JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Extraction of Methylxanthines from Guaraná Seeds, Maté Leaves, and Cocoa Beans Using Supercritical Carbon Dioxide and Ethanol

MARLENY D. A. SALDAÑA,[†] CARSTEN ZETZL,[‡] RAHOMA S. MOHAMED,*,[†] AND
G. BRUNNER[‡]

Faculty of Chemical Engineering, State University of Campinas, C.P. 6066, CEP 13083-970,
Campinas, SP, Brazil, and Arbeitsbereich Termische Verfahrenstechnik, Technical University
Hamburg-Harburg, Eisendorfer Strasse 38, D-21071 Hamburg, Germany

New experimental data on the extraction of caffeine from guaraná seeds and maté tea leaves, and theobromine from cocoa beans, with supercritical CO_2 were obtained using a high-pressure extraction apparatus. The effect of the addition of ethanol to carbon dioxide on the extraction efficiency was also investigated. Caffeine extraction yields of 98% of the initial caffeine content in both wet ground guaraná seeds and maté tea leaves were obtained. Extractions of caffeine from guaraná seeds and maté tea leaves also exhibited a retrograde behavior for the two temperatures considered in this work. In the removal of theobromine from cocoa beans, a much smaller extraction yield was obtained with longer extraction periods and consequently larger solvent requirements. The results of this study confirm the higher selectivity of CO_2 for caffeine in comparison with that for theobromine, and also the influence of other components in each particular natural product on the extraction of methylxanthines. The effect of the addition of ethanol to carbon dioxide on the extraction of methylxanthines was significant, particularly in the extraction of theobromine from cocoa beans. In general, the use of ethanol results in lower solvent and energy requirements and thereby improved extraction efficiency.

KEYWORDS: Caffeine; theobromine; guaraná seeds; maté leaves; cocoa beans

INTRODUCTION

Guaraná seeds are used in concentrated and soft drinks and are known to act as a stimulant mainly because of their rich caffeine content (4.8%) (*1*−*4*). Maté leaves, *Ilex paraguariensis,* produced and consumed mainly in South America, are also known for their stimulant and diuretic properties, attributed also to their contents of caffeine (0.5−0.8%), theophylline (0.01−0.08%), and theobromine (0.03%) (*5*−*7*). Several laboratory studies (*8*−*10*) have shown that polyphenols found in these tea leaves can inhibit the formation and growth of tumors. A high intake of maté can, however, provoke irritability, loss of sleep, cerebral depression, and nervous tremor. Cocoa beans, *Theobroma cacao*, the source of a variety of products including cocoa powder and chocolate, etc., contain significant amounts of theobromine (1.22%), and have strong diuretic, stimulant, and arterial dilating effects (*1, 11, 12*). Extraction of these methylxanthines from these natural plants is a potentially attractive process for the recovery of alkaloids with potential application as ingredients in a variety of pharmaceutical products, as well as production of higher-value methylxanthine-free products for human consumption.

Liquid extraction using solvents such as dimethyl chloride, chloroform, and water has been used for methylxanthines removal from natural plants (*13*, *14*). Chemical solvents, however, require several hours for a complete extraction (*15*) and carry with them the risk of toxic residue in the extracted products. Water, although an excellent solvent of methylxanthines, is highly nonselective and its use may result in the removal of other valuable components from the extracted product (*16*). The low critical temperature, nontoxicity, and low cost have rendered supercritical CO_2 a suitable solvent for food products (*17*−*20*). Application of supercritical fluid technology in biotechnology, and prediction of the solubility of biomolecules in supercritical solvents have been reported (*21*−*23*). Supercritical extraction is successfully used on a commercial scale for the decaffeination of coffee beans (*24*). The only data found in the open literature on the decaffeination of guaraná seeds are those of Mehr et al. (*25*). Saldaña et al. (*7*) extracted caffeine from maté leaves at 70 °C and 255 bar. The potential extraction of theobromine from cocoa beans using dry supercritical CO_2 was also reported by Sambarato (*26*).

Although carbon dioxide is an attractive alternative to current liquid solvent applications for the extraction of active components from natural products (*16*, *17*, *19*, *27*), it is not, in general, an efficient solvent of polar solutes, and the use of cosolvents to modify the solvent power of carbon dioxide is commonly

---

* Corresponding author. Fax: +55 19 788 3931. E-mail: mohamed@feq.unicamp.br.
[†] State University of Campinas.
[‡] Technical University Hamburg-Harburg.

10.1021/jf020128v CCC: $22.00

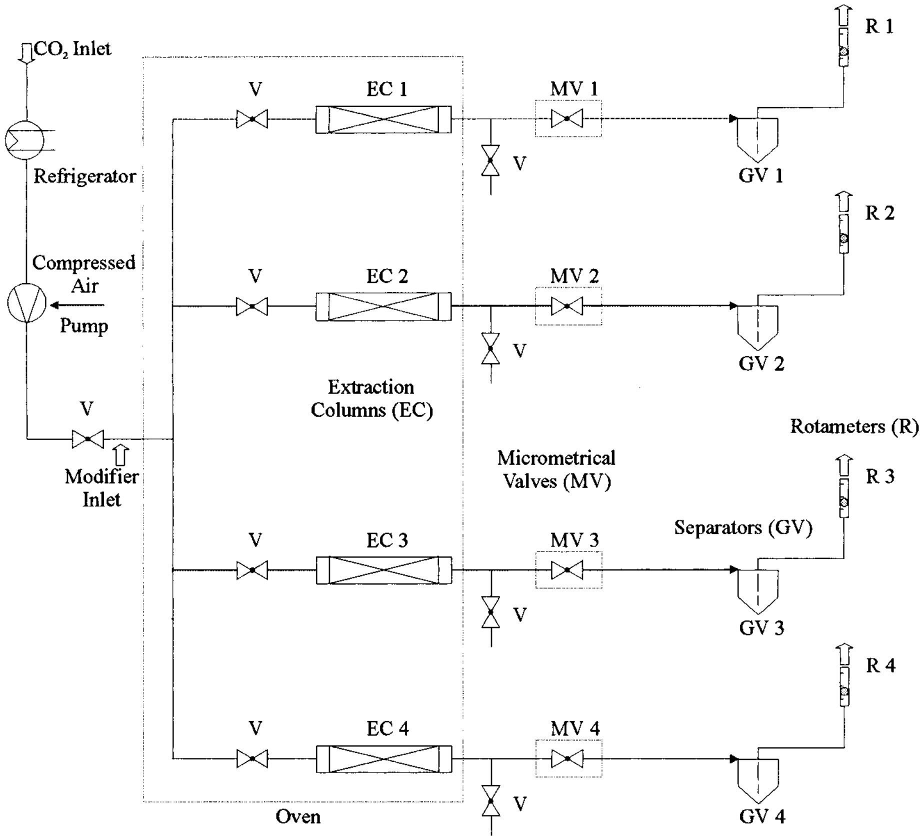

Figure 1. Microextraction plant: MV(1), micrometrical valve; EC(1), extraction column; GV(1), glass vial.

practiced. Cosolvent effects are brought about through specific chemical interaction (hydrogen bonds and acid−base interactions) or physical interactions (dipole−dipole or dipole induced dipole) between the cosolvent and solute, and also possibly interaction between the solvent and the cosolvent, affecting solvent−solute interaction (*28*). Solubility of pure methylxanthines in supercritical CO_2 (*7, 29*) or in water-saturated CO_2 (*30*) is relatively low. Li and Hartland (*31*) obtained data on the extraction of theobromine and cocoa butter from cocoa beans using supercritical carbon dioxide at 150 bar and 60 °C and pointed out ethanol as a better cosolvent for the extraction of cocoa butter than for theobromine. Johannsen and Brunner (*32*) investigated the extraction of caffeine, theophylline, and theobromine with supercritical CO_2/methanol mixtures and reported much higher solubilities of methylxanthines than those obtained with pure supercritical CO_2.

In this work, we present new experimental data on the effects of temperature and pressure on the extractability of caffeine from Brazilian guaraná seeds and maté tea leaves, as well as the extractability of theobromine from Brazilian cocoa beans using water-saturated supercritical CO_2. Li and Hartland (*31, 33*) have obtained similar low yields of theobromine using dry supercritical CO_2. We also present new data on the effect of ethanol addition as a cosolvent in the supercritical CO_2 extraction of these methylxanthines from three natural products: guaraná seeds, maté tea leaves, and cocoa beans at 200 bar and 70 °C.

MATERIALS AND METHODS

Materials. Caffeine and theobromine, 99.9% in purity, were purchased from Aldrich-Chemie (Steinheim, Germany). CO_2, 99.95% in purity, was purchased from KWD (Bad Hönningen, Germany). Maté leaves were collected from the State University of Campinas-Unicamp Experimental Plant (Campinas, Brazil). Cocoa beans and guaraná fruits were supplied in dried form by the Cardill Company S. A. (Bahia, Brazil) and Brazilian State Agricultural Research Company-Embrapa (Manaus, Brazil), respectively. The products were kept in separate and sealed plastic bags until they were ground in a mill before use. For all experiments, ground particles ranging in size from 0.630 to 1 mm, 0.315 to 0.630 mm, and 0.315 to 2 mm, were used for guaraná seeds, maté tea leaves, and cocoa beans, respectively.

Experimental Apparatus. The supercritical extraction plant (**Figure 1**), purchased from Applied Separation Inc. (Allentown, PA), is designed for working pressures and temperatures up to 690 bar and 250 °C, respectively. This microextraction apparatus allows simple and efficient extractions at supercritical conditions with flexibility to operate in a dynamic or a static mode. The plant consists of three modules: an oven, a pump, and a control and collection module. The oven module has capacity for four parallel 50-cm^3 extraction columns placed in a constant temperature environment. The pump module is equipped with a compressed air-driven pump with a constant theoretical flow capacity of 100 mL min^{-1} of liquid CO_2 or 50 standard L min^{-1} of expanded gas. The collection module is formed of 25-mL glass vials with sealed caps and septa for recovery of the extracted products, and is provided with micrometering valves and a flowmeter. Pressure and temperature are controlled in the three modules to ±3 bar and ±0.5 °C, respectively. The CO_2 flow rate is controlled to ±0.47 g min^{-1}. Caffeine and theobromine contents were quantified by HPLC using a Shimadzu LC-6A chromatograph (Kyoto, Japan) and a C18 column (4.6 × 250 mm, 5 μm) ODS−Inertsil. Isocratic solvents composed of 40% methanol in 0.5% of acetic acid in water were used at a flow rate of 1 mL min^{-1} (*7*). The xanthines were identified by a UV detector at 280 nm, and quantities were determined using a Chromjet integrator. Two chromatograms were made for each sample and the results were reproducible to within 6%.

Experimental Procedure. For each experiment, one extraction column was packed with a determined amount of ground particles of the natural product (saturated with water until reaching a humidity of 40%) mixed with 30 g of 3-mm glass spheres. Glass wool was placed at the two ends of the column to avoid any possible carry-over of solid

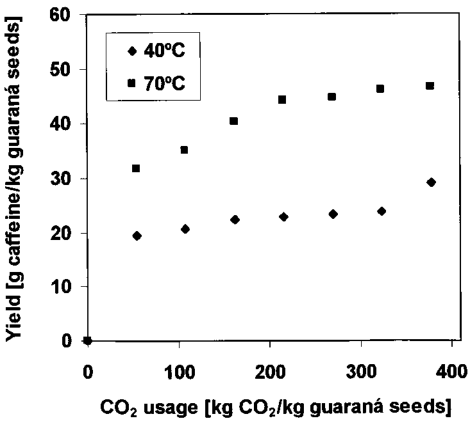

Figure 2. Extraction of caffeine from 3 g of guaraná seeds at 400 bar and 5.7 g min^{-1} water-saturated CO_2. Total = 48 g caffeine/kg guaraná seeds.

material. In another extraction column, located upstream, 40 mL of distilled water was used to allow for the saturation of the incoming CO_2 stream. When the desired temperature, as controlled by the oven, was reached in both columns, CO_2 was pumped to the water-containing column and subsequently to the extraction column until reaching the desired pressure. For experiments using ethanol/CO_2 mixtures, CO_2 and ethanol were pumped to the extraction column at flow rates that allowed the achievement of the desired ethanol concentration, until reaching the selected extraction pressure.

Once equilibrium was reached, the solvent/solute mixture was allowed to flow to the micrometering valve where the separation/precipitation of the desired solute occurred as a result of pressure reduction. After each experiment, the transfer lines were cleaned with hot water and alcohol to ensure the total collection of all extracted material. The alkaloid content was determined by HPLC.

RESULTS AND DISCUSSIONS

In **Figure 2**, the extraction curves of caffeine from a 3-g sample of wet ground guaraná seeds using water-saturated CO_2 at 40 and 70 °C, for experiments conducted at 400 bar and CO_2 flow rate of 5.7 g min^{-1}, are presented on a nondimensional basis to allow the comparison of data independent of the amount of samples used for the extraction and as possible valuable information in future scale-up studies. At 70 °C, 31.7 g of caffeine/kg of guaraná seeds were extracted using 55.9 kg of CO_2/kg guaraná. The quantity extracted reached 46.6 g caffeine/kg guaraná (97% of the initial caffeine) in 210 min of extraction (which corresponds to the use of 391.8 kg of CO_2/kg guaraná). For this same period of time (210 min), extraction at 40 °C resulted in a maximum removal of only 60.2% of the initial caffeine in guaraná seeds (29.01 g caffeine/kg guaraná).

Extraction curves of caffeine from a 2-g sample of ground maté leaves using supercritical water-saturated CO_2 at a flow rate of 5.7 g min^{-1} are presented in **Figure 3**. Similarly to that observed in the extraction of caffeine from guaraná seeds, the quantity of caffeine extracted increased with temperature. In a period of 400 min (corresponding to the use of 1080 kg CO_2/kg maté leaves), 15.5 mg of caffeine (96% of initial content that corresponds to 7.77 g caffeine/kg maté leaves) and 11.4 mg of caffeine (71%, that is 5.73 g caffeine/kg maté leaves) were extracted at 70 and 40 °C, respectively. The results shown in **Figures 2** and **3** also point clearly to the faster and more efficient extraction of caffeine from guaraná seeds than from maté tea leaves. Although there is no significant difference in the plant matrixes involved, as both products are used in the ground form, the ease or difficulty of extraction from these two plants could be attributed to possible interference by other constituents in each plant. Comparing the main components of both matrixes, maté tea leaves contain chlorogenic acids (*34*) while guaraná seeds do not (*35*). Chlorogenic acids can form a complex with caffeine, shown to occur in the case of coffee beans (*19*), making the extraction of caffeine from maté tea leaves more difficult than extraction from guaraná seeds at the same extraction conditions.

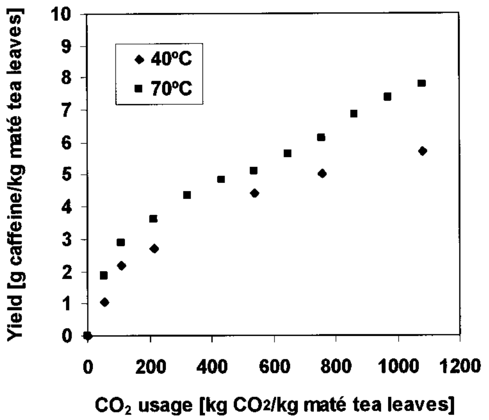

Figure 3. Extraction of caffeine from 2 g of maté at 400 bar and 5.7 g min^{-1} of water-saturated CO_2. Total = 8.1 g caffeine/kg maté tea leaves.

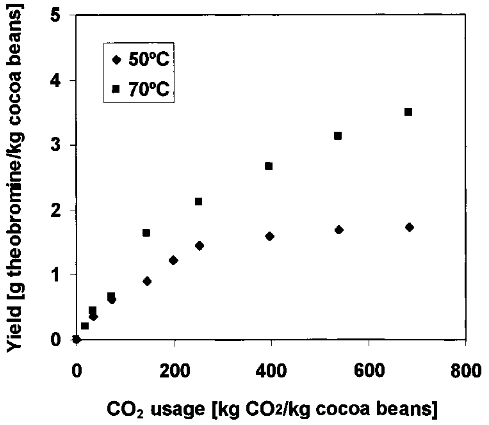

Figure 4. Extraction of theobromine from 3 g of cocoa beans at 400 bar and 5.7 g min^{-1}. Total = 12.2 g theobromine/kg cocoa beans.

The extraction yields of theobromine from a 3-g sample of cocoa beans at 400 bar and using a dry CO_2 flow rate of 5.7 g min^{-1} for the temperatures of 50 and 70 °C are presented in **Figure 4**. For these extractions, much longer periods of time than those observed in the cases of guaraná seeds and maté leaves were necessary for the extraction of theobromine from cocoa beans. More than 380 min (684 kg CO_2/kg cocoa beans) were needed for the cumulative theobromine yields to reach 14 and 29% of theobromine content, that corresponds to 1.74 and 3.49 g theobromine/kg cocoa beans, at 50 and 70 °C, respectively. Although the solubility of caffeine in supercritical CO_2 is at least twice that of theobromine (*36*), there are also substantial amounts of fat components in cocoa beans which could interfere with the extraction of theobromine from the plant matrix.

The theobromine yields are similar to those obtained by Sambarato (*26*), where only 20% of the initial theobromine content in cocoa beans was removed using supercritical CO_2 at 450 bar and 50 °C. The positive effects of temperature on the extraction of theobromine are also in qualitative agreement with those of Sebald et al. (*37*).

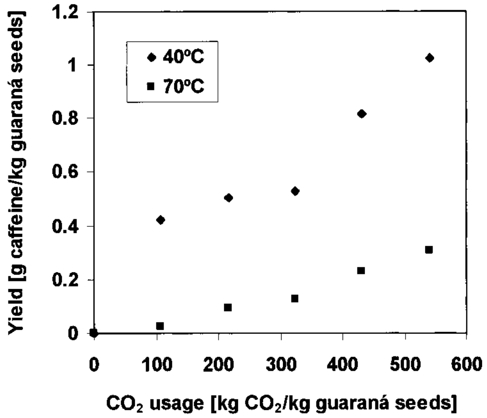

Figure 5. Extraction of caffeine from 3 g of guaraná seeds at 100 bar and 5.7 g min^{-1} of water-saturated CO_2.

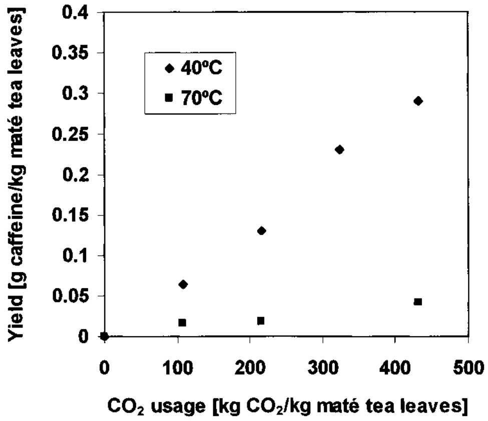

Figure 6. Extraction of caffeine from 2 g of maté leaves at 100 bar and 5.7 g min^{-1} of water-saturated CO_2.

In the extraction of the methylxanthines (caffeine and theobromine) from the three different natural plants at 400 bar (**Figures 2–4**), the increase in vapor pressure due to an increase in temperature appeared to compensate for the decrease in solubility caused by the decrease in solvent density, allowing the extraction yield to increase with temperature. On the other hand, caffeine extraction from guaraná seeds and maté tea leaves using water-saturated CO_2 at 100 bar (**Figures 5** and **6**) revealed the retrograde behavior commonly encountered in supercritical extraction systems (*4, 33, 38–40*). In this case the reduction of solvent density, and thereby the solvent power, with increase in temperature could not be overcome by the increased volatility of the methylxanthines. This result is also in agreement with the solubility values of 6.01×10^{-4} and 1.11×10^{-5} obtained using the correlation of Lentz et al. (*30*) for caffeine in humid CO_2 at 100 bar and temperatures of 40 and 70 °C, respectively.

Figures 7–9 show, respectively, the effect of pressure on the extraction of caffeine from guaraná seeds, caffeine from maté tea leaves, and theobromine from cocoa beans. An increase in extraction yield with pressure was observed for both caffeine and theobromine extractions. This incremental increase is more pronounced when increasing pressure from 100 to 200 bar than when increasing pressures from 200 to 400 bar as observed in **Figure 7**. The effect of pressure, nevertheless, was quite large and much greater than the effect of temperature for the conditions considered in this study.

Extraction at 400 bar resulted in the removal of approximately 73 and 96% of the caffeine in the original guaraná seeds (35.2 and 46.1 g caffeine/kg guaraná) in the first 60 and 180 min of operation using 108 and 324 kg CO_2/kg guaraná, respectively.

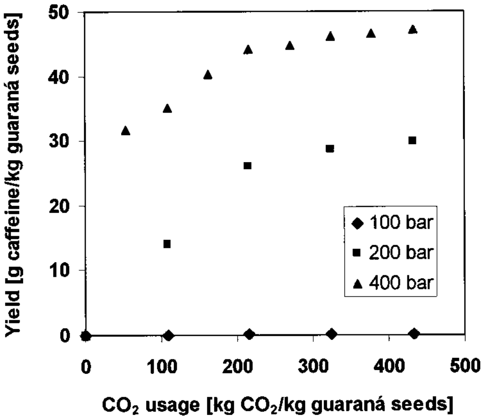

Figure 7. Extraction of caffeine from 3 g of guaraná seeds at 70 °C and 5.7 g min^{-1} of water-saturated CO_2.

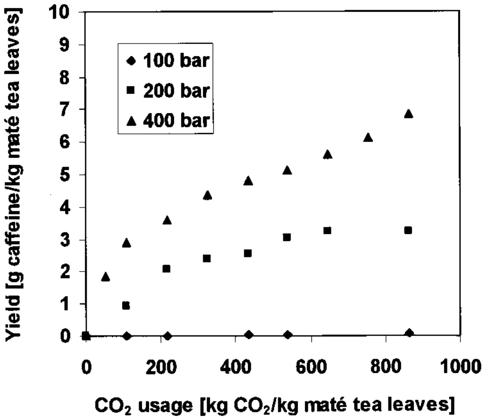

Figure 8. Extraction of caffeine from 2 g of maté tea leaves at 70 °C and 5.7 g min^{-1} of water-saturated CO_2.

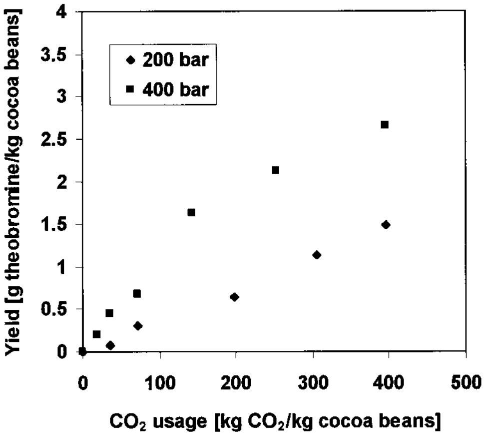

Figure 9. Extraction of theobromine from 3 g of cocoa beans at 70 °C and 5.7 g min^{-1} of dry CO_2.

At an extraction pressure of 200 bar, 29 and 60% of the initial caffeine content, that corresponds to 14 and 28.7 g caffeine/kg guaraná, were removed following 60 and 180 min of continuous extraction, using 108 and 324 kg CO_2/kg guaraná. For these same extraction time periods, only 0.17 and 0.26% of the original caffeine in seeds (0.08 and 0.12 g caffeine/kg guaraná) were removed at 100 bar. This observed influence of pressure on the amounts of caffeine extracted is consistent with the solubility values of 1.11×10^{-5}, 5.27×10^{-4}, and 7.39×10^{-4} obtained from Lentz et al. (*30*), and the values of 0.14×10^{-6}, 2.7×10^{-5}, and 10×10^{-5} obtained from the linear portions of our extraction curves for caffeine in wet CO_2 at 70 °C and pressures of 100, 200, and 400 bar, respectively.

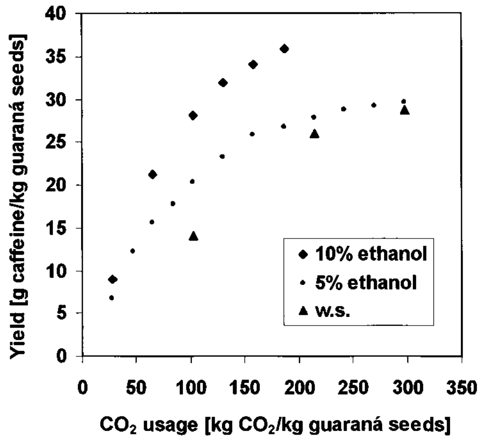

Figure 10. Extraction of caffeine from guaraná seeds at 200 bar, 70 °C, and a CO$_2$ flow rate of 5.7 g min^{-1}. w.s.: water-saturated.

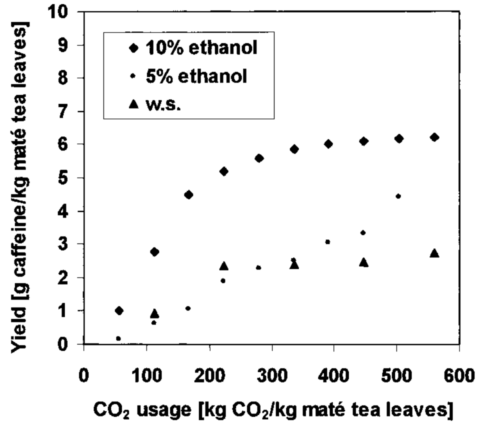

Figure 11. Extraction of caffeine from maté tea leaves at 200 bar, 70 °C, and a CO$_2$ flow rate of 5.7 g min^{-1}.

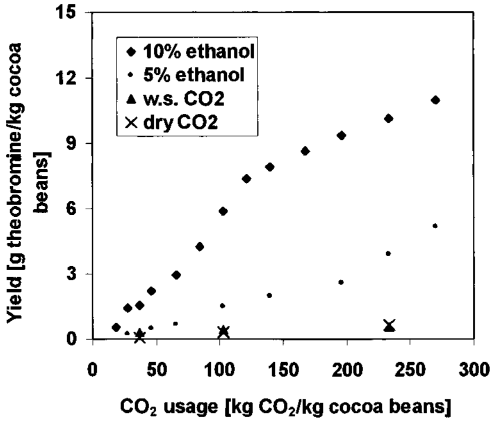

Figure 12. Extraction of theobromine from cocoa beans at 200 bar, 70 °C, and a CO$_2$ flow rate of 5.7 g min^{-1}.

This same effect of pressure can be shown in the extraction of caffeine from maté leaves and theobromine from cocoa leaves. Extraction yields of 0.08, 3.24, and 6.85 g caffeine/kg maté leaves were obtained at 100, 200, and 400 bar, respectively, when using 864 kg CO$_2$/kg maté leaves. From cocoa beans, 1.48 and 2.65 g theobromine/kg cocoa beans were obtained with the use of 396 kg CO$_2$/kg cocoa beans at 200 and 400 bar, respectively.

Figures 10−12 present the cumulative amounts of methylxanthines extracted from the three natural products using two different carbon dioxide/ethanol mixtures at 200 bar and 70 °C. The extraction curves show the influence of ethanol as a cosolvent for supercritical carbon dioxide in the extraction of methylxanthines from natural plants. The quantities of caffeine and theobromine extracted at these conditions increased with quantity of ethanol added, with this effect being more pronounced at higher ethanol concentrations.

In the first 115 min of the extraction, it was possible to remove approximately 54, 58, and 74% of the caffeine in the original guaraná seeds (which corresponds to 26.03, 27.77, and 35.84 g caffeine/kg guaraná) using, respectively, 214.59 kg of water-saturated CO$_2$, 5% ethanol/95% CO$_2$, and 10% ethanol/90% CO$_2$ mixtures per kg of guaraná seeds (**Figure 10**). A similar behavior was observed for the extraction of caffeine from maté tea leaves, as shown in **Figure 11**. In this case, the modification of the solvent with different amounts of ethanol resulted in the increase of the quantity of caffeine extracted from approximately 41 to 75% of caffeine in the original leaves (that is, 3.32 and 6.11 g caffeine/kg maté leaves) using 447.84 kg of solvent/kg maté leaves. Whereas only 30% of the original caffeine (2.46 g caffeine/kg maté leaves) was extracted using water-saturated CO$_2$, doubling the ethanol concentration resulted in an increase in caffeine extraction that is roughly proportional to the increase in the ethanol concentration.

Figure 12 shows the extraction curves for the theobromine removal from ground cocoa beans using dry, water-saturated, and ethanol/CO$_2$ mixtures containing 5 and 10% ethanol. In a period of 145 min (270.57 kg of solvent/kg cocoa beans), the amounts of theobromine extracted when using dry CO$_2$ and water-saturated CO$_2$ were about 10% of the theobromine content. The use of dry and water-saturated CO$_2$ in the extraction of cocoa beans resulted in equally low theobromine yields (**Figure 12**), in agreement with the findings of Li and Hartland (*31*). The addition of ethanol to CO$_2$ resulted in substantial increase in the extraction of theobromine from ground cocoa beans: 42 and 90% of the original theobromine content (5.15 and 10.98 g theobromine/kg cocoa beans) were removed using 5% ethanol/95% CO$_2$ and 10% ethanol/90% CO$_2$ mixtures, respectively. The observed increase in quantity of theobromine extracted from cocoa beans is in qualitative agreement with that presented by Sebald et al. (*37*), at 90 °C, using water-saturated CO$_2$ and ethanol as a cosolvent at the concentrations of 5.2, 6.8, and 9.5%, where 70, 89, and 97% of initial theobromine were obtained, respectively.

The addition of a cosolvent generally results in an increase in the quantity extracted and could possibly mean a reduction of the pressure requirement for a particular extraction, which could amount to substantial energy savings for the process in question. In the extraction of 3.12 mg theobromine/kg cocoa beans, for example, the pressure necessary for the extraction would be reduced from 400 to 200 bar and the time could be reduced from 300 to 125 min (or 540 to 233.25 kg CO$_2$/kg cocoa beans), with the use of 5% ethanol/95% CO$_2$ mixture as compared to water-saturated CO$_2$. When using 10% ethanol/90% CO$_2$ mixture, only 45 min (83.97 kg of solvent/kg cocoa beans) would be necessary to extract the same amount of theobromine at the same pressure and temperature (**Figure 12**). It is also important to note that the effect of the addition of ethanol on the extraction of methylxanthines from the three plant matrixes was not the same (**Figures 10−12**). The rate of extraction was higher for cocoa beans, followed by maté tea leaves and guaraná seeds, which could be attributed to cosolvency effects of cocoa butter in the presence of ethanol.

CONCLUSIONS

It was possible to successfully extract almost all of the initial caffeine content in wet ground guaraná seeds and maté tea leaves at 400 bar, 70 °C, and using 5.7 g min^{-1} of water-saturated supercritical CO_2. At the same conditions of temperature, pressure, and flow rate, only 29% of the original theobromine content in cocoa beans was extracted using dry CO_2. Extractions at lower pressures or temperatures required additional time and larger amounts of CO_2 to achieve the same yield.

The results revealed large influence of temperature and pressure on the supercritical extraction of methylxanthines from these natural plants.

Relatively shorter extraction periods were observed for the removal of caffeine from guaraná seeds as compared to those for caffeine extraction from maté tea leaves, probably due to the influence of other constituents such as chlorogenic acid in the plant matrix.

Extractions of caffeine from guaraná seeds and maté tea leaves at 100 bar exhibited a retrograde behavior for the two temperatures considered in this work.

The use of ethanol as a cosolvent resulted in a large increase in the amounts of methylxanthines extracted from each of the plants considered. Increasing the concentration of ethanol in the solvent mixture from 5 to 10% resulted in higher extracted methyxanthines yields from guaraná seeds, maté tea leaves, and cocoa beans. The quantity of caffeine extracted from ground maté tea leaves and guaraná seeds using supercritical CO_2 and ethanol (10% in ethanol) was two times larger than that obtained with water-saturated supercritical CO_2, whereas the quantity of theobromine extracted from ground cocoa beans using the same solvent mixture was an order of magnitude higher than that obtained with water-saturated CO_2.

The use of small amounts of ethanol as a cosolvent could have significant impact on the economic evaluation of supercritical fluid extraction of methylxanthines from natural plants, with the large decrease of pressure, and thereby energy requirements, for the process.

ACKNOWLEDGMENT

We are grateful to Cardill S.A. and Embrapa for donating the cocoa beans and guaraná seeds, respectively.

LITERATURE CITED

(1) James, J. E. *Caffeine and Health*. Academic Press Inc.: San Diego, CA, 1991; pp 24−32, 63−85.

(2) Baumann, T.; Schulthess, B. H.; Hänni, K. Guaraná (*Paullinia cupana*) rewards seed dispersers without intoxicating them by caffeine. *Phytochemistry* **1995**, *39* (5), 1063−1070.

(3) Carlson, M.; Thompson, R. D. Liquid chromatographic determination of methylxanthines and cathechins in herbal preparations containing guaraná. *J. AOAC Int*. **1998**, *81* (4), 691−701.

(4) Saldaña, M. D. A.; Zetzl, C.; Mohamed, R. S.; Brunner, G. Decaffeination of guaraná seeds in a microextraction column using water-saturated CO_2. *J. Supercrit. Fluids* **2002**, *22*, 119−127.

(5) Alikaridis, F. Natural constituents of *Ilex* species. *J. Ethnopharmacol*. **1987**, *20*, 121−144.

(6) Tormen, M. J. Economia ervateira Brasileira. In *Erva mate: Biologia e Cultura no Cone Sul*, 1st ed.; Winge, H., Ferreira, A. G., Mariath, J. E. A., Tarasconi, L. C., Eds.; Editora da Universidade UFRGS: Porto Alegre, Brazil, 1995; pp 27−40.

(7) Saldaña, M. D. A.; Mazzafera, P.; Mohamed, R. S. Extraction of purine alkaloids from maté (*Ilex paraguariensis*) using supercritical CO_2. *J. Agric. Food Chem.* **1999**, *47* (9), 3804−3808.

(8) Wang, Z. Y.; Huang, M.; Lou, Y.; Xie, J.; Reuhl, K.; Newmark, H.; Ho, C.; Yang, C.; Conney, A. Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz[*a*]anthracene-initiated SKH-1 mice. *Cancer Res.* **1994**, *54*, 3428−3435.

(9) Inagake, M.; Yamane, T.; Kitao, Y.; Oya, K.; Matsumoto, H.; Kikuoka, N.; Nakatani, H.; Takahashi, T.; Nishimura, H.; Iwashima, A. Inhibition of 1,2-dimethylhydrazine-induced oxidative DNA damage by green tea extract in rat. *Jpn. J. Cancer Res.* **1995**, *86*, 1106−1111.

(10) Hasegawa, R.; Chujo, T.; Sai-Kato, K.; Umemura, T.; Tanimura, A.; Kurokawa, Y. Preventive effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. *Food Chem. Toxicol.* **1995**, *33* (11), 961−970.

(11) Li, S.; Varadarajan, G. S.; Stanley, H. Solubilities of theobromine and caffeine in supercritical carbon dioxide: correlation with density-based models. *Fluid Phase Equilib.* **1991**, *68*, 263−280.

(12) Sotelo, A.; Alvarez, R. G. Chemical composition of wild *Theobroma* species and their comparison to the cacao bean. *J. Agric. Food Chem.* **1991**, *39* (11), 1940−1943.

(13) Baltassa, F.; Carbour, N.; Ferry, S. Étude du contenu purique de drogues a caféine: I.-Le Maté: *Ilex paraguaryensis* Lamb. *Plant. Méd. Phytothér.* **1984**, *18* (4), 195−203.

(14) Mazzafera, P. Caffeine, theobromine and theophylline distribution in *Ilex paraguaryensis. Rev. Bras. Fisiol. Veg*. **1994**, *6*, 149−151.

(15) Hulbert, G. J.; Biswal, R. N.; Walker, T. H.; Mehr, C. B.; Collins, J. L. Solid/liquid extraction of caffeine from guaraná with methylene chloride. *Food Sci. Technol. Int.* **1998**, *4* (1), 53−58.

(16) Saldaña, M. D. A. *Extração de cafeína, trigonelina e ácido clorogênico de café com CO_2 supercrítico*. M. Sc. Thesis, Universidade Estadual de Campinas, Campinas, Brazil, 1997, 186 pp.

(17) Brunner, G. Mass transfer from solid material in gas extraction. *Ber. Bunsen-Ges. Phys. Chem.* **1984**, *88*, 887−891.

(18) Peker, H.; Srinivasan, M. P.; Smith, J. M.; McCoy, B. J. Caffeine extraction rates from coffee beans with supercritical carbon dioxide. *AIChE J.* **1992**, *38* (5), 761−770.

(19) McHugh, M. A.; Krukonis, V. J. *Supercritical Fluid Extraction: Principles and Practice*; Butterworth Publishers: Boston, MA, 1994.

(20) Saldaña, M. D. A.; Mazzafera, P.; Mohamed, R. S. Extraction of caffeine, trigonelline and chlorogenic acid from brazilian coffee beans with supercritical fluids. In *Proceedings of the 4th International Symposium on Supercritical Fluids*, Sendai, Japan, 1997; pp 219−222.

(21) Schulz, K.; Mansoori, G. A. Supercritical Fluid Extraction/Retrograde Condensation (SFE/RC) with applications in Biotechnology. In *Supercritical Fluid Technology*; Bruno, T. J., Ely, J. F., Eds.; CRC Press: Boca Raton, FL, 1991; pp 451−478.

(22) Mansoori, G. A.; Schulz; K.; Martinelli, E. Bioseparation using supercritical fluid extraction/retrograde condensation. *Bio/Technology* **1988**, *6*, 393−396.

(23) Hartono, R.; Mansoori, G. A.; Suwono, A. Prediction of solubility of biomolecules in supercritical solvents. *Chem. Eng. Sci*. **2001**, *56*, 6949−6958.

(24) Brennecke, J. F. New applications of supercritical fluids. *Chem. Ind.* **1996**, *Nov.*, 831−834.

(25) Mehr, C. B.; Biswal, R. N.; Collins, J. L. Supercritical carbon dioxide extraction of caffeine from guaraná. *J. Supercrit. Fluids* **1996**, *9* (3), 185−191.

(26) Sambarato, D. *Esperienze di Estrazione della Teobromina da Cacao com Anidride Carbonica Supercritica*, Ph.D. Thesis, University of Milan, Italy, 1984; pp 85−115.

(27) Fillipi, R. P. CO_2 as a solvent: application to fats, oils and other materials. *Chem. Ind.* **1982**, *19*, 390−394.

(28) Ekart, M. P.; Bennett, K. L.; Ekart, S. M. Cosolvent interactions in supercritical fluid solutions. *AIChE J*. **1993**, *39* (2), 235−248.

(29) Johannsen, M.; Brunner, G. Solubility of the xanthines caffeine, theophylline and theobromine in supercritical carbon dioxide. *Fluid Phase Equilib.* **1994**, *95*, 215−226.

(30) Lentz, H.; Gehrig, M.; Schulmeyer, J. Dynamic solubility measurements of caffeine in carbon dioxide and in carbon dioxide saturated with water. *J. Phys. B* + *C* **1986**, *70*, 139−140.

(31) Li, S.; Hartland, S. Influence of cosolvents on solubility and selectivity in extraction of xanthines and cocoa butter from cocoa beans with supercritical carbon CO_2. *J. Supercrit. Fluids* **1992**, *5*, 7−12.

(32) Johannsen, M.; Brunner, G. Measurements of solubilities of xanthines in supercritical carbon dioxide plus methanol. *J. Chem. Eng. Data* **1995**, *40* (2), 431−434.

(33) Li, S.; Hartland, S. A. A new process for extracting cocoa butter and xanthines with supercritical CO_2. *J. Am. Oil Chem. Soc.* **1996**, *73* (4), 423−429.

(34) Clifford, M.; Ramirez-Martinez, J. R. Chlorogenic acid and purine alkaloids contents of maté (*Ilex paraguariensis*) leaf and beverage. *Food Chem*. **1990**, *35*, 13−21.

(35) Henman, A. R. *Vida Natural: O guaraná*. Global Editora e Distribuidora Ltda.: Rio de Janeiro, Brazil, Sept. 1986; 77 pp.

(36) Martin, A.; Paruta, A. N.; Adjei, A. Extended Hildebrand solubility approach: methyxanthines in mixed solvents. *J. Pharm. Sci.* **1981**, *70* (10), 1115−1120.

(37) Sebald, J.; Schulmeyr, J.; Gehrig, M. Removal of xanthines from cacao. In *High Pressure Chemical Engineering*; von Rohr, R., Trepp, Ch., Eds.; Elsevier Science BV: Amsterdam, The Netherlands, 1996; pp 333−338.

(38) Stahl, E.; Schilz, W. Mikroanalytische untersuchungen zur loslichkeit in uberkritischem kohlendioxdid.. *Talanta* **1979**, *26*, 675−679.

(39) Friedrich, J. P.; Pryde, E. H. Supercritical CO_2 extraction of lipid-bearing materials and characterization of the products. *J. Am. Oil Chem. Soc.* **1984**, *61* (2), 223−228.

(40) Friedrich, J. P.; List G. R.; Heakin, A. J. Petroleum-free extraction of oil from soybean with supercritical CO_2. *J. Am. Oil Chem. Soc*. **1982**, *59*, 282−292.

Received for review January 31, 2002. Revised manuscript received May 27, 2002. Accepted May 29, 2002. We appreciate the financial support received from the Brazilian Research Funding Agencies FAPESP and CNPq.

JF020128V