JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical Composition and Extraction Yield of the Extract of *Origanum vulgare* Obtained from Sub- and Supercritical CO_2

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In this work sub- and supercritical CO_2 were used to obtain extracts from two origanum samples, one commercial, and another cultivated under agronomic control. The experiments were performed in the temperature range of 293−313 K and from 100 to 200 bar in pressure, employing around 26 g of origanum samples. Results show that the commercial sample provides a higher yield of extract if compared to the other sample. It is also achieved that a raise in temperature at constant pressure leads to an increase in the extraction yield despite solvent density changes. Chemical analyses were carried out in a GC-MSD, allowing the identification of around 24 compounds by use of the library of spectra of the equipment and injection of some standard compounds for both commercial and cultivated origanum samples. It was also found that the distribution of chemical components as a function of extraction time differs appreciably between the origanum species. The chromatographic analysis permitted the identification of thymol and cis-sabinene hydrate as the most prominent compounds present in commercial oregano sample and carvacrol and cis-sabinene hydrate in the cultivated *Origanum vulgare*.

KEYWORDS: Chemical analysis; sub- and supercritical fluid extraction; extraction kinetics; origanum; CO_2 extract; GC-MSD

INTRODUCTION

In recent years, the development of new separation techniques has gained increasing importance in the chemical and food industries due to the imposed environmental and public health regulations toward solvent-free. It is well-known that carbon dioxide is an appropriate solvent for supercritical extraction purposes in food industry because it is nontoxic, nonflammable, nonexplosive, readily available, and has a low critical temperature that avoids degradation of thermolabile compounds. The advantages of using near critical carbon dioxide extraction prevail when small raw material amounts and high quality products are processed.

Among several raw materials, origanum has been recognized as one of the most used vegetables all over the world, with abundant occurrence in East Europe, in Middle Asia, and South and North America (*1*, *2*). Some properties such as antimicrobial, antioxidant, and antimutagenic activities have been attributed to the essential oil from origanum (*3*−*5*). However, the chemical composition of the oil as well as the extraction yield may vary depending on the species under investigation and the extraction method employed. Several oregano species are characterized by the presence of two main chemotypes, thymol and carvacrol. Another intermediate type would contain high content of two monoterpene hydrocarbons, γ-terpinene or *p*-cymene. However, some species were found with high values of linalool and others monoterpenes and sesquiterpenes (*6*−*10*).

Some papers report equilibrium distribution coefficients of key components of *Origanum vulgare* in SC−CO_2 (*11*) and others characteristics of the extracts of oregano (*12*−*15*). However, a systematic study related to sub and supercritical carbon dioxide extraction from different origanum samples, reporting the extraction kinetics, the extraction yield obtained, and mainly, the chemical composition of the extract as a function of temperature and pressure, is not satisfactorily described in the literature.

The present paper is part of a project aiming the total characterization of some kinds of Labiatae species of plants, mainly *Marjoran* and *Origanum*, as can be seen in the references (*16*−*19*). In this context, the goal of this work is to assess the

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10.1021/jf030575q CCC: $27.50
Published on Web 04/16/2004

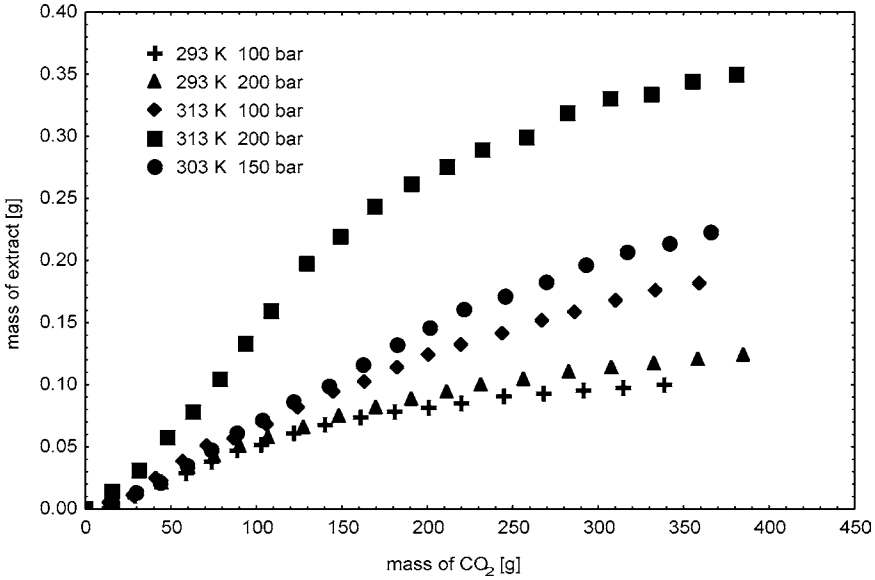

Figure 1. Extraction curves obtained from commercial origanum samples with sub- and supercritical CO_2 extraction (experimental conditions, see **Table 1**).

influence of temperature and pressure (solvent density) on the characteristics of the extracts obtained from sub- and supercritical CO_2 extraction of two origanum samples, a commercial and another cultivated under agronomic control. For this purpose, a semi-batch laboratory unit operated in the temperature range of 293−313 K and from 100 to 200 bar at a constant CO_2 flow rate of 1 g min^{-1} was employed. Chemical analyses were conducted in a GC-MSD (Shimadzu, model QP 5050A). The extraction yield, extraction kinetics, and CO_2-extract chemical composition are reported in this work.

EXPERIMENTAL SECTION

Materials. The commercial origanum samples were purchased from free market. *Origanum vulgare* L. (seeds from Denmark) was cultivated under agronomic control in the Agricultural Experimental Station (FEPAGRO, RS, Brazil) and were collected in the winter season. Voucher specimens were identified by Dr. Sergio Bordignon and deposited at the Herbarium BLA (Brazilian Laboratory of Agrostology) under number BLA 17251. Both origanum species were dried at ambient temperature, crushed manually, and stored under nitrogen atmosphere.

Carbon dioxide (99.9% purity) was kindly provided by White Martins S. A. The analytical standards α-pinene, *β*-pinene, *p*-cymene, camphene, α-terpinene, limonene, *γ*-terpinene, linalool, terpineol-4, α-terpineol, carvacrol, thymol, and biphenyl are purchased from Aldrich (Palo Alto, CA). A stock solution of each standard was prepared at 1000 mg L^{-1} in dichloromethane (Merck, pa grade, bi-distilled) and stored under refrigeration.

Sub- and Supercritical Extraction Procedure. The experiments were performed in a laboratory scale unit, which was described in a previous paper (*19*). Typically, amounts of around 26 g of dried origanum leaves and flowers were used in each experiment. The CO_2 extract was collected in a glass tube, and the mass of the extract was weighed. The experiments were accomplished in approximately 400 min, isothermally and at constant pressure. A whole experimental run lasted, in general, 10 h, including all steps involved: sample weighing, temperature stabilization (baths, extractor), depressurization, etc.

An experimental 2^2 factorial design was established so as to investigate the influence of the process variables on the extraction yield and on the extract chemical composition. The experimental range investigated was 293−313 K in temperature and 100−200 bar in pressure. Triplicate runs were performed for all conditions leading to an overall standard deviation of the extraction yield of about 0.02.

Extract Characterization. The extracts were analyzed with a gas chromatograph with a mass spectrometric detector (GC-MSD Shimadzu, Model QP 5050A), using a capillary column DB-5 (methyl siloxane with 5% phenyl groups) with 30 m of length, 0.25 mm of internal diameter, and 0.25 *μ*m of film thickness. The main GC/MSD conditions are flow rate of 1 mL min^{-1} (helium as carrier gas), electronic impact mode of 70 eV, injection in the split mode (ratio 1:30), interface at 280 °C. The following oven temperature program was used: 50 °C heating to 100 °C at 2 °C min^{-1}, to 145 °C at 3 °C min^{-1}, and to 280 °C at 5 °C min^{-1} (25 min hold). The identification of compounds was made by comparing the mass spectra obtained with those from the Wiley library (compounds tentatively identified) and by additional comparison of GC retention time of standards compounds. Biphenyl was used as internal standard.

Quantification. The method used was the internal standardization, and it was calculated through the comparison between the relative areas of the peaks (related to the internal standard). As carvacrol and thymol are the main compounds in the extracts, the quantitative comparison is made only for these two compounds. A 50 mg L^{-1} solution of standards thymol, carvacrol, and biphenyl (internal standard) was prepared in dichloromethane,0.5 *μ*L was injected in GC/MS, and detection was done mainly in the selected ion monitoring (SIM) mode (at *m*/*z* 135 and 150 for thymol and carvacrol and 154 for biphenyl). To a 500-mg L^{-1} solution of CO_2 extract of oregano in dichloromethane was added 50 mg L^{-1} of biphenyl, and the mixture was submitted to the same chromatographic analysis as the standard solution. All analyses were made in triplicate at least.

Semiquantitative data were also obtained by comparing the relative areas of peaks obtained for four samples, supercritical CO_2 extract from *Origanum vulgare* L., supercritical CO_2 extract from commercial oregano, hydro distillation (essential oil) from *Origanum vulgare* L., and hydro distillation (essential oil) from commercial oregano. These data are used only for a rapid comparison of distinct methodologies, hydro distillation (essential oil) and CO_2 extraction.

RESULTS AND DISCUSSION

Extraction of Commercial Oregano Samples. First, the effect of temperature and pressure on the yields of the extraction process was evaluated using the commercial origanum sample. The yield is defined here as the weight percentage of the oil extracted with respect to the initial charge of the raw material in the extractor.

Table 1 presents the extraction yield for the five experimental conditions (runs) studied in this work. It can be noted from this

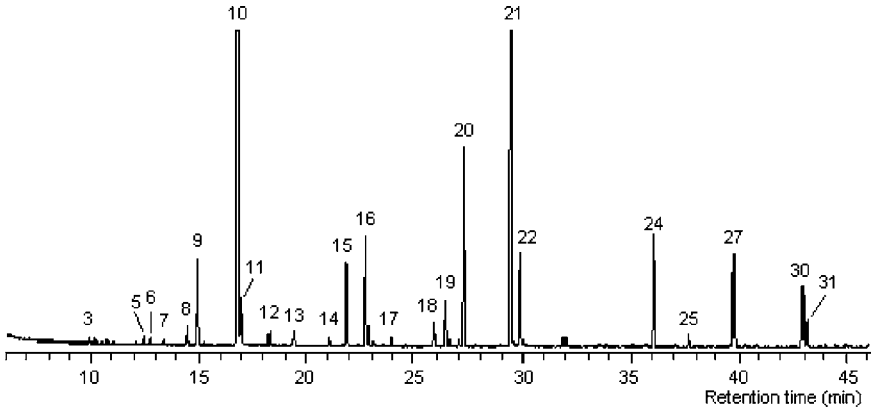

Figure 2. Typical chromatogram of the commercial oregano extract obtained at 150 bar and 303 K. Peaks identification according to **Table 2**. Chromatographic conditions described in the text.

Table 1. Physical Properties, Extraction Yield, and Characteristic Parameters Employed in the Sub- and Supercritical CO_2 Extraction of a Commercial Oregano Sample

run	*T* (K)	*P* (bar)	solvent density[a] (g cm^{-3})	extraction yield[b] (%)
1	293	100	0.8553	0.38 ± 0.01
2	293	200	0.9378	0.46 ± 0.01
3	313	100	0.6164	0.67 ± 0.02
4	313	200	0.8408	1.32 ± 0.03
5	303	150	0.8475	0.82 ± 0.02

[a] Estimated from reference (*20*). [b] $n = 3$.

table that a rise in temperature at constant pressure leads to an increase of the extraction yield, despite large solvent density changes (estimated from Angus et al. (*20*) correlation). Although to less extent, a rise in pressure at constant temperature also leads to an increase of the extraction yield due to the enhancement of the solvent power (*21*).

In **Figure 1** is presented the experimental extraction curves for all conditions shown in **Table 1**. The extraction curves are characterized by a linear part followed by the decreasing and zero extraction rates (*21*). One can also observe from this figure that the solubility (the slope of the linear part − constant extraction rate of the extraction curves) increases with increasing temperature at constant pressure, thus indicating that all the solubility values are located in the right side of the crossover point (*21*). Then, in the range investigated, no competition between temperature (vapor pressure of essential oil) and pressure (solvent power) is verified.

Qualitative Analyses. According to the literature, oregano extract is in fact a very complex mixture of hydrocarbon, oxygenated, and aromatic compounds (*8*−*10*). In general, the oxygenated compounds comprise the major fraction and carvacrol or thymol, isomers of phenol terpene compounds are the main constituents. The results obtained in this work show that the extract analyzed through GC-MSD of commercial oregano samples (see **Figure 2**) for all experimental conditions presented in **Table 1** led to the presence of almost the same compounds, namely 5 monoterpenes, 10 terpene alcohols, 2 phenols, 3 sesquiterpenes, and 4 other oxygenated compounds, overall representing 33.3% of terpenes and 66.7% of nonterpenes. **Table 2** presents the components identified in the extracts of commercial origanum. The compounds were identified by injection of standards (std) and/or by comparing the mass spectra (ms) with the equipment library.

In this table, it is also possible to compare the relative concentration (semiquantitative analysis) of the compounds based on the peak area percentages. The estimated concentration was obtained by comparison of the peak areas for the same injection volume, same dilution, and in the same signal scale (attenuation). Comparing the relative amount of each compound, they were classified in **Table 2** as: ND (not detected), T (traces), and +, ++, +++, ++++ (increasing values of peak areas). It can be noticed that *cis*-sabinene hydrate (peak 10), thymol

Table 2. Volatile Compounds Identified in Four Analyzed Samples of Origanum Extracts: (A) Supercritical CO_2 Extract from *Origanum vulgare* L.; (B) Supercritical CO_2 Extract from Commercial Origanum; (C) Essential Oil (hydro distillation) from *Origanum vulgare* L.; and (D) Essential Oil from Commercial Origanum

peak order	identification method[a]	compound	relative amount[b] A	B	C	D
1	ms	α-thujene	ND	ND	ND	T
2	std, ms	α-pinene	ND	ND	T	T
3	std, ms	β-pinene	T	T	T	ND
4	ms	1-octen-3-ol	ND	ND	++	T
5	std, ms	*p*-cymene	ND	T	+	++
6	std, ms	limonene	T	T	ND	ND
7	std, ms	camphene	ND	T	++	ND
8	std, ms	γ-terpinene	ND	T	++	++
9	ms	*trans*-sabinene hydrate	++	++	ND	+
10	ms	*cis*-sabinene hydrate	++++	++++	ND	+
11	std, ms	linalool	+	++	T	ND
12	ms	*p*-menth-2-en-1-ol	T	T	ND	++
13	ms	*trans*-pinene hydrate	T	T	ND	+
14	ms	borneol	ND	T	T	ND
15	std, ms	terpineol-4	+	+++	+	+++
16	std, ms	α-terpineol	+	+++	T	++
17	ms	*cis*/*trans*-piperitol	ND	T	T	+
18	ms	thymol methyl ether	ND	+	T	+
19	ms	carvacrol methyl ether	ND	+	+	+
20	ms	linalyl acetate	+++	+++	T	+
21	std, ms	thymol	+	++++	+	++++
22	std, ms	carvacrol	++++	++	++++	+
23	ms	carvacryl acetate	ND	ND	+	T
24	ms	β-caryophyllene	T	++	++	++
25	ms	α-caryophyllene	ND	T	+	ND
26	ms	α-humulene	ND	ND	+	+
27	ms	germacrene-B/γ-humulene	T	++	ND	+
28	ms	β-bisabolene	ND	ND	+	+
29	ms	δ-cadinene	ND	ND	+	+
30	ms	spathulenol	T	++	++	++
31	ms	caryophyllene oxide	T	+	++	++

[a] Compounds were identified by injection of standards (std) and/or by comparing the mass spectra with the equipment library (ms). [b] Relative amount obtained by comparison of the relative peak areas for the same injection volume, same dilution, and in the same signal scale: T = trace; ND = not detected; +, ++, +++, ++++ = increasing values of peak areas.

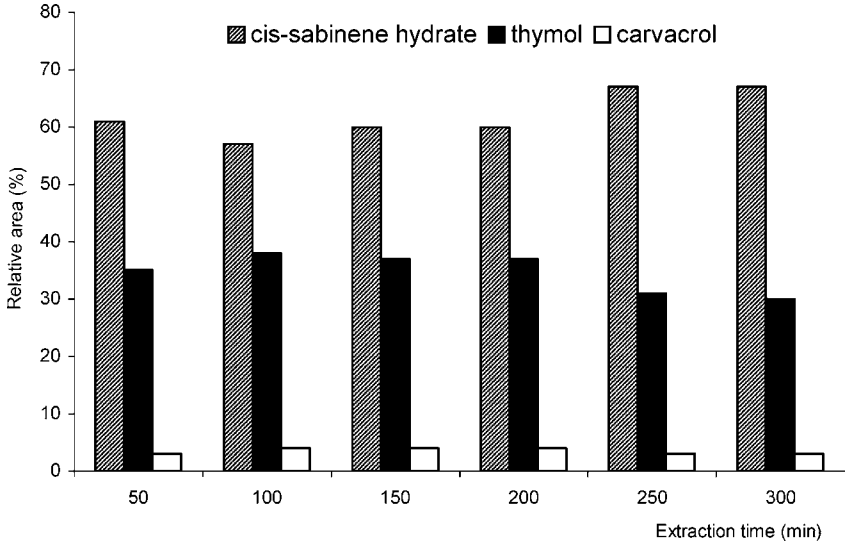

Figure 3. Relative area for *cis*-sabinene hydrate, thymol, and carvacrol present in the extract from commercial oregano as a function of extraction time. Extraction condition: 150 bar and 303 K.

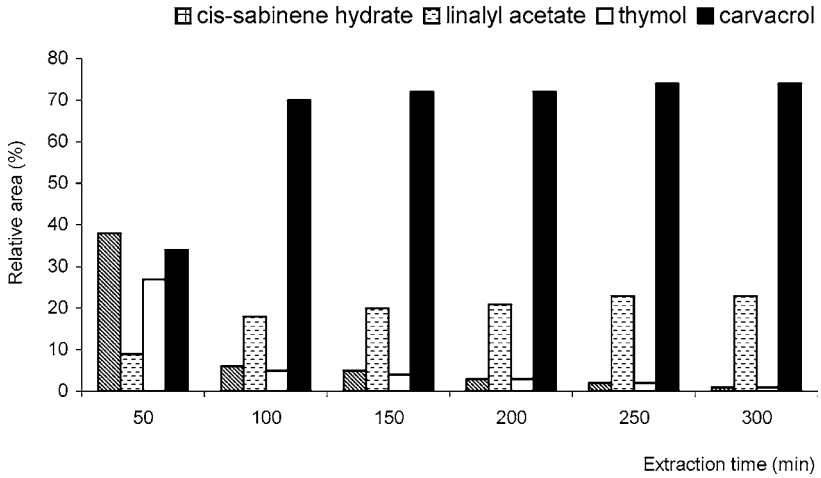

Figure 4. Relative area for *cis*-sabinene hydrate, linalyl acetate, thymol, and carvacrol present in the extract of *Origanum vulgare* as a function of extraction time. Extraction condition: 150 bar and 303 K.

(peak 21), or carvacrol (peak 22) and linalyl acetate (peak 20) are the major constituents, followed by β-caryophyllene (peak 24), 4-terpineol (peak 15), and α-terpineol (peak 16).

Comparing these results with those obtained from hydro distillation in a Clevenger apparatus (*18*), it is clear that the method of extraction may lead to quite different extracts, especially with regard to the hydrocarbon terpene fraction. The results presented in **Table 2** are in complete agreement with the literature (*14*, *15*), as both hydrodistillation and supercritical CO_2 extraction techniques produce almost the same main volatile compounds but with negligible monoterpene hydrocarbons for the second case. According to Temelli et al. (*22*) an attractive point of supercritical extraction is to obtain extracts rich in aroma compounds with the lowest possible monoterpene concentration, because they do not contribute much to the flavor of essential oils and also because they are very sensitive to heat and light and may decompose into undesirable substances.

Quantitative Analyses. Table 3 presents the influence of the extractions conditions in the concentration of the major compounds within the phenol class, thymol, and carvacrol, of commercial oregano. It can be noted that the extraction condition of 100 bar and 313 K leads to the highest thymol concentration (∼46 mg L^{-1}) for commercial oregano samples. Indeed, the experimental condition of 200 bar and 313 K resulted in the greatest extraction yield but a lower thymol concentration than the one observed at 100 bar and 313 K, once the highest working pressure would favor the extraction of higher molecular weight compounds.

Table 3. Concentration of Thymol and Carvacrol (mg L^{-1}) in the Commercial Origanum Extracts Obtained from Sub- and Supercritical CO_2 Extraction

	runs[a]				
compound	1	2	3	4	5
thymol	25.32	25.75	46.13	25.82	36.82
carvacrol	2.88	2.73	4.61	2.45	3.31

[a] Runs 1–5, see **Table 1**.

This result is in agreement with literature, where it is verified that the pressure range of 80−120 bar and temperature near 313 K are required for the appropriate extraction of compounds responsible for the essential oil flavor (*23*).

Furthermore, from an engineering point of view, an important aspect that should also be taken into account is the productivity of each compound in addition to the extraction yield and the concentration of compounds in the extract. Here, the productivity of compound is defined as the extraction yield multiplied by

Table 4. Productivity (Yield × Concentration) (mg L^{-1}) of Thymol and Carvacrol in the Commercial Origanum Extracts Obtained from Sub- and Supercritical CO_2 Extraction

	runs[a]				
compound	1	2	3	4	5
thymol	9.61	11.84	30.90	34.08	30.19
carvacrol	1.09	1.26	3.08	3.23	2.71

[a] Runs 1–5, see **Table 1**.

the concentration of the compound, as presented in **Table 4**. When runs 1, 4, and 5 (approximately the same density, see **Table 1**) are compared, it can be noticed that an increase in temperature leads to an increase of thymol and carvacrol content in the extract, mainly from 293 to 303 K. The weak effect of pressure can be seen when one compares runs 1 and 2 (293 K) or runs 3 and 4 (313 K), which just corroborate the strongest effect of temperature on the extraction of origanum samples with sub and supercritical CO_2.

Cultivated and Commercial Origanum Samples. Samples of cultivated and commercial origanum were submitted to sub- and supercritical CO_2 extraction at 150 bar and 303 K. The results in terms of overall extraction yield for cultivated and commercial samples were 0.47 and 0.82%, respectively, showing that there is a great difference between the origanum samples. The chromatographic analyses of the cultivated origanum extracts revealed the presence of almost the same compounds found in the commercial sample (see **Table 2**) but with a higher carvacrol concentration (~33 mg L^{-1}) when compared to thymol (~3.5 mg L^{-1}), while the commercial sample provided an opposite result, ~3.3 mg L^{-1} for carvacrol and ~37 mg L^{-1} for thymol.

According to the literature, there are 39 species used all over the world as spices or pharmaceutical called origanum. Therefore, the classification of origanum should be carried out based on the phenolic compounds concentration (carvacrol and thymol). Nevertheless, just a few researchers have found origanum with a significant thymol concentration (*14*, *24*). For the cultivated sample, carvacrol is the major phenol constituent, which confirms the origin of this plant as *Origanum vulgare*.

Figure 3 shows th normalized percentage area for some compounds (*cis*-sabinene hydrate, thymol, and carvacrol), based on the normalized area obtained from the GC-MSD, from the extraction of commercial origanum at 150 bar and 303 K as a function of extraction time. This is another kind of semiquantitative comparison of the extracts. The normalized relative area, using the same volume of injection and the same dilution factor, can be considered proportional to the concentration of the compound. The extracts were collected at each 50 min along 6 h and analyzed according to the method previously described (*19*). It can be noted from this figure that thymol is the major phenol compound, while *cis*-sabinene hydrate is the major constituent of the whole analysis. For this origanum sample, the compound's concentration does not vary appreciably with the time of extraction.

The same comparison is made by the cultivated sample, and the results are shown in **Figure 4**. The compounds analyzed are *cis*-sabinene hydrate, linalyl acetate, thymol, and carvacrol. It is noted that only at the first 50 min is the area for *cis*-sabinene hydrate greater than that of carvacrol. After this short time interval, both *cis*-sabinene hydrate and thymol become progressively less noticeable and the extract becomes more concentrated in carvacrol. This result might be very important if one considers the possibility of obtaining a variety of extracts with different composition profiles as a function of extraction time.

The extraction yield for the origanum species is shown to vary appreciably and be strongly dependent on temperature within the variables range studied. Great differences in chemical concentration of the major constituents in the origanum essential oils were observed, and the extraction time may exert a great influence on the distribution of chemical components.

ACKNOWLEDGMENT

We thank FEPAGRO for technical assistance.

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Received for review August 6, 2003. Revised manuscript received February 1, 2004. Accepted February 1, 2004. Financial support provided by CNPq, CAPES, and FAPERGS.

JF030575Q