

COMPOSITIONAL CHANGES ON CHLOROPHYLLS AND CAROTENOIDS AS A FUNCTION OF SUPERCRITICAL CARBON DIOXIDE AND SUBCRITICAL PROPANE EXTRACTION OF OREGANO LEAVES

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In this work, the structural and compositional changes on chlorophylls and carotenoids as a function of supercritical carbon dioxide and sub-critical propane extraction of oleoresin from oregano leaves were studied. The results indicated that the maximum oleoresin yield could be obtained with SC-CO₂ at 400 bars and 35 or 55°C and sub-critical propane at 50 bars. Pigment solvating capacity of SC-CO₂ increased with the increase of extraction pressure. Great variation between raw material, oleoresin and residues (powders after extraction) was noticed in their pigment composition. Chlorophyll b and a and pheophytin a were the dominant pigments in raw material with small quantities of other derivatives. After extraction of oregano with SC-CO₂ at 35°C and pressures higher than 200 bars, epimerisation and oxidation of chlorophylls took place in the residues. The content of artefacts was increased proportionally to the increase of the extraction pressure. SFE at 55 °C and 100 bars (long-time extraction) resulted in a great conversion of chlorophyll to Mg-free derivatives (pheophytins). The brownish green coloured-oleoresins contained only pheophytins indicating that rapid release of Mg and substitution of hydrogen atoms on the molecules accompany solubilization of such pigment by SC-CO₂ and subcritical propane.

INTRODUCTION

Oregano is used worldwide as a natural flavour and medicinal plant. The leaves of the plant contain, in addition to the essential oils, considerable amounts of biologically active carotenoids and tocopherols. The product is usually used as a powder of dried leaves or oily extract alone or mixed with sesame. Recently, supercritical fluid extraction (SFE) has become superior over traditional extraction methods, using organic solvents, in the extraction of oils from plant products [1-3]. As a general principle of extraction of plant oils by non-sequential SFE, the solvation power of the solvent should be maximised by ascertaining the optimum conditions [4,5].

The objective of the present work was the extraction of oregano by SC-CO₂ and sub-critical propane with quantification of chlorophylls, carotenoids and tocopherols in oils and residues that can be used as natural colours or fodders.

MATERIALS AND METHODS

Food grade CO₂ (99.5%) and propane (99.95%) were purchased from Messer Griesheim Hungaria (Budapest). All analytical and HPLC grade organic solvents used in the analysis of pigments and antioxidants were from Reanal (Budapest, Hungary), while the standard materials used for identification and quantification of the effective compounds were obtained from Sigma (St. Louis, MO). Freshly ground oregano (local variety) was obtained from the local markets of Amman, Jordan.

Extraction procedure

The extractions were performed with a high-pressure, flow-up stream extraction apparatus, which has been previously described [6]. A membrane pump (Model EL-1 from Lewa Herbertott, Leonberg, Germany), pumped the solvent to the extractor at a flow rate of 1.0-1.5 l/min. The solvent passes through a buffer vessel into the thermostated column. A back-pressure valve regulator adjusted the pressure in the extractor. The solute-rich compressed gas was expanded to atmospheric pressure through a heated needle valve. The extracts were received in a cool container and left to stand for 20 min to warm allowing the solvent to evaporate. For various time intervals, the stable weight of the extracts was measured. The mass of solvent used was determined by applying Peng-Robinson equation [7] after the normalization of the volume. The density of SC-CO₂ was calculated by applying Bender equation [8].

SFE studies were conducted to extract pungent paprika with SC-CO₂ at 35-55°C and pressure of 100-400 bars. In the case of sub-critical propane, the extraction was carried out at 25°C and pressure of 30-50 bars.

Analytical methods

To analyse the chlorophylls and carotenoids in ground oregano leaves, 0.5-1.0 g were extracted by shaking with 50 mL of 2: 1:1 dichloroethane-acetone-methanol for 15 min. after mechanical shaking, the mixture was filtered through filter paper, and the solvent was evaporated under a vacuum at 35-40 °C using rotary evaporator. In case of oleoresin, 50-100 mg were dissolved in 5-10 mL of the HPLC eluent using water bath ultrasonic wave (Tesla, Check Republic) for 30 sec followed by filtration before injection into the HPLC column.

The high-performance liquid chromatographic (HPLC) analyses were carried out using a Beckman (Fullerton, CA) model 114 solvent delivery pump, a Model 420 controller and a model 166 variable wavelength ultraviolet-visible detector for the detection of chlorophylls and carotenoids. The signals of the detectors were recorded and integrated by a Shimadzu C-R3A integrator.

The chlorophylls and carotenoids were separated on a Lichrosorb C-18, 6µm, 250x46 mm column using a mobile phase consisting of 39:52:5:4 acetonitrile-isopropanol-methanol-water at a flow rate of 0.9 mL/min [9]. The effluents were monitored at 450 and 655nm.

Authentic standards for chlorophylls were prepared by thin-layer chromatography on cellulose layer [10]. The procedure included extraction by acetone and separation on Silica gel (TLC aluminium sheets without fluorescence indicator, Merck, Darmstadt, Germany) with light petroleum (60-80°) – pyridine (9:1 v/v) as the developing solvent. Each color band was scrapped of the plate and its spectrum and quantity were spectrophotometrically measured. Also, standard β-carotene (Sigma, catalogue no. C 4582) and lutein (Sigma, catalogue no. X 0625) were used for their identification and quantification. In qualitative analysis, scanned spectra and retention times of the individual compounds were compared with those of standard materials.

Results And Discussion

Extraction by SC-CO₂ and propane

The extraction curves of pungent paprika oleoresin were obtained by plotting solvent use-up (solvent/solid ratio) versus extract yield (g/100g paprika). At constant temperature, the applied pressure affected oil solvating capacity of SC-CO₂ (**Figure 1**). At a constant pressure,

the extraction curves can be characterized by an initial steep linear increase of oil solubility as a function of increased volume of solvent passing through the sample in the extractor. Then the curve reaches a plateau and approaches the maximum yield of oleoresin. The tangent of the straight line gives the equilibrium solubility of paprika oil in SC-CO₂ (g extract/100g CO₂).

As a function of pressure rises from 100 to 400 bars, the solubility of oil in SC-CO₂ repeatedly increased. The maximum yield was 5.2 g oleoresin from 100 g ground oregano. Under the used conditions the ratio of solvent/oregano needed for the complete recovery of the oil was approximately 6, which is moderate as compared to the 8-11 found for SFE of paprika using the same solvent and extraction conditions [11].

Figure 1 also shows the extraction curve of oregano oil by propane at sub-critical conditions. The relationship between yield and solvent use could be characterised by a steep linear increase followed by a steady state to give the maximum yield. From the tangent of the linear relationship, the initial equilibrium solubility was estimated to be 5.1 g extract/100 g propane. This value is well above the 2.8 found with CO₂ at 35°C and 400 bars. The extraction curves at 30 and 50 bars run close to each other indicating that complete extraction of oleoresin by propane can be approached even at low pressures. The maximum oleoresin yield with propane was 5.8g/100 g paprika. The considerable difference between SC-CO₂ and sub-critical propane was in the solvent use, which was about 3 times lower with propane. In addition, great differences were found between the two types of extraction solvents in terms of color intensity of extracts.

Compositional changes on pigments

The HPLC analysis of photosynthetic pigments indicated that ground oregano contains violaxanthin, lutein, neolutein and β -carotene as the major carotenoids and chlorophylls a and b as well as their Mg-free derivatives (pheophytins) as dominant green-coloured pigments. The presence of oxidised chlorophylls, at low level, in the starting material may revealed the mild post-harvest treatments (drying) of oregano leaves.

Table 1 shows the effect of extraction conditions on the carotenoid content of oleoresins. Over the applied range of pressure, SC-CO₂ extracted hardly any of the pigments from ground oregano. With an increase of pressure from 100-400 bars, the amounts of carotenoids recovered in oil increased greatly at either temperature applied. However, the recovered amount did not exceed 50% of the initial content of carotenoids in the starting materials (ground oregano). These results indicate that solvating power of CO₂ at different conditions is much lower than the association of the different carotenoids with the tissues of oregano leaves. The highest recovery was recorded for β -carotene, whose content in the extract was about 50% of that in starting material.

As a function of pressure and temperature, all trans to cis isomerisation of carotenoid compounds (mainly lutein in the residues) was recorded. The concentration of cis isomers increased parallel to the increasing pressure irrespectively of the temperature applied. The concentration of cis lutein increased from 4 μ g/g in the starting material to 64 μ g/g in the residues after extraction at 400 bars and 35 °C. These results agree with those of Tuma and Schneider [12], who studied the changes in isomerisation of carotenoids as a function of time, pressure and temperature in a static solubility mode using SC-CO₂. The authors stated that pressure dependency of isomerisation was between 20 and 40 Mpa and at higher pressure (60 MPa) the formation of cis isomer decreased. Solubility of cis isomers of both lutein and β -carotene in SC-CO₂ was improved by increasing the extraction temperature to 55°C.

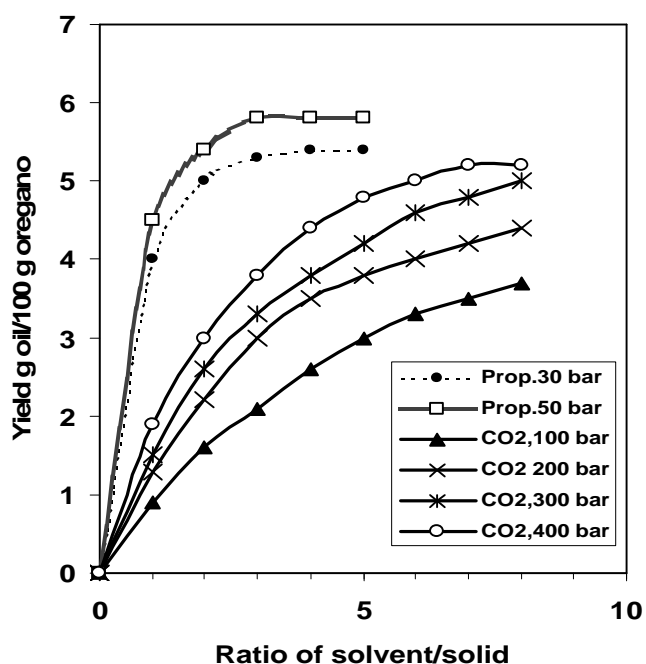


Figure 1. Extraction of oregano with SC-CO₂ at 35°C and sub-critical propane at 25°C

Table 1: Effect of extraction conditions on the carotenoid content of oleoresin and residues from ground oregano.

Extraction conditions			Carotenoid concentration mg/g powder				
<i>P. bar</i>	<i>Temp. °C</i>	<i>Solvent</i>	<i>Violaxanthin</i>	<i>Lutein</i>	<i>Cis-lutein</i>	<i>b-carotene</i>	<i>Cis-b-carotene</i>
In residues							
100	35°C	CO ₂	22	154	20	39	13
200	35°C	CO ₂	19	119	34	17	5
300	35°C	CO ₂	17	115	45	16	3
400	35°C	CO ₂	10	98	64	11	3
100	55°C	CO ₂	11	140	6	31	13
200	55°C	CO ₂	14	125	34	23	6
300	55°C	CO ₂	15	112	38	12	4
400	55°C	CO ₂	14	110	55	6	3
50	25°C	Propane	46	104	66	4	Nd
In oleoresin							
100	35°C	CO ₂	< 1	< 1	< 1	6	Tr
200	35°C	CO ₂	2	2	1	7	4
300	35°C	CO ₂	4	5	4	8	5
400	35°C	CO ₂	7	6	4	12	8
100	55°C	CO ₂	1	1	1	3	1
200	55°C	CO ₂	3	2	1	3	1
300	55°C	CO ₂	8	5	5	13	6
400	55°C	CO ₂	11	8	8	23	12
50	25°C	Propane	1	1	1	30	7
Control *			49	178	4	46	15

Nd = not detected, Tr = traces

*The control was prepared by extracting the ground oregano by a mixture of 1,2-di-chloroethane-acetone-methanol (2:1:1).

Table 2: Effect of extraction conditions on the chlorophyll content ($\mu\text{g/g}$ powder) of residues and oleoresin from ground oregano

Pigments	SC-CO ₂								Propane	Control*
	35°C				55°C					
	100 bar	200 bar	300 bar	400 bar	100 bar	200 bar	300 bar	400 bar		
In residues										
Phytol-free	6.1	8.2	9.5	15.0	3.8	10.2	13.1	9.2	11.2	4.2
Oxidised Ch.b	2.6	12.0	12.6	14.0	1.4	6.2	7.7	7.5	7.1	1.1
Ch.b	37.2	13.8	13.6	4.4	9.1	17.2	17.5	28.1	11.2	41.3
Oxidised Ch.a	6.6	21.1	29.2	67.3	3.5	19.6	16.1	15.2	42.3	6.3
Ch.a	64.0	53.5	49.6	32.4	11.2	50.4	59.2	51.0	42.5	75.1
Ch.á	2.1	3.3	4.6	5.0	0.4	3.0	2.9	3.3	3.5	2.8
Oxidised Ph.b	7.2	5.4	5.1	3.8	8.3	7.5	6.5	6.1	4.2	8.0
Ph.b	13.4	12.9	11.9	10.5	13.3	12.4	12.8	12.3	10.3	15.0
Oxidised Ph.a	9.6	9.1	7.9	6.5	4.5	9.0	10.3	8.8	7.0	12.4
Ph.a	55.3	56.0	50.2	51.1	81.0	58.8	61.7	59.5	43.3	65.3
P.Ph.b	11.9	8.5	8.6	7.7	14.0	10.2	8.7	10.1	6.7	12.4
P.Ph.a	Nd	0.5	Nd	Nd	2.2	0.5	0.8	0.4	Nd	Nd
In oleoresin										
Phytol-free	0.1	0.2	0.3	0.3	tr	tr	tr	tr	tr	
Ch.b	tr	0.3	0.6	0.7	tr	0.6	1.6	2.4	Nd	
Ch.a	0.1	0.3	0.4	0.6	0.1	1.2	1.8	2.4	0.3	
Ph.b	0.2	0.6	0.8	1.1	0.1	0.4	1.0	1.2	1.8	
Ph.a	1.1	2.6	3.3	3.8	0.9	1.4	1.7	2.4	8.8	
P.Ph.b	0.1	0.3	0.5	0.6	0.2	0.7	1.8	2.5	1.6	
P.Ph.a	0.2	1.5	2.1	2.4	0.3	2.3	5.9	18.8	0.5	

*The control was prepared by extracting the ground oregano by a mixture of 1,2-di-chloroethane-acetone-methanol (2:1:1).

Ch. = Chlorophyll, Ph. = Pheophytin, P.Ph. = Pyropheophytin

Nd = not detected

With sub-critical propane, the pigment recovery ranged between 2.2 and 44% with the more hydrophobic fractions being more easily extracted. Like SC-CO₂, sub-critical propane promoted all trans to cis isomerisation of lutein in the residues after extraction.

Compositional changes on chlorophylls as a function of SC-CO₂ and sub-critical propane extraction are shown in **Table 2**. At 35°C, there was a remarkable increase in the content of oxidised chlorophylls and phytyl-free derivatives in the residues after SC-CO₂ and sub-critical propane extractions. The artefact formation was proportional to the increase in the extraction pressure. The same held true for extraction with SC-CO₂ at 55°C except that the maximum level of the artefacts was at pressure of 200-300 bars. Also, epimerisation of chlorophyll a was activated to some extent particularly at high pressures.

In oil, even with propane extraction, traces or small quantities of chlorophylls could be determined. The major pigment was composed of mainly pheophytins, of which pyropheophytin a was formed and recovered during the extraction process. Solubility of different pheophytins in SC-CO₂ was affected by pressure and temperature of extraction. Although pyropheophytin a could not be detected in the ground oregano and propane-extracted oil, it was found at considerable concentration in oleoresin extracted by CO₂ at 55°C and high pressures. This revealed that this compound is formed with a high pressure and temperature-dependent mechanism.

It could be concluded that SC-CO₂ and sub-critical propane can hardly extract chlorophylls from ground oregano leaves. To produce chlorophyll-rich extract with minimum artefact formation from such a product it seems necessary to use suitable modifiers that assist break the chemical links between the pigment and plant tissues.

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