CO₂-isopropyl alcohol, the caffeine concentration in the extracts initially increased slightly with pressure and subsequently decreased although there was an overall increase of the accumulated mass extracted. Saldaña (1997) studied the extraction of caffeine from coffee beans with water saturated supercritical CO₂ and observed that the total extraction of caffeine decreased with the increase in pressure (solvent density), despite an increase in the total mass extracted. This extraction behavior with pressure was also observed by He et al. (2003) and attributed to the lack of selectivity on the extraction of squalene from amaranth grains using supercritical CO₂ as a solvent in a pressure range of 10 to 30 MPa and temperatures ranging from 40 to 70°C. The reported data show that the extraction ratio increased from 15 to 20 MPa and decreased with pressures from 25 to 30 MPa.

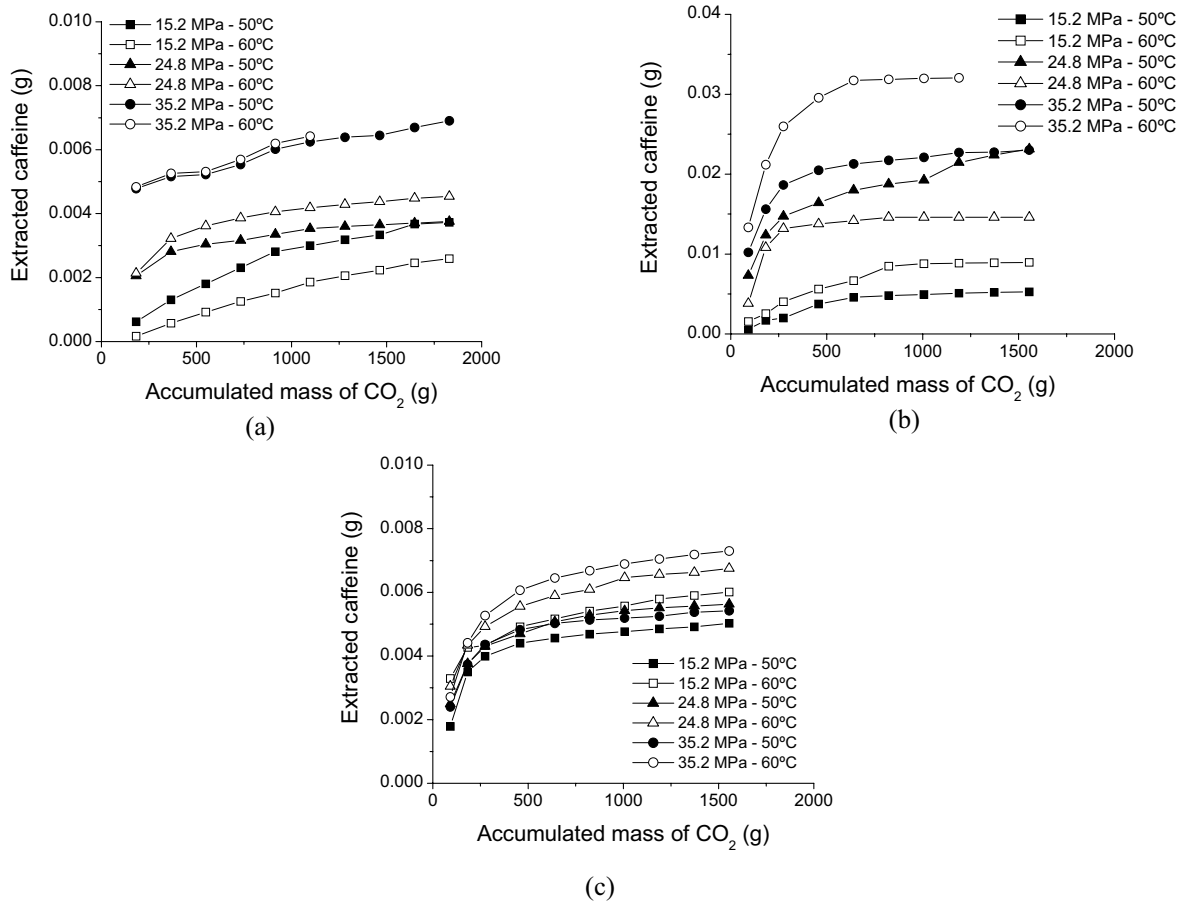


Figure 4: Caffeine extraction curves. (a) pure CO₂, (b) CO₂/ethanol and (c) CO₂/isopropyl alcohol.

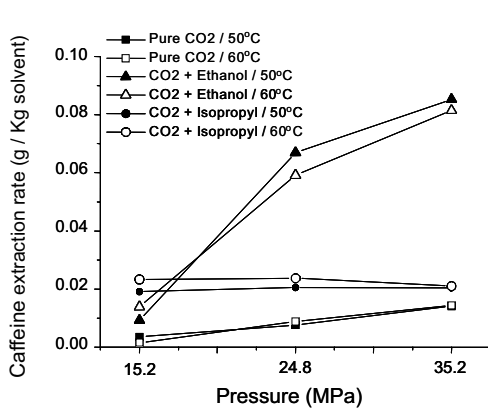


Figure 5: Caffeine extraction rate as a function of pressure.

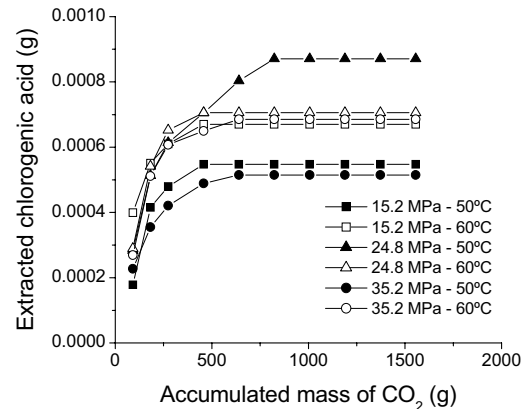


Figure 6: Chlorogenic acids extraction curves using CO₂/isopropyl alcohol as solvent.

Chlorogenic Acid

Extraction experiments using pure CO₂ and CO₂-ethanol revealed that chlorogenic acids were only present as traces in the extracts. Chlorogenic acids were only detected in the extracts when the mixture CO₂-isopropyl alcohol was used as a solvent (Fig. 6). The amount extracted is very small indicating a low solvent efficiency, although liquid isopropyl alcohol has been found a good solvent for conventional extraction of chlorogenic acids from coffee beans (Clifford, 1985). The low concentration of isopropyl alcohol used could be responsible for the inefficient extraction.

Specifically for chlorogenic acids the overall poor extraction efficiency can be attributed to the competition of these molecules with more polar components present in coffee beans (ex. sugars, phenols) as presumed by Azevedo (2005). Had the extraction process been continued for more time, it might have been that the chlorogenic acids would be extracted after the exhaustion of the other competitive components present in the coffee beans.

The effect of pressure on the extraction of chlorogenic acids was the same as observed for caffeine. These results are also in agreement with the assumption of a loss of solvent selectivity as pressure is increased (Brunner, 1983). The change on temperature did not produce a retrograde behavior in the extraction of chlorogenic acids.

CONCLUSIONS

An effective extraction of coffee green bean oil was obtained at 35.2 MPa, even when only supercritical CO₂ was used as a solvent. The addition of ethanol and isopropyl alcohol as co-solvents resulted in an increase in the oil extraction, leading to yields of 93 and 99%, respectively. The effect of co-solvents was associated with the modifications of intermolecular interaction forces and with the increase in solvent density. Increases in temperature showed a moderate retrograde behavior at 15.2 MPa when CO₂ and CO₂-ethanol were used as solvent. Coffee oil has higher affinity with the solvents when compared to caffeine and chlorogenic acids. The low yields for caffeine and chlorogenic acids are probably due to the inability of the solvents to break down the caffeine-chlorogenic acid complex at low co-solvent concentration.

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