Supercritical Fluid Extraction and High Performance Liquid Chromatographic Determination of Caffeine in *Ilex Paraguariensis* St. Hill.

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The supercritical extraction process was employed to obtain the extracts and decaffeinated herb from *Ilex paraguariensis*, using carbon dioxide as solvent. The products were obtained from dry leaves of the plant by means of a supercritical extraction pilot process. The hot infusion prepared with leaves of *Ilex paraguariensis*, known for its stimulating properties, is traditionally consumed in South America and it has importance in the economy and cultural life. The extracts of mate tea leaves are recognized as a rich source of saponins and alkaloids. Extraction of these compounds is potentially attractive because of their economic value, as well as for the production of methylxanthine-free products for human consumption. In this way, one of the aims of this study was to evaluate the content of caffeine in the extract and in the herb. The herb (before and after processing) and the extract were analyzed by High Performance/Pressure Liquid Chromatography (HPLC). From the experimental data it was possible to evaluate the effects of temperature and pressure on the extract yield and caffeine concentration in the extract and in the herb after the extraction from mate tea leaves. The extraction procedure was performed on pilot-scale unit of supercritical extraction, carried out at in the range of pressure (10.76 to 19.20 MPa) with temperature varying from 308 to 337 K.

INTRODUCTION

Ilex Paraguariensis St. Hill. knowing popularity by mate is a symbol product of the state of Rio Grande do Sul, Brazil. The mate tea leaves is widely consumed in Brazil, Paraguay, Uruguay, Argentina, and another countries of South America, having a large cultural and economical importance. The beverage is prepared compacting an amount of milled parts of plant, leaves and stem, in a vessel where is completed with hot water. The Brazilian popular name of the infusion of mate tea is *chimarrão*. Recently this substance is used for medicaments, soluble tea and canned drinks [1]. The decaffeination of the grains and leaves can be economically attractive because the possibilities of obtainment of decaffeinate product and caffeine, a bioproduct used in sodas and medicaments [2, 3]. The mate contains metilxantines, more specifically, a mass concentration about 0.2- 2.0 % of caffeine, 0.3 % of theobromine, traces of theophilline and 10.0 – 16.0 % of chlorogenic acid [4, 5]. The aqueous extract of the *Ilex paraguariensis* St. Hill is a beverage rich in antioxidants, amino acids, saponins and metilxantines [6]. The presence of these compounds is a limiting factor of consume of mate by a part of population justifying the partial removal of them to specify levels from the legislation [7]. Another hand the subproducts of decaffeination can present commercial interest. Many relevant properties like diuretic, anti-inflammatory, anti-rheumatic and stimulating are attributed to the mate [3, 8]. In small doses, caffeine reduces the fatigue but it is prejudicial if an excess is consumed. Extraction of these compounds is potentially

attractive because of their economic value, as well as for the production of caffeine-free products for human consumption.

The proposed process, the supercritical extraction with carbon dioxide, does not let residuals in the product and it is classified like clean technology in comparison to the traditional processes with toxic solvents [9, 10]. Traditionally, the processes of supercritical extraction are divided in three scales: laboratorial, pilot, and industrial, in each one the objectives are well defined in function of the uses. In this work the process take a place in a pilot plant to obtain parameters for the scale-up to industrial process [11].

A supercritical extraction process was carried out to acquire extracts from *Ilex Paraguariensis* using CO_2 as a solvent at different conditions of pressure and temperature. Experimental data are presented about the effects of temperature and pressure on the yield of extracts from mate tea leaves, performed in an automated pilot-scale unit of supercritical extraction, carried out at 12, 15, 17 and 20 MPa with temperature ranging from 313.15K to 343.15K, and with a constant solvent flow rate (0,4 kg/h). Caffeine was analyzed in the extracts resulting from the supercritical extraction under different conditions. The chemical analysis was performed by HPLC.

MATERIALS AND METHODS

Extraction process

The raw material, dry and milled leaves, was provided by Madrugada Alimentos Ltda. The leaves were dried at 373.15 K by 15 minutes and the moisture content decrease from 5.6% to 1.5%. The average particle diameter was $1x10^{-3}$ m. A sample (0.2 kg) was used for extraction of the oil in a Pilot Equipment [11].

The schematic diagram of the experimental apparatus can be seen in Figure 1. It included a air driven liquid pump (Maximator G35-CO_2) for solvent delivery, a 500 mL high-pressure extraction vessel, and a separator flask. The extraction vessel was supplied with a heating jacket and an automated temperature controller.

Heating tapes were used throughout the apparatus to maintain a constant temperature in the extraction section. To ensure a constant and steady solvent delivery the pump head was cooled by a circulating fluid, which passed through a chiller. Flow rates and accumulated gas volumes passing through the apparatus were measured using a flowmeter assay, (Sitraus F C Massflo 2100 – Siemens, with accuracy of < 0.1%). Ke (USA) micrometering valves were used for flow control throughout the apparatus. Heating tapes with automated temperature control were also used around this valve to prevent both freezing of the solvents and solid solute precipitation following depressurization. Pressure in the extractor was monitored with a digital transducer system, Novus 8800021600, acquired from Novus Produtos Eletrônicos (Brazil) with a precision of ±1.0 bar. The temperature controller was connected to thermocouples (PT-100, with an accuracy of 0.5K).

An experimental planning was made using the factorial 2^2 resulting in 11 experiments viewed in the diagram showed at Figure 2. The central is the point corresponding to the condition the 15 MPa and 323.15 K. The method proposes 3 repetitions in the middle point and simple analysis in an others points (without repetitions).

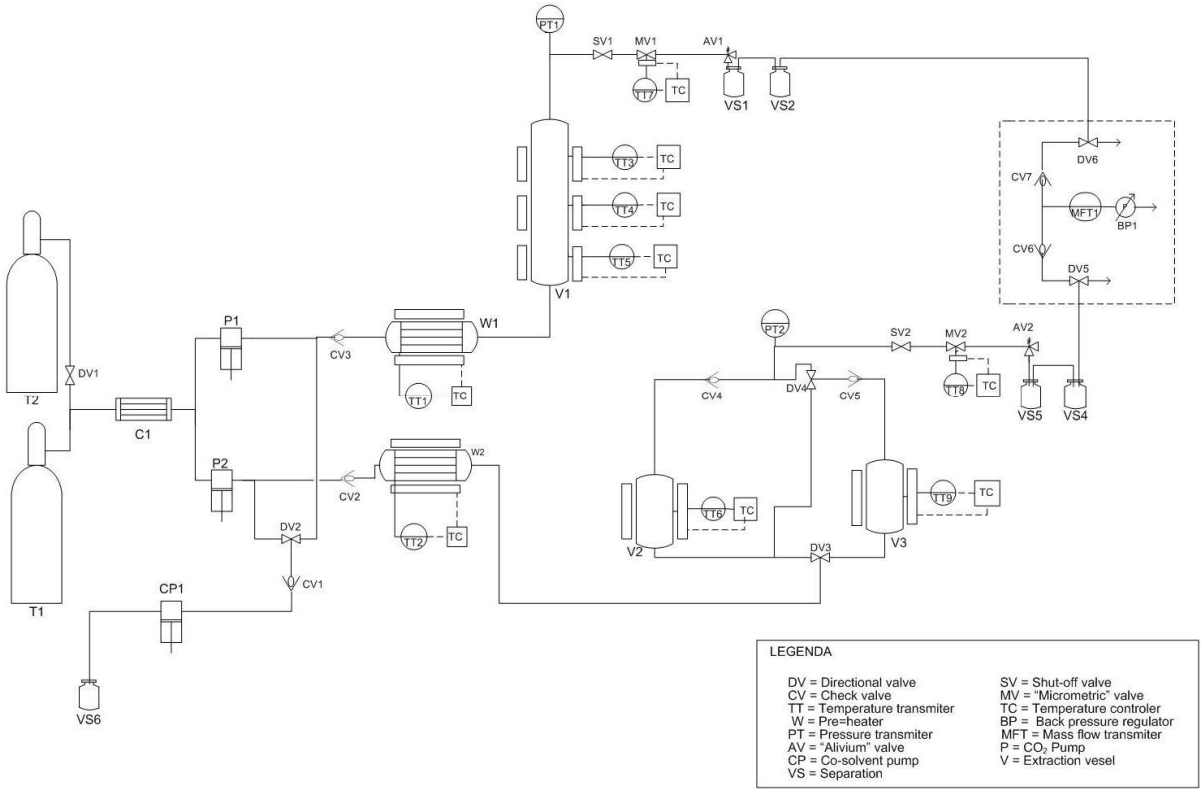

Figure 1: Flowchart of the supercritical extraction pilot scale equipment

Caffeine Analysis

The amount of caffeine in the mate before and after supercritical extraction, and in the extract obtained was determinate by High Performance Liquid Chromatography (HPLC- Agilent Technologies 1200 Series). The method for sample preparation used was proposed by Filip et al. [12]. Mate leaves, before the processing, used in caffeine quantification was milled until to obtain a fine powder (diameter < 0,32 x 10^{-3} m). A sample of 5 g of mate leave was boiled with 70 mL of water for 20 min. Then it was cooled at room temperature, filtered and the volume adjusted to 100 mL. 5 mL of this solution was diluted to 10 mL, for the determination of caffeine. For analysis of supercritical extracts, they were boiling with 40 mL of water for 20 min.

Samples of mate before and after the supercritical extraction was prepared and chromatographed in duplicate. The extract sample was analyzed twice by HPLC. Before analysis, the samples were filtered vacuum with a membrane (0.45 µm) and mixed for 5 min in the ultra-sound bath.

The separation was achieved by applying a gradient using a solvent A (water : acetic acid 98:2) and solvent B (methanol : acetic acid 98:2). For caffeine the gradient was from 17% B to 20 % B in 10 min; 20% B (isocratic) for 5 min; 20% B to 23% B in 10 min and 23% B to 100% B in 5 min with a flow rate of 1.0 mL/min. Identification and quantitation was carried out by simultaneous detection with a UV Agilent 1220 Series detector at 273 nm for caffeine.

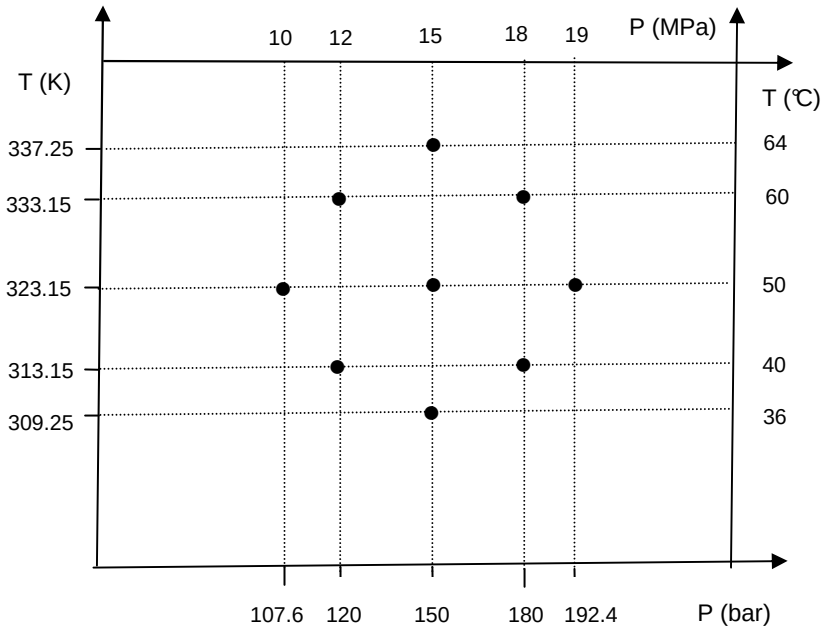

Figure 2: Schematic diagram of the experimental planning

For the validation of quantitative analysis, small amounts of standard caffeine (purity of 99%, from Sigma-Aldrich) were weighed and diluted with water and methanol (7:3). A calibration curve was constructed to show the relation between the peak area and the content of caffeine. Each caffeine concentration was analyzed three times. The caffeine concentration experimental data were adjusted by linear correlation and coefficient (R^2) obtained was the 0.9992.

RESULTS

Experimental results of the supercritical extraction were obtained at the following range of pressure and temperature: 10.76 to 19.20 MPa and 308.9 to 337.3 K, according with the experimental planning (Figure 2). The extract global yield (extract mass/plant mass) of mate leaves, expressed as weight, is listed in Table 1.

Table 1: *I. paraguariensis* extract yield obtained by CO_2 supercritical

P (MPa)	T (K)	Yield (% p/p)
15.00	323.2	1.030
15.00	323.2	1.110
15.00	323.2	0.917
19.20	323.2	0.701
10.76	323.2	0.489
15.00	337.3	0.292
15.00	308.9	0.468
18.00	333.2	0.765
18.00	313.2	0.588
12.00	333.2	0.021
12.00	313.2	0.200

Caffeine was analyzed in the mate unprocessed, in the mate after processing and, in the extracts resulting from the supercritical extraction under different conditions by HPLC. The supercritical extract chromatographic analysis is showed in the Figures 4, 5 and 6, exhibiting the selectivity of carbon dioxide for caffeine.

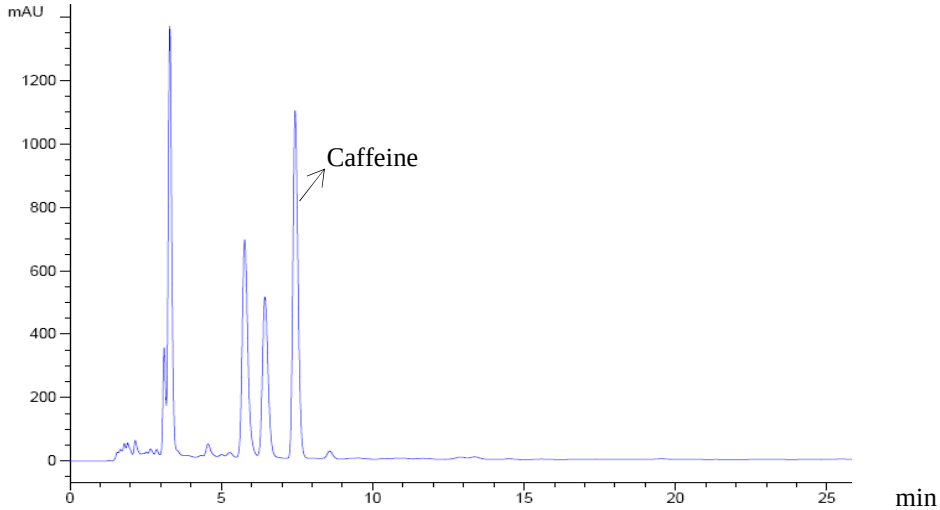

Figure 4: HPLC profile of caffeine of unprocessed mate leaves

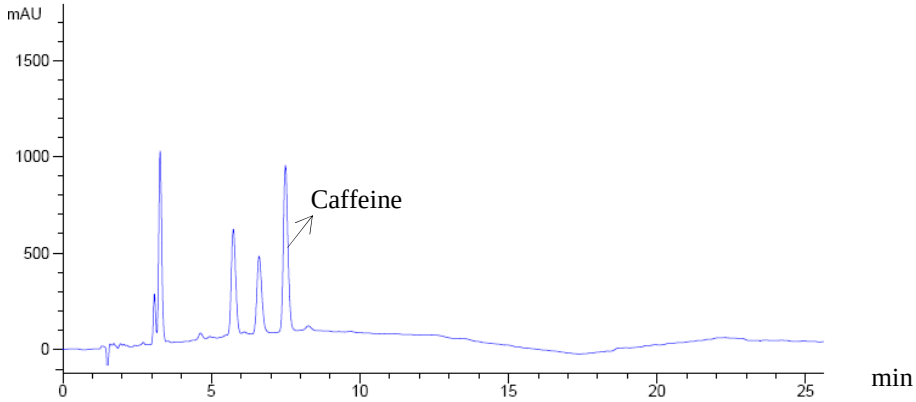

Figure 5: HPLC profile of caffeine of processed mate leaves (15.00MPa/ 323.15K).

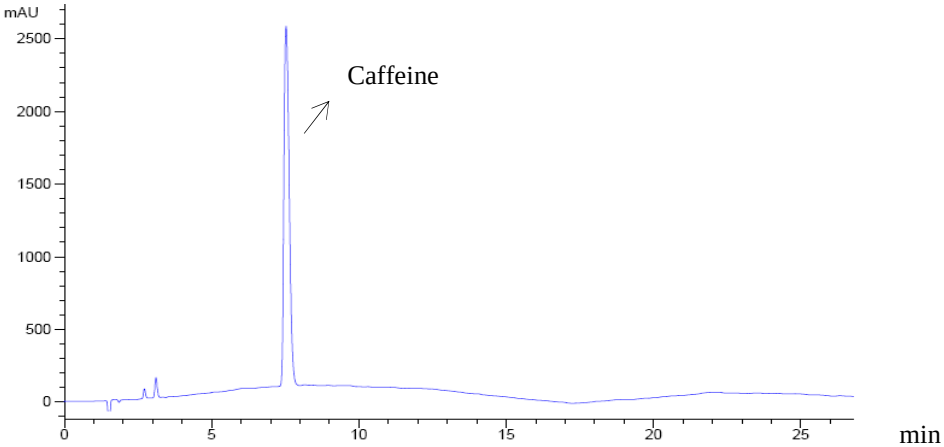

Figure 6: HPLC profile of caffeine from supercritical extract of mate leaves (15.0MPa/ 323.15K).

The caffeine content in the supercritical extract was determinate by mass balance (difference between mass of caffeine in the unprocessed mate and processed mate) and by High Performance Liquid Chromatography for to compare the results. Table 2 shows the results for caffeine quantification.

Table 2: Weight and composition of caffeine – EA: mate leaves unprocessed; MD: mate leaves after supercritical extraction; E: supercritical extraction extract of mate leaves.

		HPLC Analysis		Mass balance		HPLC Analysis
P (MPa)	T (K)	MA (g)	MD (g)	E (g)	E (% w/w)	E (g)
15.00	323.15	1.719 ± 0.001	1.028 ± 0.018	0.691	44.58	0.676 ± 0.019
15.00	323.15	1.146 ± 0.001	0.736 ± 0.018	0.410	36.94	0.394 ± 0.019
15.00	323.15	1.146 ± 0.001	0.666 ± 0.018	0.480	52.34	0.399 ± 0.019
19.24	323.15	1.146 ± 0.001	1.015 ± 0.031	0.131	18.69	0.115 ± 0.022
10.76	323.15	1.146 ± 0.001	0.922 ± 0.006	0.224	45.81	0.179 ± 0.008
15.00	337.15	1.146 ± 0.001	0.969 ± 0.000	0.177	60.62	0.102 ± 0.004
15.00	308.15	1.146 ± 0.001	0.972± 0.007	0.174	37.24	0.156 ± 0.008
18.00	333.15	1.146 ± 0.001	1.029 ± 0.022	0.117	5.61	0.098 ± 0.018
18.00	313.15	1.146 ± 0.001	1.042± 0.004	0.104	13.59	0.086 ± 0.020
12.00	333.15	1.146 ± 0.001	1.136± 0.001	0.010	47.61	ND
12.00	313.15	1.146 ± 0.001	1.029± 0.001	0.117	58.5	ND

ND: Not Detected
E (% w/w): mass fraction of caffeine related with extract mass

The caffeine content in the unprocessed mate (1.146 ± 0,001 %) is similar to those cited in the literature [13, 14, 15]. The best experimental condition for decaffeination associated with higher caffeine concentration in the extract is 15.0 MPa/323.15K. The maximum value for the caffeine concentration in the extract (60.62%) was obtained at 15.00 MPa/337.15 K, but in this condition the global yield (0.292%) is one of the smallest (Table 1).

The difference of mass obtained from mass balance and from HPLC can be attributed for the fact of adherence of the extract in the walls of tube from extraction vessel to expansion valve. This fact was observed after two extractions for the same conditions of pressure and temperature, where the global yield in the second one decreased drastically. After this, the equipment was cleaned before each extraction. When the expansion occurs, the extract was deposited not only in the bottom of the flask but also in the walls, which it can collaborated

for the mass of caffeine in the extract obtained by HPLC was smaller than the mass determined from the mass balance.

CONCLUSION

The present work shows that supercritical extraction with dioxide carbon is one possibility to extract caffeine from *Ilex paraguariensis* St. Hill. The maximum extract yield obtained by CO_2 supercritical and the higher caffeine concentration in the extract occur in the same condition: 15.0 MPa and 323.15K. The results of caffeine analysis in the mate unprocessed, in the mate after processing and, in the extracts resulting from the supercritical extraction under different conditions showed the selectivity of carbon dioxide for caffeine.

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