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Extraction of Purine Alkaloids from Maté (*Ilex paraguariensis)* Using Supercritical CO_2

Marleny D. A. Saldaña,[†] Rahoma S. Mohamed,*[,†] Martin G. Baer,[†] and Paulo Mazzafera[‡]

Faculty of Chemical Engineering and Institute of Biology, State University of Campinas, C.P. 6066,
CEP 13083-970, Campinas, SP, Brazil

Experimental data for the supercritical CO_2 extraction of purine alkaloids (caffeine, theobromine, and theophylline) from ground herbal maté tea (*Ilex paraguaryensis*) using a high-pressure apparatus are presented. Caffeine, theophylline, and theobromine were identified in the extracted fractions using HPLC. Results indicated a much higher CO_2 selectivity for caffeine in comparison with those for theophylline and theobromine. Solubilities of pure compounds in carbon dioxide were also determined at 313.2, 323.2, 338.2, and 343.2 K, and pressures ranging from 14 to 24 MPa. Caffeine solubility exhibited a retrograde behavior with temperature while theophylline and theobromine manifested a normal behavior at conditions explored in this study. Solubilities in binary CO_2/purine alkaloid model systems were much higher than those obtained during extraction of maté tea, demonstrating the difficulty of using binary data in predicting complex multicomponent behavior.

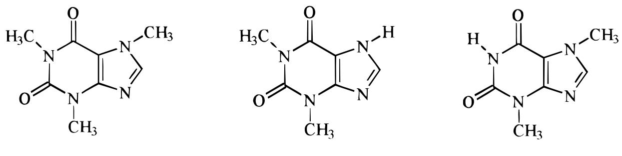
Keywords: *Supercritical fractionation; maté tea; caffeine; theophylline; theobromine; methylxanthines*

INTRODUCTION

Maté, *Ilex paraguaryensis*, a member of the denominated "holly" plants, is a native plant of South America. Most of the maté produced is locally consumed, with Argentina being the largest producer and consumer (Alikaridis, 1987; Tormen, 1995).

Caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), and theobromine (3,7-dimethylxanthine), whose chemical structures are shown in Figure 1, are natural components of maté tea leaves, coffee, guaraná, and cocoa beans (Baltassa et al., 1984; Suzuki and Waller, 1988). For the human body, these alkaloids act as stimulants of the central nervous, muscle, and circular systems (James, 1991).

Decaffeination of natural beans and leaves can be economically attractive in obtaining a decaffeinated product and caffeine, a valuable byproduct commonly used in cola drinks and pharmaceutical products (Mazzafera and Carvalho, 1991). Maté tea is a beverage that is prepared by the infusion of green dried maté leaves and traditionally consumed by the Gauchos of South America, residing in Argentina, southern Brazil, Paraguay, and Uruguay (Tormen, 1995). Estimates of caffeine intake due to maté tea consumption exceed by far intakes recorded in the literature from other beverages containing this alkaloid (Mazzafera, 1997). Similar to coffee decaffeination, caffeine removal of maté leaves could be performed using organic solvents (dimethyl chloride) or water (Saldaña, 1997). However, chemical solvents bring with them a risk of toxic residue in the extracted products, and the use of water results in a nonselective extraction with a consequent loss of valuable flavor components (Mazzafera and Carvalho, 1991).



A. Caffeine B. Theophylline C. Theobromine

Figure 1. Purine alkaloids chemical structures.

The low critical temperature (31 °C), nontoxicity, and low cost have rendered supercritical carbon dioxide a more suitable solvent for food products (McHugh and Krukonis, 1994; Saldaña, 1997).

While there are various patents on the use of supercritical CO_2 as a solvent for caffeine extraction from coffee beans and tea leaves (Saldaña et al., 1997; Peker et al., 1992; Brunner, 1984; McHugh and Krukonis, 1994), very little is known about the extraction of other important methylxanthine constituents of pharmaceutical value and pronounced physiological effects, such as theophylline and theobromine. Data on the solubility of these purine alkaloids in supercritical fluids is rather scarce in the open literature. The works of Johannsen and Brunner (1994) and Li et al. (1991) on binary methylxanthine/CO_2 systems were the only ones encountered.

The main objective of this work was to obtain experimental information on the extractability of the methylxanthines: caffeine, theophylline, and theobromine from ground herbal maté tea leaves, using supercritical CO_2. Individual solubilities of pure methylxanthines in model supercritical carbon dioxide/methylxanthine systems were also measured at various temperatures and pressures and compared to those in complex maté/carbon dioxide systems.

MATERIALS AND METHODS

Materials. Caffeine, theophylline, and theobromine, 99% purity, were purchased from Sigma Chemical Co. (St. Louis,

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* To whom correspondence should be addressed. E-mail: mohamed@feq.unicamp.br. Fax: +55 19 289 4717.

[†] Faculty of Chemical Engineering.

[‡] Biology Institute.

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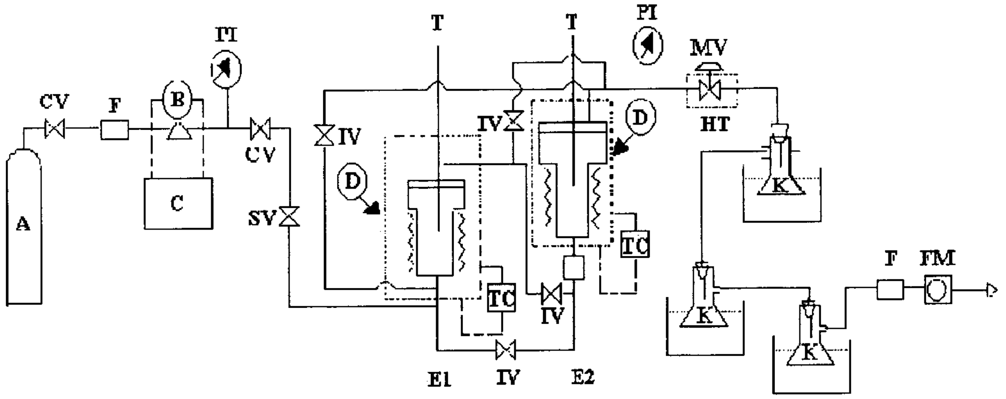

Figure 2. Experimental apparatus: (A) CO_2 cylinder, (B) pump, (C) chiller, (CV) check valve, (D) heating jacket, (E1) simple extractor, (E2) extractor with window, (F) line filter, (FM) flow meter, (HT) heating tape, (IV) isolation valve, (K) separator flasks in cooling bath at 0 °C, (MV) micrometering valve, (PI) pressure indicator, (TC) temperature controller, and (T) thermocouples.

MO). Carbon dioxide, bone dry, 99.9% purity, was donated by White Martins (Campinas, SP, Brazil). Maté tea leaves, produced by Ximango Erva-Maté Ltda. (Ilópolis, Rio Grande do Sul, Brazil) was purchased in a local market. This product, ready for maté infusion, is composed of dry and coarsely ground leaves.

Sample Preparation and Analysis. Before the extraction, samples of maté tea leaves (30 g) were granulometrically classified using standardized sieves. The sample was separated in fractions as a function of particle size. Moisture content of the sample was determined by drying at 105 °C for 24 h. Compositions of methylxanthines in extracted products were determined by HPLC (Shimadzu, Japan) with a Nucleosil C_{18} column (Supelco, 4 × 150 mm, 5 μm), using 20% methanol (HPLC grade) in 0.5% aqueous acetic acid as isocratic solvent. The flow rate was 1 mL min^{-1}, and the compounds eluting from the column were detected with a UV monitor set at 280 nm. The peak areas registered by a recorder/integrator were compared with those obtained with pure standards.

Experimental Apparatus. The experimental apparatus used was a semicontinuous-flow high-pressure system purchased from Autoclave Engineers (Erie, PA), and designed for working pressures up to 37 MPa (370 bar) at 200 °C (Figure 2). This apparatus is the same one used earlier by Neves et al. (1996). The major components of the apparatus include positive liquid displacement pumps for solvent delivery, high-pressure extraction vessels, and three separator flasks in series. Flow rates and accumulated gas volumes passing through the apparatus were controlled with micrometering valves from Autoclave Engineers (Erie, PA) and measured with a flow computer measuring device from EG&G Instrument Flow Technology (Phoenix, AR). Heating tapes were used to maintain constant temperature in the extraction section and in valves to prevent freezing of solvents or solid solute precipitation following depressurization. Pressure in both extractors were monitored with a digital transducer system, Heise Series 901A RTS, acquired from Dresser Industries (Stratford, CT) with a precision of ± 0.03 MPa (± 0.3 bar). Extractor temperatures were controlled to ± 0.5 °C.

Pure Methylxanthines Solubility Measurements. The solubilities of caffeine, theophylline, and theobromine in supercritical carbon dioxide as a function of temperature and pressure were first determined. These are binary model systems that could provide information essential for the comprehension and thermodynamic modeling of the complex multicomponent real system, e.g., maté leaves/carbon dioxide. To measure the pure alkaloid solubility in supercritical CO_2, each methylxanthine (12 g) was mixed with glass beads (4 mm in diameter) and placed in extractor E1. Liquid CO_2 was slowly delivered into the extractor until the extraction pressure at the specified temperature was reached. Following a 2−3 h period to reach equilibrium, the heated micrometering valve was slowly opened while pressure was maintained constant in the extractor. CO_2 flow rates of 0.9−1.2 g min^{-1} were used in all experiments reported in this work. These flow rates were similar to those reported in the literature (Mohamed et al., 1998; Jimmy Yun et al., 1991) and are much lower than the maximum space velocity of 2.0 min^{-1} suggested in the literature (Fillipi, 1982) in order to avoid mass transfer limitations in supercritical extraction systems. Furthermore, experiments were also repeated at different flow rates (0.5−2.5 g min^{-1}). The variation in flow rate was found to have no effect on the measured solubilities and thereby confirming achievement of equilibrium solubility. Glass wool plug in the exit of the extractor was used to prevent any carry-over of methylxanthine particles. The effluent of the heated valve (low-pressure CO_2, now in gaseous state, and precipitated methylxanthine) were introduced into the cooled and tarred flasks for the recovery of precipitated methylxanthines. The second and third flasks contained deionized water to ensure complete recovery of all precipitated alkaloid. At the end of each experiment, the tubing located down stream of the micrometering valve was washed with hot water, added to the contents of the second and third flasks, and the whole sample was analyzed for the alkaloid by HPLC. The total amount of CO_2 used was recorded by the gas flow meter.

Extraction of Methylxanthines from Maté Tea Leaves. In a typical experiment liquid CO_2 was pumped into the ground maté leaves sample (10 g, 10% moisture content) in extractor E1 until the specified extraction pressure was reached at the specified temperature. After a 3-h period to reach equilibrium, the saturated supercritical fluid was depressurized while pressure was maintained constant in the extractor and a precipitated fraction was collected in the tarred separator flasks placed in a cooling bath maintained at 0 °C. Collected samples were weighed and analyzed for purines using HPLC. The three separator flasks were replaced at intervals and extracted fractions were collected for each 61.5 g of carbon dioxide passing through the extractor for the first 7 h. Subsequently, fractions were collected for each 369.2 g of CO_2 passing through the extractor. A total of 14 fractions were continuously collected during the extraction carried out at 25.5 MPa and 343.2 K. Tubings and valves throughout the apparatus were cleaned with chloroform at the end of each experiment.

RESULTS AND DISCUSSION

The granumetrically classified sample was separated in four fractions as a function of particle size. In this work, a finely divided fraction with an average diameter of 0.046 mm and a moisture content of 10% was used in all experiments.

Pure Methylxanthines Solubility Measurements. To verify the reliability of equilibrium data obtained with our apparatus, caffeine solubility was obtained at 313.2 K (Figure 3a) and compared to those reported by Johannsen and Brunner (1994). Good agreement be-

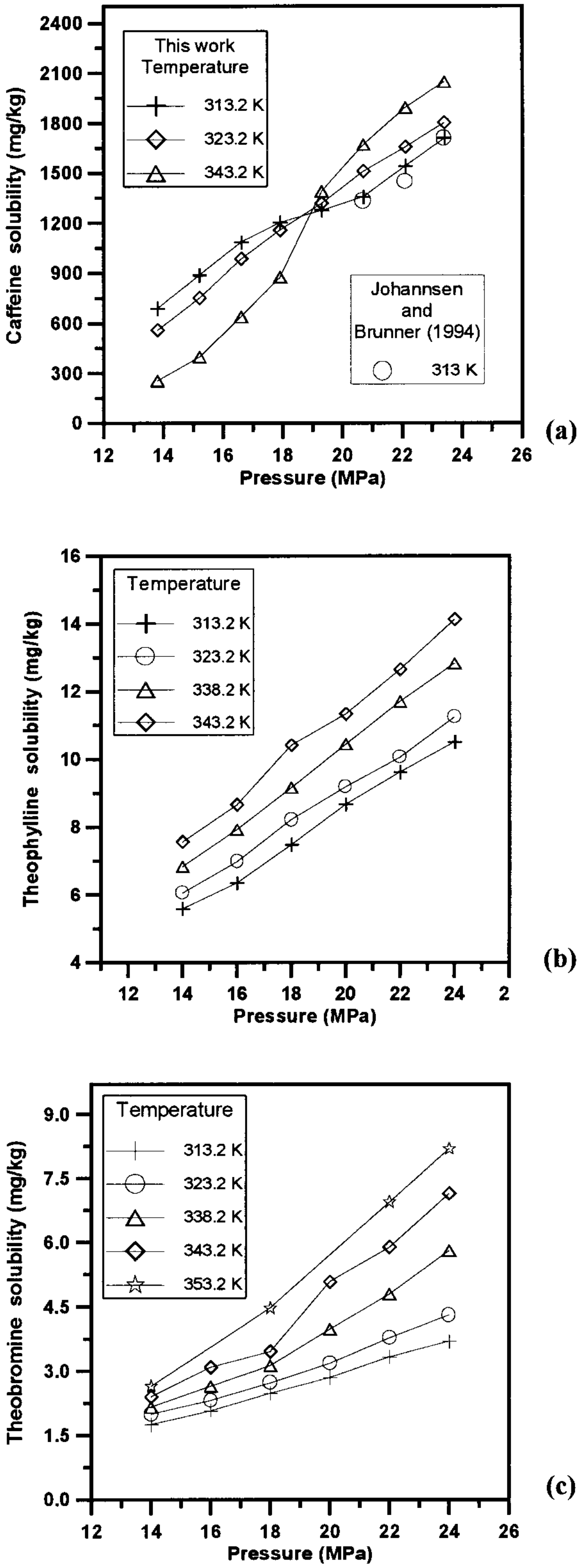

Figure 3. Caffeine (a), theophylline (b), and theobromine (c) solubilities at different pressures and temperatures.

tween our data and reported values was observed. New data at 323.2 and 343.2 K were also obtained (Figure 3a). Under these conditions, caffeine solubility exhibited a retrograde behavior (a decrease in solubility with increase in temperature) at pressures lower than 19 MPa. These results were in qualitative agreement with those obtained by Stahl and Schilz (1979). At pressures above 19 MPa, the normal behavior of increase in solubility with increase in temperature was observed.

Solubilities of pure theophylline and theobromine in supercritical CO_2 at 313.2, 323.2, 338.2, and 343.2 K and pressures from 14 to 24 MPa were also obtained (Figure 3, parts b and c). All experimental data reported were average values of four independent experiments with a standard deviation of ±6%. The solubilities of theophylline and theobromine were found to be much lower than those of caffeine and increased with pressure at all temperatures investigated. For these systems, the increase in vapor pressure due to increase in temperature appeared to compensate for the decrease in solubility caused by the reduction in supercritical fluid density.

Pure theobromine solubilities at 338.2 K were found to be in close agreement with the values reported by Li et al. (1991), using a similar dynamic extraction method but lower than those reported by Johannsen and Brunner (1994), who employed a static method. Data at other temperatures represented new contributions to the literature.

Johannsen and Brunner (1994) pointed out that dynamic methods often tend to underestimate equilibrium solubilities due to nonachievement of equilibrium conditions. Due to the very low solvent flow rates used and the good agreement between measured caffeine solubilities and those reported by Johannsen and Brunner (1994) using the static methods, it is unreasonable to believe that equilibrium conditions were closely approached in this work. Because of the low solubilities of theophylline and theobromine, reported values would be sensitive to the method of measurement, sample collection, and analysis.

When comparing parts a–c of Figure 3, we may conclude that solubilities of caffeine in supercritical carbon dioxide are much higher than those of theophylline and are about 2 orders of magnitude higher than those for theobromine. As pointed out by Martin et al. (1981), the ideal behavior of these methylxanthines in liquids, at 25 °C is such that caffeine is more soluble than theophylline and both being an order of magnitude more soluble than theobromine. This behavior is qualitatively similar to that observed in supercritical carbon dioxide. A possible explanation of this behavior can be found in the differences of the chemical structures of these methyxanthines (Figure 1). We see that the difference in structure between the three methyxanthines is that caffeine has three methyl groups while theophylline and theobromine have two methyl groups and one hydrogen attached. As pointed out by Li et al. (1991), the presence of a hydrogen atom (an electron acceptor), and the negative atoms: ring nitrogen and carbonyl oxygen, allows the formation of hydrogen bonding between theobromine as well as theophylline molecules. The effects of these additional cohesive forces are macroscopically manifested in the higher melting temperatures, 547 and 621 K, and enthalpies, 7097 and 9819 cal mol^{-1} for theophylline and theobromine, respectively as compared to caffeine (512 K and 5044 cal mol^{-1}) and ultimately the lower observed solubilities of theophylline and theobromine in supercritical carbon dioxide in comparison with those of caffeine. The differences in melting temperature and enthalpies and ultimately in solubility between theophylline and theobromine in supercritical carbon dioxide is attributed to the greater extent of hydrogen bonding in theobromine due to the localization of the hydrogen between the two carbonyl oxygen atoms.

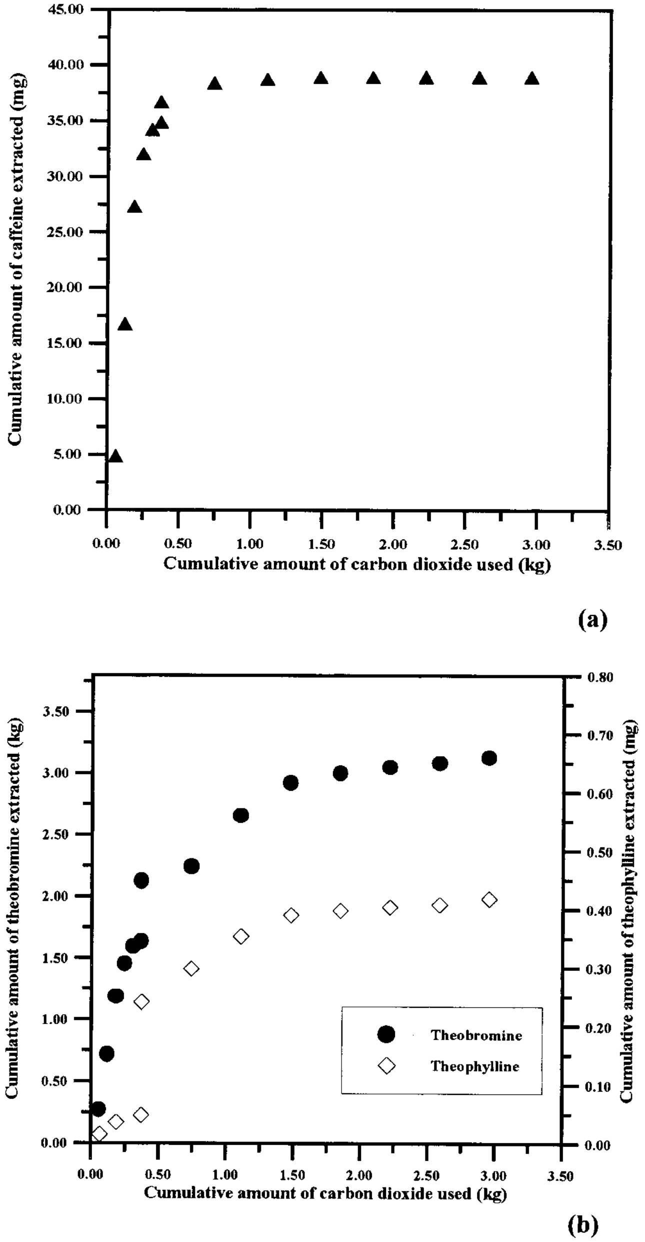

Figure 4. Caffeine (a) and theobromine and theophylline (b) extraction curves during fractionation of maté tea at 343.2 K and 25.5 MPa and CO_2 flow of 0.9−1.2 g min^{-1}.

Extraction of Methylxanthines from Maté Tea Leaves. A supercritical extraction curve for maté tea at 343.2 K and 25.5 MPa, representing the cumulative amounts of extracted caffeine as a function of cumulative amounts of carbon dioxide used, is shown in Figure 4a. High caffeine removal rates were obtained in the early stages of the extraction. Extraction rates, at later stages, diminished with time as shown by changes in the inclination of extraction curve. A similar qualitative behavior was observed for theophylline and theobromine extraction as shown in Figure 4b. These extraction curves clearly show three distinct regions: solubility dependent, intermediate, and diffusion controlled, as defined by the three distinct inclinations in the curve. This is in qualitative agreement with the behavior pointed out by Hedrick et al. (1992).

An examination of Figure 4a,b reveals the higher selectivities of CO_2 for caffeine followed by theobromine and theophylline. The cumulative amounts of methylxanthines extracted from 9 g of dry ground maté tea using a cumulative amount of 2.95 kg of carbon dioxide at 343 K and 25.5 MPa were 38.77, 3.13, and 0.42 mg of caffeine, theobromine, and theophylline, respectively. These amounted to 4308, 348, and 47 mg of caffeine, theobromine and theophylline, per kg of dry maté tea, respectively. Considering that different maté leaves were used, these values are in good accordance with the values of 5371, 340, and 142 mg kg^{-1} reported by Mazzafera (1994).

After about 7 h of extraction, 94, 68, and 57% of extracted caffeine, theobromine and theophylline in plant matrix, respectively, had been recovered. By the 11th fraction, 99.9% of caffeine, 96% of theobromine, and 95% of theophylline had been removed. Fractions obtained at the late stages became richer in theobromine and theophylline and could provide an interesting way for the separation of extractable methylxanthines into fractions of varying concentrations.

It is important to observe that supercritical CO_2 could effectively remove this moisture during the extraction, given sufficient time. For the flow rates, temperature and pressure conditions used in this experiment, analysis of maté tea at the end of the extraction showed that only 3.6% of moisture was removed in the first 7 h when 94% of caffeine had already been extracted (Figure 4a). Furthermore, when 99.4% of caffeine, 82.7% of theobromine, and 80.8% of theophylline are extracted, the moisture content is reduced by only 10% of its original value. Peker et al. (1992) also showed that concentration histories at small times showed little differences between experiments performed with dry CO_2 and humidified supercritical CO_2.

Another interesting observation is with respect to the solubility of methylxanthines in model binary dioxide/methylxanthine systems as compared to amounts of methylxanthines extracted in carbon and carbon dioxide/maté tea systems at the same conditions (343.2 K and 25.5 MPa). The binary solubilities of 2047.6, 14.13, and 7.15 mg of caffeine, theophylline, and theobromine per kilogram of CO_2, respectively, are substantially higher than the amounts obtained during the extraction of maté tea (94.1, 0.13, and 4.44 mg of caffeine, theophylline, and theobromine per kilogram of CO_2, respectively, in the solubility-dependent region, as obtained using the linear part of the extraction curves). This could be attributed to component interactions and possible cosolvent effects in naturally occurring mixtures and demonstrates the difficulty in using binary data to predict the extraction of these substances from natural and complex structures.

Finally, as suggested by one of the reviewers of this work, it is important not to ignore the role of moisture in substantially improving the efficiency of caffeine extraction and decreasing the extraction time. It is, however, equally important to recognize that soaking the ground mate leaves in water before the extraction would lead to the loss of caffeine, chlorophyll, and other water-soluble substances from the extracted product. If caffeine is not the product of interest but rather the decaffeinated product that is of interest as it is in the majority of cases, the quality of this decaffeinated product would be compromised if the operation is carried out with maté tea soaked in water.

CONCLUSIONS

Solubilities of caffeine in supercritical carbon dioxide are about 2 orders of magnitude higher than those of theobromine and theophylline.

After 7 h of extraction at 343.2 K and 25.5 MPa and a CO_2 flow rate of 0.9−1.2 g min^{-1} 94, 68 and 57% of all extractable caffeine, theobromine and theophylline were successfully removed from maté tea.

Amounts of pure methylxanthines dissolved in supercritical CO_2 were found to be much higher than those obtained during the extraction of ground maté tea, in the solubility-dependent region, demonstrating the difficulty of using pure-component solubility data in predicting the extraction of these substances from natural products.

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