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The Effects of Temperature and Pressure on the Characteristics of the Extracts from High-Pressure CO₂ Extraction of *Majorana hortensis* Moench

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The aim of this work is to assess the influence of temperature and pressure (solvent density) on the characteristics of the essential oil obtained from high-pressure carbon dioxide extraction of marjoram (commercial marjoram samples available in the free market and *Majorana hortensis* Moench, cultivated in South Brazil under rigorous agronomic conditions). The extracts were analyzed in terms of the liquid yield (extract/raw material, wt/wt) and distribution of volatile chemical components. The experiments were performed in a laboratory-scale unit using the dynamic method in the temperature range of 293.15–313.15 K, from 100 to 200 bar in pressure. Chemical analyses were carried out in a GC/MSD. Results show that an increase in temperature leads to a rise in the extract liquid yield despite large changes in solvent density. Chromatographic analyses permitted the identification of *cis*-sabinene hydrate, terpineol-4, α -terpineol, and *cis*-sabinene hydrate acetate as the main volatile compounds present in both commercial and cultivated samples.

KEYWORDS: Chemical analysis; high-pressure extraction; extraction kinetics; marjoram; essential oil; GC/MSD

INTRODUCTION

Marjoram comprises several aromatic *labiatae* herbs belonging to different species. The best known is *Origanum majorana* L. (syn. *Majorana hortensis* Moench, *M. vulgaris* Miller), native on Cyprus and South Turkey. It is also cultivated extensively as sweet marjoram, an annual herb, in several areas of Europe, North Africa, America, and Asia. The dried leaves of sweet marjoram are widely used by food industry as flavoring agents for dressings, soups, and in formulation of vermouthe and bitters, among others (1–3). The essential (volatile) oil of sweet marjoram has been known since antiquity due to its biological activity, notably antibacterial, antifungal, and antioxidant properties, thus making the investigation of the flavor composition of this raw-material of great interest (4, 5). Some studies have shown that the aroma composition may vary with the origin of plants and cultivating conditions (6, 7). For instance, some essential oils of marjoram have been obtained with high contents of monoterpene alcohols whereas in others phenols are the major constituents. In some oils, terpineol-4 is the major component, alone or together with other monoterpene alcohols such as *cis*-

and *trans*-sabinene hydrate and α -terpineol. The essential oil is commonly defined by the method of preparation, namely, the separation of volatile substances by distillation at atmospheric pressure and elevated temperature (8). Other methods such as simultaneous steam distillation extraction (SDE) and supercritical fluid extraction (SFE) can also produce high-quality essential oils from herbaceous materials (8, 9). For instance, Jiménez-Carmona et al. (10) have used subcritical water extraction (CSWE) to extract the essential oil from marjoram under dynamic conditions and have concluded that this method, when compared with hydrodistillation, provides a more valuable essential oil, because the oxygenated fraction content is larger than the terpenic one.

Some studies concerning the application of high-pressure carbon dioxide to the extraction and fractionation of marjoram are available in the literature (9, 11). In a recent review, Reverchon (12) pointed out that SFE of flavors and fragrance from vegetable matters presented in the literature are focused mainly on the analytical aspects and thus there is a lack of a comprehensive study regarding the influence of process parameters on SFE of essential oils.

This work is aimed at investigating the influence of temperature and pressure (solvent density) on the characteristics of the extracts obtained from high-pressure carbon dioxide extraction of two marjoram samples, a commercial and a cultivated

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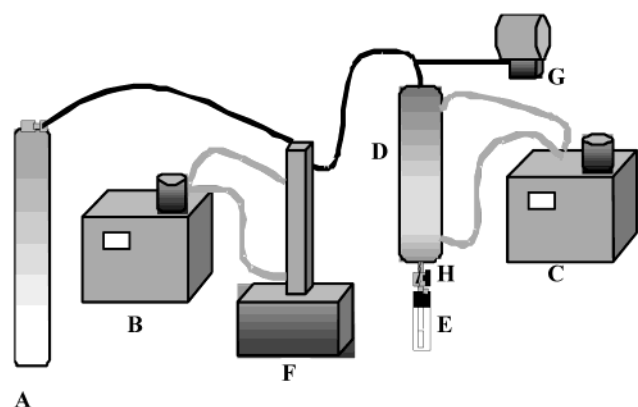


Figure 1. Schematic diagram of the high-pressure extraction apparatus: (A) CO₂ reservoir; (B, C) thermostatic baths; (D) extraction vessel; (E) collector vessel with a glass tube; (F) high-pressure pump; (G) absolute pressure transducer; (H) electrical heater.

one under agronomic control. The experiments were performed by following an experimental saturated design (two variables and two or three levels) in a semibatch laboratory unit operated in the temperature range of 293.15–313.15 K, from 100 to 200 bar, with a constant CO₂ flow rate of 1 g min⁻¹. Chemical analyses were conducted in a GC/MSD (Shimadzu, Model QP 5050A). The liquid (extract/raw material, wt/wt), extraction kinetics, and essential oil chemical composition are reported in this paper.

EXPERIMENTAL PROCEDURES

Material. Samples of commercial marjoram (dried leaves) were purchased in the free market and used without further treatment. The *Majorana hortensis* Moench samples, cultivated under rigorous agronomic control, were kindly provided by FEPAGRO, Viamão, RS, Brazil. These samples were collected in the winter, dried at room temperature, crushed manually, and stored under nitrogen atmosphere. The carbon dioxide (99.9% purity) was purchased from White & Martins. The analytical standards α -pinene, camphene, β -pinene, α -terpinene, *p*-cymene, limonene, 1,8-cineol, γ -terpinene, terpinolene, linalool, terpineol-4, α -terpineol, carvacrol, thymol, and biphenyl were from Aldrich, Palo Alto, CA. For each standard, solutions were prepared (1000 mg L⁻¹) using dichloromethane (MERCK) and stored under refrigeration.

Apparatus and Experimental Procedure. The experiments were performed in a laboratory scale unit, schematically presented in **Figure 1**, which consists basically of a CO₂ reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 0.2 dm³ jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of ± 0.12 bar, a collector vessel with a glass tube, and a cold trap. Amounts of around 25 g of dried marjoram leaves and flowers were charged into the extraction vessel. The CO₂ was pumped at a constant flow rate of 1 g min⁻¹ 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 1 h to allow the system to stabilize. Afterward, the essential oil was collected by opening the micrometering valve and the CO₂ 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 preestablished limit. The experiments were accomplished in approximately 400 min, isothermally 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 saturated design using two levels for temperature and three levels for pressure was established so as to assess the influence of the process variables on the liquid yield and on the

Table 1. Extraction Yield, Physical Properties, and Characteristic Parameters

run	<i>T</i> (K)	<i>P</i> (bar)	solvent density (g cm ⁻³)	extraction yield (%) ^a
1	293.15	100	0.8553	0.64 \pm 0.013
2	293.15	150	0.9044	0.64 \pm 0.013
3	293.15	200	0.9378	0.66 \pm 0.013
4	313.15	100	0.6164	0.73 \pm 0.014
5	313.15	150	0.7807	1.38 \pm 0.028
6	313.15	200	0.8408	1.45 \pm 0.029
7	303.15	150	0.8475	1.07 \pm 0.021

^a *n* = 3.

extract composition. The experimental range investigated was 293.15–313.15 K in temperature and from 100 to 200 bar in pressure. Triplicate runs were performed for all conditions, leading to an overall standard deviation of the liquid yields of about 0.019.

Extract Characterization. The extracts were analyzed with a gas chromatograph interfaced with a mass selective detector, GC/MSD (Shimadzu, Model QP 5050A), using a capillary column DB-5 (30 m \times 0.25 mm \times 0.25 μ m), flow rate of 1 mL min⁻¹, in electronic impact mode of 70 eV, split mode (split ratio 1:30), at 280 °C interface temperature, with the following column temperature gradient programming: 50 °C (0 min) to 2 °C min⁻¹ to 100 °C to 3 °C min⁻¹ to 145 °C to 5 °C min⁻¹ to 280 °C (25 min). The identification and quantification of some compounds, mainly terpineol-4 and α -terpineol, were accomplished through the analytical standards and biphenyl as internal standard by comparing the mass spectra and GC retention time. The other compounds were identified by comparing the mass spectra obtained with those from the Wiley library.

RESULTS AND DISCUSSION

Extraction of Commercial Marjoram Samples. Initially, the effect of temperature and pressure on the extraction yield was evaluated using the commercial marjoram sample. Here, the yield is defined 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 liquid yield along with the experimental conditions investigated in this study. This table also shows the large variation on the solvent density, which was calculated from Angus correlation (13). The general analysis of the results presented in this table reveals that a rise in temperature at constant pressure leads to an increase of the extraction yield despite large solvent density changes and a rise in pressure at constant temperature also leads to an increase of the extraction yield due to the enhancement of the solvent power (14). However, comparing runs 1, 6, and 7 (almost same density values) or coming from run 3 to 6 (same pressure, increasing temperature, decreasing solvent density) clearly shows that the temperature has a much more pronounced effect on the extraction yield as compared to pressure (density).

Figure 2 presents the experimental extraction curves for all conditions shown in **Table 1**. These profiles are similar to the ones obtained in drying processes: the extraction is initially linear and then becomes asymptotic (14). It seems from this figure that the solubility (the slope of the linear part of the extraction curves) increases with increasing temperature at constant pressure (150 and 200 bar), which means that, for these pressure levels, the solubility values are located in the right side of the inflection point (14). The thermodynamic solubility is dependent on temperature and pressure (solvent density), and thus, the appreciable decrease in density observed at 100 bar from 293 to 313 K is responsible for the slight reduction in solubility despite the enhancement in the liquid yield.

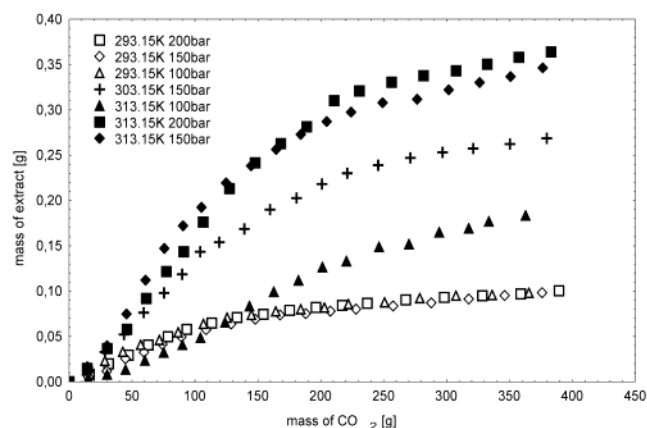


Figure 2. Extraction curves obtained from commercial marjoram samples with CO₂ at high pressures.

Chromatographic Analyses of Commercial Marjoram Extracts. Qualitative Analyses. According to the literature, marjoram essential oil is in fact a complex mixture of hydrocarbon, oxygenated, and aromatic compounds (9, 11, 12). For instance, Reverchon (9) has found that oxygenated terpenes comprised the major fraction, with *cis*-sabinene hydrate, *cis*-sabinene hydrate acetate, terpineol-4, and α -terpineol as the major constituents. The commercial marjoram sample extracts for which experimental conditions were presented in Table 1 were analyzed through GC/MSD. In summary: 5 monoterpenes, 8 terpene alcohols, 1 phenol, 3 sesquiterpenes, and 4 other oxygenated compounds were found, overall representing 23.81% of monoterpenes and 76.19% of nonmonoterpenes. Table 2 presents the components identified in the extracts of commercial marjoram. The compounds were identified by injection of standards and/or by comparing the mass spectra with the equipment library. In the first case it was labeled std in the table and in the second one, ms.

In this table it is also possible to compare the relative concentration of the compounds on the basis of the peak area percentages. The estimated concentration was obtained by comparison of the peak areas for the same injection volume and in the same signal scale (attenuation). With the aim of 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 13) and *cis*-sabinene hydrate acetate (peak 21) are the major constituents, followed by terpineol-4 (peak 18), *trans*-sabinene hydrate (peak 11), and α -terpineol (peak 19).

According to Reverchon et al. (9), *cis*-sabinene hydrate and its acetate are mainly responsible for the aroma of marjoram oil whereas terpineol-4 and α -terpineol are considered to be the products resulting from the decomposition of these unstable components.

An interesting effect that can also be observed in this table is that the contribution of monoterpenes (peaks 1–10) is very small, almost negligible, for the extract of commercial marjoram. According to Temelli et al. (15) an attractive point of supercritical extraction is to obtain extracts with the lowest possible monoterpene concentration because monoterpenes 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. Thus, supercritical CO₂ extraction may offer some important advantages with respect to applications in food industry, transport costs, and storage.

Table 2. Volatile Compounds Identified in the Three Samples of Marjoram Essential Oil Analyzed: (A) Carbon Dioxide Extract at High Pressures from *Majorana Hortensis* Moench, (B) Carbon Dioxide Extract at High Pressures from Commercial Marjoram, and (C) Steam Distillation Oil from *Majorana Hortensis* Moench

peak order	identification method ^a	compound	est concn ^b		
			A	B	C
1	std, ms	α -thujene	nd	nd	+
2	std, ms	α -pinene	t	t	t
3	std, ms	β -pinene	t	t	nd
4	ms	sabinene	nd	nd	++
5	ms	β -mircene	nd	nd	+
6	ms	α -phellandrene	nd	nd	t
7	std, ms	α -terpinene	t	t	++
8	ms	camphene	t	t	nd
9	std, ms	<i>p</i> -cymene	nd	nd	++
10	std, ms	γ -terpinene	t	t	+++
11	ms	<i>trans</i> -sabinene hydrate	++	++	+
12	ms	terpinolene	nd	nd	+
13	std, ms	<i>cis</i> -sabinene hydrate	+++	+++	+++
14	ms	linalool	t	t	t
15	ms	<i>p</i> -menth-2-en-1-ol	t	t	+
16	ms	terpineol-1	t	t	+
17	ms	menthone	nd	nd	t
18	std, ms	terpineol-4	++	++	++++
19	std, ms	α -terpineol	++	++	+++
20	ms	<i>cis/trans</i> -piperitol	t	t	+
21	std, ms	<i>cis</i> -sabinene hydrate acetate	+++	+++	+
22	ms	linalyl acetate	+	+	+
23	std, ms	α -terpineol acetate	t	t	nd
24	std, ms	carvacrol	t	t	nd
25	ms	neryl acetate	nd	nd	t
26	std, ms	β -caryophyllene	+	+	+
27	ms	germacrene-b	+	+	+
28	ms	spathulenol	t	t	+
29	ms	caryophyllene oxide	t	t	t

^a Compounds were identified by injection of standards (std) and/or by comparing the mass spectra with the equipment library (ms). ^b Estimated concentration obtained by comparison of the peak areas for the same injection volume and in the same signal scale: t = trace; nd = not detected; +, ++, +++, +++++ = increasing values of peak areas.

Table 3. Concentration of Terpineol-4 and α -Terpineol (mg L⁻¹) in the Extracts of Commercial Marjoram Obtained from Carbon Dioxide Extraction at High Pressures

compound	runs ^a						
	1	2	3	4	5	6	7
terpineol-4	5.21	6.57	7.22	14.14	13.37	13.21	16.06
α -terpineol	3.94	4.76	5.39	9.84	7.75	7.64	9.53

^a Runs 1 to 7: see Table 1.

Quantitative Analyses. In Table 3 the concentration of the compounds terpineol-4 and α -terpineol is calculated using biphenyl as an internal standard. It was observed that the extraction condition of 150 bar and 303.15 K (run 7) led to the highest terpineol-4 and α -terpineol concentrations, of 16.06 and 9.53 mg L⁻¹, respectively, for commercial marjoram samples. Recalling the results presented in Table 1, it is interesting to notice that this is not the experimental condition where the extraction yield is maximized within the experimental range investigated. Of course, this result means that an attempt to maximize the process extraction yield does not necessarily imply in the enrichment of target compounds. According to Nykänen (6), there are two main chemical types of marjoram, dependent on the concentration of either alcohol or phenol monoterpenes. Generally, terpineol-4, alone or together with other alcohol terpenes, such as *trans*- and *cis*-sabinene hydrate and α -terpi-

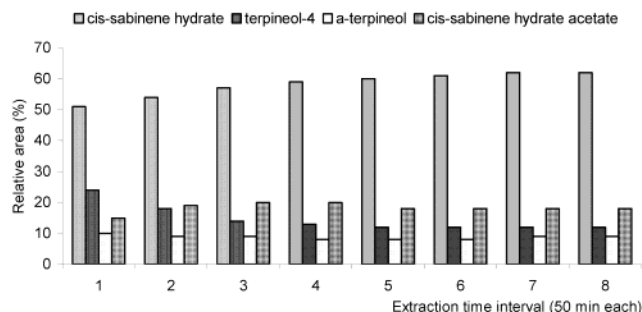


Figure 3. Relative area for *cis*-sabinene hydrate, terpineol-4, α -terpineol, and *cis*-sabinene hydrate acetate present in the commercial marjoram extract obtained at 150 bar and 303.15 K.

neol, are the major constituents. Sarer et al. (3) have found carvacrol with a concentration as high as 5% in marjoram essential oil.

Effect of Extraction Time. To assess the influence of extraction time on the characteristics of the extracts, a commercial sample of marjoram was submitted to CO₂ extraction at 150 bar and 303.15 K, collecting extract samples at each 50 min and afterward proceeding with the chemical analyses via GC/MSD, as described previously. **Figure 3** depicts the normalized percentage area for some compounds (based on the normalized area obtained from the GC/MSD) as a function of extraction time. It can be noted that *cis*-sabinene hydrate and *cis*-sabinene hydrate acetate are the major constituents of the essential oil for almost all time intervals. It is worth noticing from this figure that the chemical distribution of the compounds is only slightly affected over the time course of the extraction.

Cultivated and Commercial Marjoram Samples. With the aim of comparing the chemical composition profiles of cultivated and commercial marjoram samples, 25 g of the former sample was submitted to CO₂ extraction at 150 bar and 313.15 K (resulting in 1.28%, wt/wt). The results of the chromatographic analyses were shown in **Table 2** and permitted the identification in the cultivated marjoram sample the same compounds found in commercial sample, with no significant concentration differences.

Further, we have compared the volatile profiles of marjoram essential oil obtained from CO₂ extraction with the one coming from steam distillation in a Clevenger apparatus (16). The results of the chromatographic analysis of essential oil obtained by steam distillation of cultivated marjoram samples were also presented in **Table 2**.

Comparing the results presented in **Table 2**, it is clear that the method of extraction produces quite different extracts, especially with regard to the hydrocarbon terpene fraction. Some compounds such as α -thujene, sabinene, β -mircene, α -phellandrene, *p*-cymene, terpinolene, menthone, and neryl acetate are present only in the essential oil produced by Clevenger extraction. As mentioned before, supercritical fluid extraction might be in some cases a more attractive technique over conventional separation methods, especially when focused on the quality of the products obtained.

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