EFFECTS OF FIELD VARIABLES ON THE EXTRACTION YIELD AND CHEMICAL COMPOSITION OF MATE LEAVES OBTAINED FROM HIGH-PRESSURE CO_2

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Mate (*Ilex paraguariensis*) is an important natural product in the economic and cultural context of South Brazil with many attributed relevant properties like anti-inflammatory, therapeutic, anti-rheumatic, stimulating and diuretic. Is it a common sense that field variables such as fertilize types and light intensity affect the flavor and yield of leaves of mate plant. In this context, this work is aimed at investigating the influence of field variables on the extraction yield and chemical composition of the extracts of mate leaves obtained from CO_2 at high pressures. For this purpose, a controlled field experiment was built at Industria e Comércio de Erva-Mate Barão Ltda, Barão de Cotegipe, Brazil, where the plants were cultivated under agronomic control. The extraction experiments were performed in a semi-batch laboratory-scale unit and the extract chemical analyses were conducted in a GC/MSD. The extraction yield, extraction kinetics and essential oil chemical composition are reported in this work. The results show that the liquid yield and chemical composition of the extracts are directly affected by the agronomic variables.

INTRODUCTION

Mate (*Ilex paraguariensis*) is an important natural product in the economic and cultural context of South Brazil with many attributed relevant properties like anti-inflammatory, therapeutic, anti-rheumatic, stimulating and diuretic [1-4]. To take a glance at the mate market, we shall consider that only at the Alto Uruguai gaúcho and extreme west catarinense regions one can find more than 40 mate processing industries and about 180 thousand medium and small properties crowded together in a narrow area dedicated almost exclusively to cultivate this raw material [5]. Considering the fact that all of those industries direct their efforts to produce the same based-product comminuted mate leaves for teas and the recent availability of this raw material from other countries [6], it is not surprising that the strong competition established has required company investments towards producing higher-value products.

Despite the importance of mate tea leaves in the social and economic context of South Brazil, the literature is very scarce on works focusing on the extraction of volatile compounds of this raw material from supercritical fluid extraction. Saldanã et al. [2,7] presented a study on the extraction of methylxantines from mate tea leaves using CO_2 as solvent and ethanol as cossolvent in the temperature range of 40 to 80^{o}C and pressures up to 40 MPa. However, an investigation regarding the effect of temperature and solvent density on the extraction yield and on the distribution of chemical components of the extracts has not been presented in the literature. Furthermore, despite the common sense that field variables such as fertilize types and light intensity affect the flavor and yield of leaves of mate plant, the literature is very scarce on studies focusing the influence of these variables on the extraction yield and chemical composition of the extracts of mate tea leaves obtained from $SCCO_2$.

In this context, this work is aimed at investigating the influence of field variables on the extraction yield and chemical composition of the extracts of mate leaves obtained from CO_2 at high pressures. For this purpose, a controlled field experiment was built at Industria e Comércio de Erva-Mate Barão Ltda, Barão de Cotegipe, Brazil, where the plants were cultivated under agronomic control. The extraction experiments were performed in a semi-batch laboratory-scale unit and the extract chemical analyses were conducted in a GC/MSD. The extraction yield, extraction kinetics and essential oil chemical composition are reported in this work.

MATERIALS AND METHODS

Sample collect and preparation

Mate tea leaves samples, were obtained from an agronomic controlled experiment in Ervateira Barão Ltda, Brazil, where mate plant were cultivated under light intensity and fertilizing control. The mate samples were dried to around 3%, crushed and classified with respect to particle size (12-35mesh), and then stored under nitrogen atmosphere.

Apparatus and extraction procedure

The experiments were performed in a laboratory-scale unit, which consists basically of a CO_2 reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 0.1 dm^3 jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of $\pm$ 0.012 MPa, a collector vessel with a glass tube and a cold trap, as previously described by Rodrigues et al. [8]. The experiments were conducted in the pressure range of 10 to 25 MPa and from 20 to 40^{o}C in temperature at constant CO_2 mass flow rate of 2gmin^{-1}. Typically, amounts of around 25g of comminuted mate tea leaves were charged into the extraction vessel. The CO_2 was pumped into the bed, which was supported by two 300 mesh wire disks at both ends, and was kept in contact with the herbaceous matrix for at least one hour to allow the system stabilization. Afterwards, the essential oil was collected by opening the micrometering valve and the CO_2 mass flow was accounted for by the pump recordings. After that, the mass of the extracted oil was weighed, the glass tube was re-connected to the equipment and this procedure was performed until no significant mass was extracted or, as in some cases, the extraction period exceeded a pre-established limit. The experiments were accomplished in approximately 400 minutes, isothermally at constant pressure. Triplicate runs were performed for all conditions leading to an overall standard deviation of the extraction yields of about 0.05.

Extract characterization

The extracts were analyzed through a GC/MSD (Shimadzu QP 5050A), using a capillary column DB-5 (30m x 0.25mm x 0.25μm). Column temperature was programmed from 70 to

310°C at 3°Cmin^{-1}. Helium was the carrier gas and injection port temperature was 290°C and detector temperature 320°C. 1μL of the samples (40000 ppm in CH_2Cl_2) was injected in the split mode (1:10) and the components were identified by matching their mass spectra with those of Wiley library database and by comparison of retention times with authentic standards. In all samples was added 100 ppm of an internal standard (biphenyl) and the concentration of each component was calculated based on the ratio between the peak area of the component by the peak area of the internal standard.

RESULTS AND DISCUSSION

Extraction of mate leaves samples

Figure 1 presents the effect of temperature and pressure on the liquid yield (defined here as the weight percentage of the oil extracted with respect to the initial charge of the raw material in the extractor). It can be noted that at the lowest pressures (10 MPa), an increase in temperature leads to a decrease in the liquid yield due to the pronounced density reduction. At higher pressures (25 MPa), an enhancement in temperature from 20 to 40°C, increases the yield. In this region, the thermal effects overcome the reduction of density when the temperature is decreased resulting in an increase in the liquid yield.

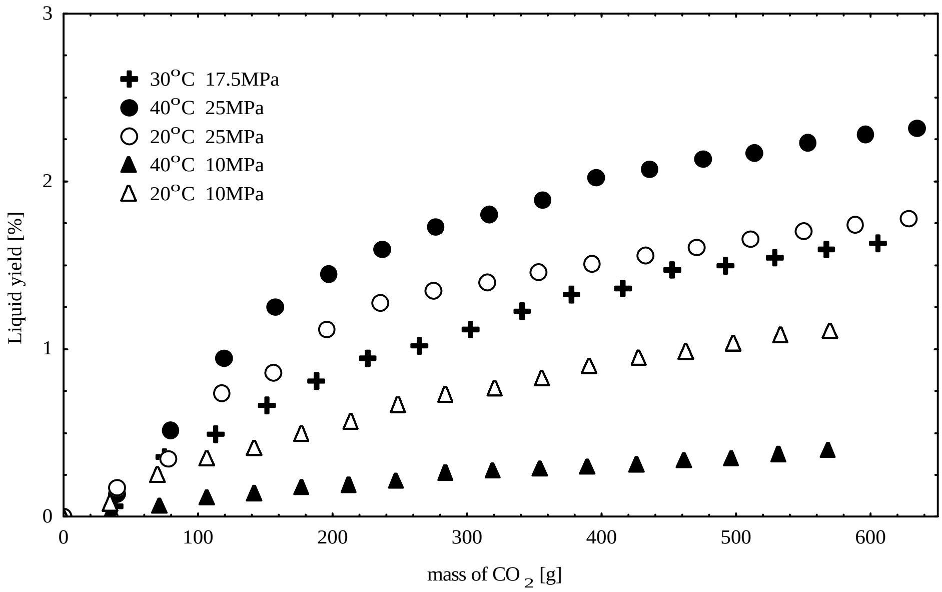

Figure 1. Effects of temperature and pressure on the extraction kinetics of mate tea leaves from carbon dioxide at high pressures.

To evaluate the effects of field variables, the extraction conditions were fixed at 17.5MPa and 30°C. Plants under sun (without covering) were selected to investigate the fertilizing type. Some plants (line 1) have no fertilizer addition; plants from line 2 received the addition of nitrogen sources; from line 3 with potassium sources and from line 4 with sources of nitrogen and potassium simultaneously. Figure 2 presents the effects of fertilizing type on the kinetics of the extraction of mate tea leaves. One can observe that when the plants were fertilized with nitrogen sources, the highest yields were achieved. An interesting aspect that can be noted is the negative synergism observed when the two sources of fertilizing were applied in the plants.

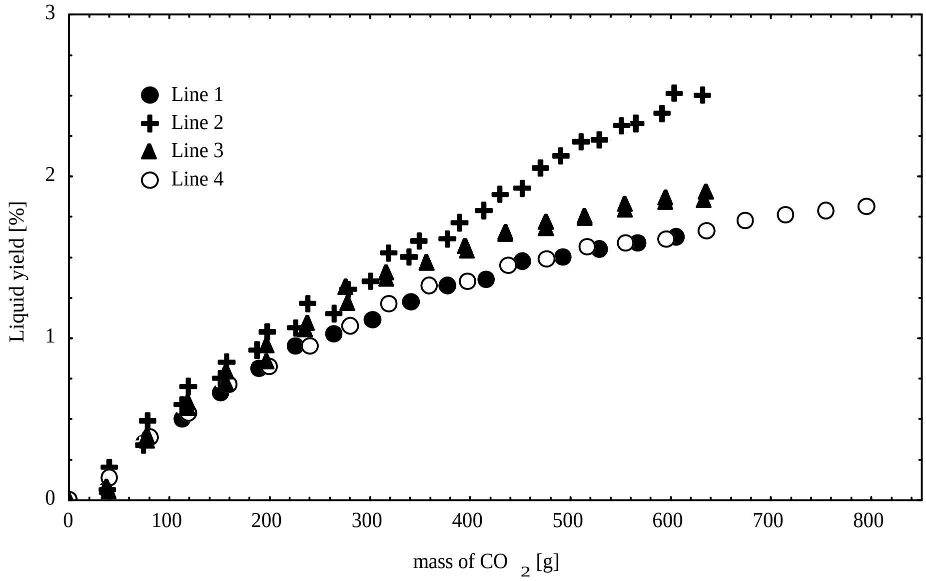

Figure 2. Kinetics of carbon dioxide extraction of mate tea leaves with different types of fertilizing. Plants under sun, extracted at 30°C and 17.5 MPa.

Figure 3 presents the effects of light intensity on the kinetics of the extraction of mate tea leaves from carbon dioxide. It can be observed that, independent of the fertilizer applied in the plants, the presence of sun in direct incidence on the plant resulted in higher yields of volatile matter.

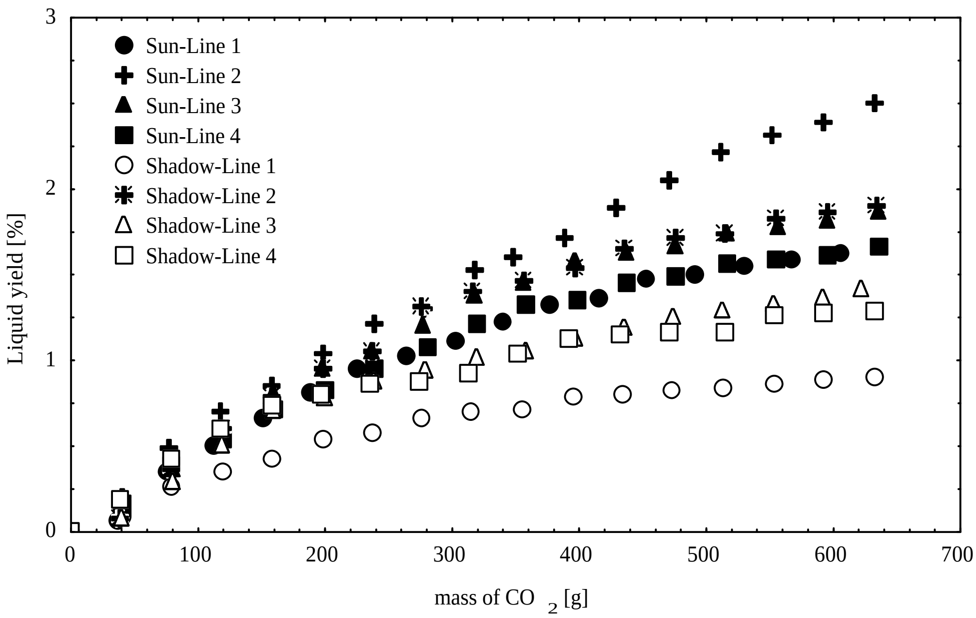

Figure 3. Effects of light intensity on the extraction kinetics of mate tea leaves with carbon dioxide. Samples extracted at 30°C and 17.5 MPa.

Chromatographic analyses of the extracts

The chemical profiles of the samples were identified by the ratio of peak areas of each compound by the peak area of the internal standard. To evaluate the effects of the extraction and agronomic variables in a statistical basis, an analysis of variance were employed along with a Tukey test at a confidence level of 95%. Table 1 presents the effects of temperature and density on the chemical profile of the extracts of mate tea leaves where the compositions presented reflect the means of at least three analyses. In each extract, around 30 compounds were identified and, for sake of concision, in Table 1, only the major compounds are presented. An inspection of this table reveals that the highest contents of the components occurred at 40°C. This fact is due to the enhancement in the compounds vapor pressures, facilitating the extraction of the volatile material. The density presented an opposite trend, where the lowest density produces an increasing in the concentration of the more volatile compounds. It is important to note that the components present in Table 1 can be considered lights in comparison with triterpenes and wax that occur in mate tea leaves. An enhancement in density can promote the extraction of this heavier material decreasing the concentration of the more volatile compounds.

Table 1: Effects of temperature and pressure on the chemical profile of the extracts of mate tea leaves. Plants without sun covering and without additional fertilization.

	Temperature			**Density***		
Compounds†	**20**	**30**	**40**	**High**	**Intermediate**	**low**
Caffeine	10.188 [b]	7.488 [b]	18.061 [a]	11.911 [b]	9.441 [b]	20.552 [a]
teobromine	0.151 [ab]	0.106 [b]	0.237 [a]	0.227 [a]	0.142 [a]	0.206 [a]
Phytol	1.379 [b]	0.994 [b]	2.828 [a]	1.672 [b]	1.222 [b]	3.505 [a]
ethyl ester hexadecenoic acid	0.086 [a]	0.054 [a]	0.103 [a]	0.132 [a]	0.068 [a]	0.089 [a]
squalene	30.122 [b]	25.782 [b]	52.485 [a]	36.078 [b]	28.284 [b]	62.537 [a]
dotriacontane	2.424 [b]	2.310 [b]	5.668 [a]	3.006 [b]	2.978 [b]	5.813 [a]
vitamin E	16.192 [ab]	13.181 [b]	19.864 [a]	20.160 [a]	15.255 [a]	18.151 [a]
stigmasterol	4.624 [a]	5.270 [a]	5.644 [a]	6.145 [a]	5.265 [a]	4.461 [a]

* high (≈0.95 g/cm^3); intermediate (≈0.86 g/cm^3); low (≈0.65 g/cm^3);

† equal letter means that there is no statistical difference at 5% (Tukey test)

Table 2 presents the chemical profile of mate tea leaves as a function of agronomic variables, where the extraction conditions were kept constant at 17.5MPa and 30°C. One can observe from this table that the light intensity has a significant effect on the distribution of most part of the compounds. A remarkable effect on the content of caffeine can be observed. The type of fertilizing added to the plants present a significant effect on the content of some compounds like the steroid stigmasterol and in some alkanes like dotriacontane and squalene, although in other components like caffeine and vitamin E no significant difference occured.

Table 2. Effects of agronomic variables on the chemical profile of the extracts of mate tea leaves. Mate tea leaves extracted at 30°C and 17.5MPa.

Compounds[†]	Light intensity		Fertilization[*1]			
	sun	shadow	L1	L2	L3	L4
caffeine	8.402[b]	22.371[a]	17.678[a]	13.367[a]	13.228[a]	17.272[a]
teobromine	0.167[a]	-	0.053[b]	0.117[a]	0.069[ab]	0.095[ab]
phytol	0.902[b]	4.285[a]	1.750[b]	2.137[b]	2.058[b]	4.431[a]
ethyl ester hexadecenoic acid	0.089[a]	0.035[b]	0.029[a]	0.090[a]	0.101[a]	0.027[a]
squalene	26.172[a]	26.787[a]	20.256[b]	41.378[a]	26.687[ab]	17.597[b]
dotriacontane	1.810[b]	3.290[a]	3.310[a]	1.918[c]	1.755[d]	3.217[b]
vitamin E	9.278[a]	7.777[a]	9.801[a]	9.087[a]	8.503[a]	6.721[a]
stigmasterol	3.486[b]	4.399[a]	5.128[a]	3.535[b]	3.309[b]	3.797[ab]

[*] L1- without fertilizing; L2- nitrogen source; L3- potassium source; L4- nitrogen and potassium sources;

[†] equal letter means that there is no statistical difference at 5% (Tukey test)

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