CHARACTERIZATION OF THE ROSEMARY EXTRACT (*ROSMARINUS OFFICINALIS L.*) OBTAINED WITH SUPERCRITICAL CO$_2$, STARTING WITH THE THEORETICALLY STAGED DETERMINATION OF THE EXTRACTION.

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Abstract

Rosemary (*Rosmarinus officinalis L.*), as a good source of antioxidants compounds, was the raw material to obtain extracts using CO$_2$ in supercritical state as solvent. Two factors were varied to determine their influence in the extract yield: the extraction temperature and the solvent (CO$_2$) flow rate. A randomized 2^2 factorial experimental design was employed. The levels for each factor were 40 and 60°C and 8.3 and 15 mL/min for temperature and flow rate, respectively. The extraction pressure was fixed at 4,500 psi (3.10 Mpa), given that literature reports that the antioxidant compounds of rosemary are obtained between 4000 and 5000 psi [11]. The theoretical stages for extraction were calculated and determined that 3, 4 and even 5 stages were needed; the number of stages being inversely proportional to the CO$_2$ flow rate.

The rosemary extracts composition was determined by GC/MS. Of more than 100 compounds separated, 94 were identified and 58 quantified. The concentration of the three main compounds were used to calculate the statistical influence of the mentioned factors and the theoretical stages needed for extraction.

Introduction

Rosemary (*Rosmarinus officinalis* L.) produces antioxidants similar to synthetic ones (BHA, BHT, BTHQ). The antioxidant activity of its extracts is originated by polyphenolic compounds, mainly rosmarinicus acid, carnosol, rosmanol and carnosic acid [2]. Those chemicals are obtained through the preparation of oleoresins and other extracts. [12] obtained rosemary extracts using supercritical CO$_2$ at pressures between 4 000 and 5 000 psi and temperatures between 35 an 100°C. The antioxidant properties of the extracts were reported to be better than that of extracts obtained by solvent extraction[4, 9].

The oleoresins and extracts from plants are prepared as a liquid-solid separation known as percolation and a later concentration of the percolate. Traditionally, the percolation process is carried out at countercurrent. Even in an extraction battery where the solid phase is not physically moved from one stage to the next, the charge is treated by successive amounts of liquid, of decreasing concentration, as if the solid were displaced countercurrent. In this way, percolation can be calculated as a number of ideal stages as well as the efficiency of extraction[1,5].

This work shows the dependency of extraction temperature and of solvent flow rate in the yield and composition of the extract; with the data collected the theoretical number of stages will be calculated

Materials and Methods

Rosemary (*Rosmarinus officinalis* L.) was gathered in La Barca, Jalisco (Mexico). It was dried at the shadow until its moisture reached 8-10%. The plant was analyzed for its essential oil and ashes content. Samples of 75 g were extracted by supercritical $CO2$ in a Newport Scientific, Inc. Model 46-19345, USA. A randomized 2^2 experimental design [7] was used to evaluate the extraction yield dependency on temperature (40 and 60°C) and on solvent flow rate. The apparatus has an extractor vessel with capacity of 1,2 L and a 0.8 L separator vessel. Both, temperature and solvent flow rate, can be automatically controlled. The extraction temperature was varied according to the experimental design, while the separation temperature was maintained at 40°C.The extraction and separation pressure were fixed at 4,500 and 500 psi, respectively.

To analyze the extracts, 0.1 g was dissolved in 1,1 mL of n-pentane. The solution was then filtered through a 0.45 μm nylon membrane. Aliquotes of 0.1 μL were automatically injected into a GC/MS (HP 5890/5972). The column was a crosslinked methylsiloxane (HP-1), 50 m long, 0.33 mm film. The carrier was Helium at 0.8 mL/min. The injection and detection temperatures were 250 and 280°C, respectively. The oven temperature was programmed from 75°C to 280°C (20 min at constant temperature) at 2.5°C/min. The MS adquired data by electron impact (EI) at 70 eV. For compounds identification a data station HP MSD ChemStation version B.01.00 was used, as well as by comparison to the spectra library Wiley 275L. Confirmation of identification used injection of standards, Kovats' indexes and data reported in literature.

Results and Discussion

The average yield of extracts was 4.83%. The analysis of variance for the yields, at the 95% confidence level, showed no statistical differences between levels of the two factors: the CO_2 flow rate and temperature.

Table 1. Analysis of Variance for the yields of extract obtained from rosemary

Source	Sum of Squares	Degree of freedom	Mean Square	F- Ratio	P-Value
A. Temperature	0.9248	1	0.9248	3.47	0.1359
B. CO_2 flow rate	0.3528	1	0.3528	1.32	0.3140
AB	0.045	1	0.045	0.17	0.7022
Error	1.0658	4	0.26645		
Total	2.3884	7			

With the data obtained, the number of theoretical steps were determined as if the extraction were solid-liquid (percolation). Two experiments showed experimental equilibrium lines (flow rate 8.3 and 15 mL/min and 40°C, in both). Calculations showed that the theoretical steps were 5 and 3, respectively, with a strong inverse dependency on the flow rate of solvent. The menor number of stages at greater flow rate implies that the plant material could still be extracted. Evidence of this can be seen in Figure 1 where asynthotic values are quickly reach at flow rate of 8.3 mL/min, while at 15 mL/min the data have not flatten out at similar times.
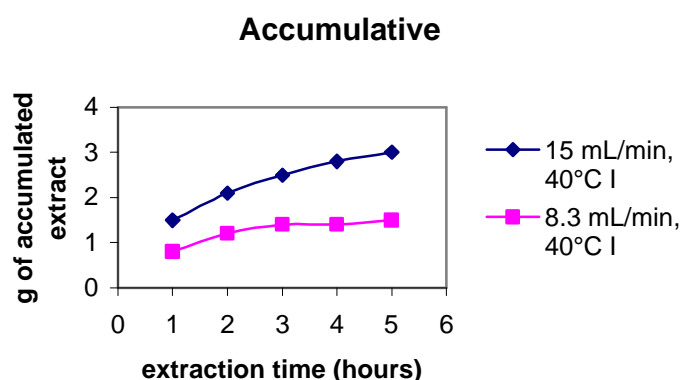
Accumulative

Figure 1. Extract collected as a function of time.

The composition of the extracts was determined by GC-MS. Of 100 separated compounds, 94 were identified and 58 quantified. The main fraction, 43%, was formed by terpenic compounds, namely alpha-pinene, beta-pinene, paracimene, and L-borneol. Hydrocarbons of high molecular weight, like tetra-cosane, accounted for 20%. The rest, 37%, was a mixture of fat acids, alcohols, cetones, etc.

Three main components (alpha-pinene, linalool and camphene) of the extracts were monitored by GC [10]. Its remarkable the influence of the solvent flow rate on the extraction of the compounds. While at 8.3 mL/min most of the compounds are extracted at the second hour, at 15 mL/min the extraction just begins (Figure 2). The authors assume that the transport phenomena are quite different in each case: at 8.3, having a greater residence time, allows diffusion compete with dissolution of the compounds; on the other hand, a smaller residence time (flow rate 15 mL/min) permits the dominance of dissolution over diffusion.
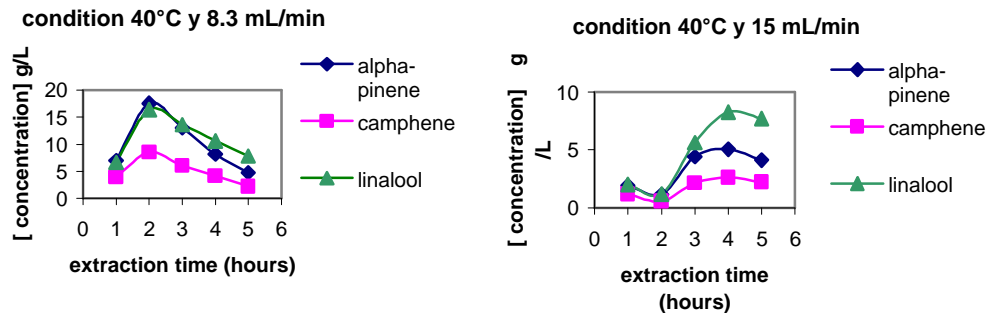
Figure 2. Profile of extraction of the three main components in extracts.

With the results obtained in this work, we consider that even when the yield of extracts have no statistical difference in both factors, the concentration of some compounds in the extracts differs as the flow rate changes.

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