SOLUTE-SOLUTE AND SOLUTE-MATRIX INTERACTIONS IN THE SUPERCRITICAL FLUID EXTRACTION FROM PLANTS

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Phase equilibrium controls supercritical fluid extraction from plants in the first extraction period when mass transfer resistance is low; this period is decisive for the economics of the process. We have studied phase equilibrium during the CO_2 extraction of different compounds from several plants and in this work we present the results for the extraction from stinging nettle (*Urtica dioica*) leaves and roots. The solutes occurr in the solid phase in two forms, as free solute and as solute interacting with matrix. According to their polarity they form two groups, the polar compounds and the low-polar compounds that prevail in the extract due to the non-polar character of the solvent. Phase behaviour of major components practically is not affected by the presence of minor components. Equilibrium fluid-phase concentration of the examined minor low-polar component was, surprisingly, dependent on the equilibrium of major component rather than on the solubility of the pure component in CO_2. On the other hand, the equilibrium fluid-phase concentration of minor polar components was most dependent on the solvent composition (we used ethanol in different concentrations as carbon dioxide modifier) and on the extraction temperature, and it was independent of the phase equilibrium of major, low-polar components.

The consequence for the supercritical fluid extraction was that the extraction of minor low-polar components was synchronised with the extraction of major components, it was relatively fast, and the percentage of minor low-polar components in extract could not be significantly varied by changes in extraction pressure and temperature. The extraction of polar components was slower than that of low-polar compounds. It could be, however, as well as their percentage in the extract, controlled in a wide range primarily by changes in the ethanol concentration in the solvent and in the extraction temperature.

INTRODUCTION

In supercritical fluid extraction from plants the driving force is the difference between the concentration in the bulk of supercritical solvent and the equilibrium concentration at the solvent-solid interface. Phase equilibrium controls the extraction process particularly in the first extraction period when mass transfer resistance is low, and this period is decisive for the process economics. As the extracted plants consist of many components, usually hundreds of them, which are present in the plant in a wide range of concentrations from tens percent to trace amounts, phase equilibrium is being established in a complex multi-component system. The equilibrium of a compound extracted from plant may be affected by matrix, it is by the insoluble part of the plant, and/or by co-extracted compounds. Its equilibrium fluid-phase concentration may therefore substantially differ from its solubility measured in a two-component system compound + solvent, though the same pressure and temperature are applied.

In our studies on extraction of phytocompounds with dense carbon dioxide we focussed attention on these aspects of equilibrium. The extraction operating conditions were adjusted to measure fluid-phase concentrations at equilibrium or very close to equilibrium. In interpretation of experimental concentrations and their changes in the course of extraction we distinguish between major and minor components and between low-polar and polar compounds. Effect of ethanol added to the solvent as a modifier is studied, too.

EXPERIMENTAL

Material. Air-dried stinging nettle parts (leaves or roots) were ground and sieved, and the fraction of 0.2-0.4 mm size was used in extraction experiments. Most of the experimental runs were performed after several months of storage of the ground material. Carbon dioxide in the quality for food industry and ethanol for spectroscopy were used.

Equipment (see Figure 1). CO_2 and ethanol were pumped into the equipment independently and mixed before they entered the extractor of 12 ml volume filled with 1-4 g of ground nettle placed between the layers of glass beads serving as solvent flow distributor. The solution leaving the extractor was depressurised in the valve (9) and the extract was collected in pre-weighed glass trap (10). By switching the valve (8) the flow of the solvent through the extractor was interrupted, and before a sample of extract was taken by changing the trap (10) for an empty one, the valve (9) and connecting line to trap (10) were rinsed with ethanol.

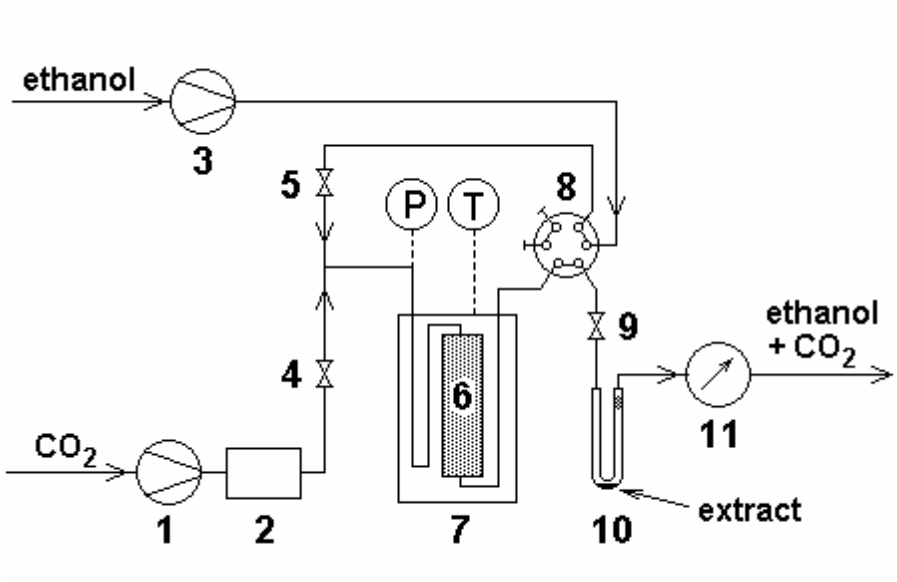

Figure 1 : Scheme of experimental set-up. (1) Compressor, (2) pressure regulator, (3) high-pressure pump, (4), (5) shut-off valves, (6) extractor, (7) water bath, (8) six-port valve, (9) heated micrometer valve, (10) trap, (11) gas meter.

The operating conditions were extraction temperature of 45-60 °C for supercritical solvent and 25 °C for liquid solvent, extraction pressure of 20-28 MPa for supercritical solvent and 10-25 MPa for liquid solvent, and ethanol concentration in the solvent of 0-9.4 wt. %. The solvent flow rate was 0.5 g/min.

The extract samples were analysed for pigments β-carotene, lutein, and chlorophylls a and b in leaves and for β-sitosterol and scopoletin in roots. Extraction curves were plotted as

the extraction yield, *e*, g/g dry mass, versus the specific amount of the solvent passed through the extractor *q*, g/g dry mass.

When plotted in one graph, the extraction curves measured at the same operating conditions but with different charges of extracted material in the extractor overlapped. It is a proof that equilibrium was established at the extractor outlet and the fluid-phase equilibrium concentration can be read from the slope of extraction curves. The quick achievement of equilibrium is a result of fast mass transfer from the particle core to its surface, which is enabled by breaking cell walls during the vegetable material grinding to very small particles.

EQUILIBRIUM RELATIONSHIP

To describe the phase equilibrim of both free solute and the solute interacting with matrix, we apply the relationship proposed by Perrut et al. [1]

$$y^*(x) = y_s \text{ for } x > x_t; \quad y^*(x) = Kx_t \text{ for } x \le x_t; \quad Kx_t < y_s, \tag{1}$$

where x is the solid-phase concentration, g/g dry mass, and y^* is the equilibrium fluid-phase concentration, g/g solvent. A discontinuity exists (see Figure 2) at the transition concentration, x_t, which is equal to matrix capacity for interaction with the solute. At solid-phase concentrations lower than x_t all solute interacts with matrix and phase equilibrium is determined by partition coefficient, K. At the concentrations higher than x_t the solid phase contains also free solute whose equilibrium fluid-phase concentration is equal to the solubility, y_s. Letters A-D indicates four regions of initial equilibrium concentrations corresponding to four types of extraction curves. The extraction curves measured in this work were of type B; their slopes indicated in the first part solubility of free solute and in the second part partition coefficient for the solute interacting with matrix.

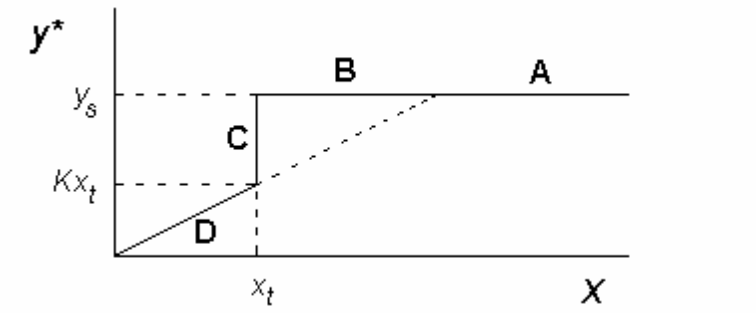

Figure 2 : Phase equilibrium relationship [1]

EXTRACTION OF OLEORESIN

The major components in the extract from leaves are cuticular waxes. The equilibrium extraction curves measured for oleoresin (total extract) from stinging nettle leaves in this work are compared in Figure 3 with equilibrium extraction curves for cuticular waxes from peppermint leaves at similar operating conditions [2]. The curves for waxes consist of the first section of the slope 0.63-0.73 g/kg, which corresponds to the solubility of free waxes in CO_2, y_s, and the second section of the slope 0.025-0.030 g/kg, which corresponds to the solubility of waxes interacting with matrix. The second slope is, on the assumption of plug flow, equal to Kx_t. It is evident that the slopes of the stinging nettle curves are practically identical with those measured for peppermint waxes, except for the short initial section of fast extraction of a compound that is present in nettle leaves in the concentration of 0.35 g/100 g matrix. The

compound is very probably fatty oil, whose CO_2 extraction from stinging nettle leaves was examined by Rafajlovska et al. [3]. Figure 3 shows also the yields from freshly ground stinging nettle leaves that are increased by volatile compounds, missing in the ground leaves after several months of storage.

The extraction curves from stinging nettle roots consisted of two sections. The first section had the slope of approximately 2 g/kg corresponding to the solubility of free solute and the second section was a curved line corresponding to the extraction of solute interacting with matrix, when intensive axial mixing occurs in the extraction layer.

In the examined range of temperatures and pressures the slopes of oleoresin extraction curves varied only slightly, increasing with increasing temperature and to a smaller extent with increasing pressure. When ethanol was added to the solvent, the total extraction yield was higher due to co-extracted polar compounds, but the shape of extraction curves remained unchanged.

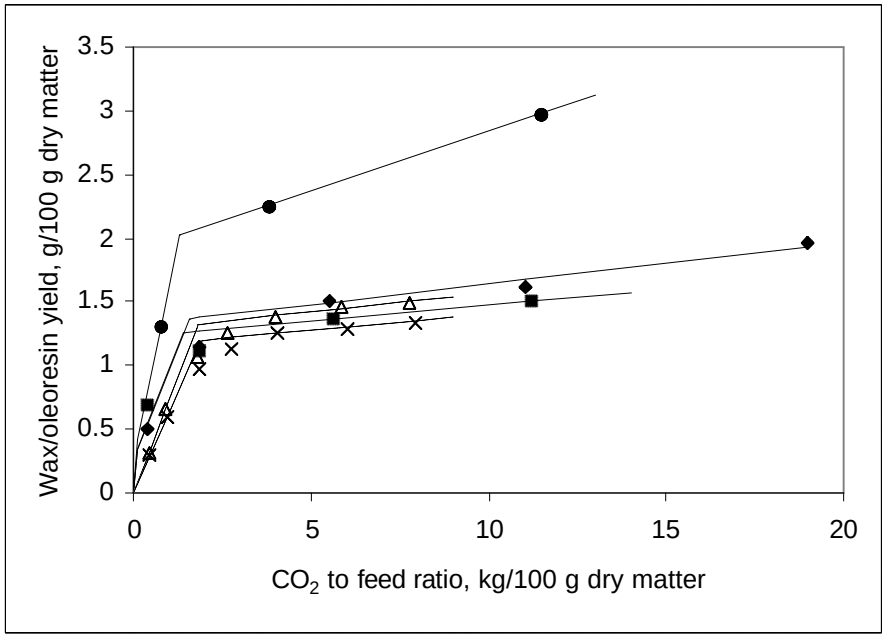

Figure 3 : Extraction from leaves with pure CO_2 at 40 °C. Oleoresin from stinging nettle leaves, 28 MPa, this work: (■) 0 % ethanol; (◆) 4.3 % ethanol; (●) initial experiment with the leaves containing volatile compounds, 4.3 % ethanol; (——**)** fitted curves. Waxes from peppermint leaves, pure CO_2 [2]: (×) 24.5 MPa; (△) 30 MPa; (----) fitted curves.

EXTRACTION OF MINOR LOW-POLAR COMPOUNDS

The non-polar and low-polar compounds whose extraction was examined were β-carotene from leaves and β-sitosterol from roots. β-Carotene content in the leaves was 18-24 mg/100 g dry mass in dependence on the storage time. From previous work [4] we knew that its solubility in CO_2 measured in the two-component system varies considerably with extraction conditions, e.g. at 40 °C and 20 MPa it is equal to 1.6 mg/kg and at 60 °C and 28 MPa it is equal to 7.1 mg/kg. The initial rate of β-carotene extraction from leaves, however, was 7.2-9.7 mg/kg in the whole range of conditions. It was synchronised with the extraction of oleoresin, as we show in Figure 4 where the experimental data from extraction runs carried out under different operating conditions are plotted together. The shift of the straight line suggests that β-carotene is extracted very slowly in the beginning when the substance

tentatively identified as fatty oil is co-extracted. Then it is extracted together with cuticular waxes at constant mass ratio 9.3:(1000-9.3), which is either equal or almost equal to the initial ratio of both substances in the leaves. When the oleoresin extraction yield is close to 1.3 g/100 g dry mass, the extraction of oleoresin and β-carotene is slowed down simultaneously and thus no change is visible on the graph. Thus, cuticular waxes dissolved in carbon dioxide act as β-carotene entrainer. When the extraction of β-carotene is finished, the extraction of oleoresin slowly continues, especially at higher ethanol concentrations in the solvent, but this fraction of oleoresin may be composed of different compounds than cuticular waxes.

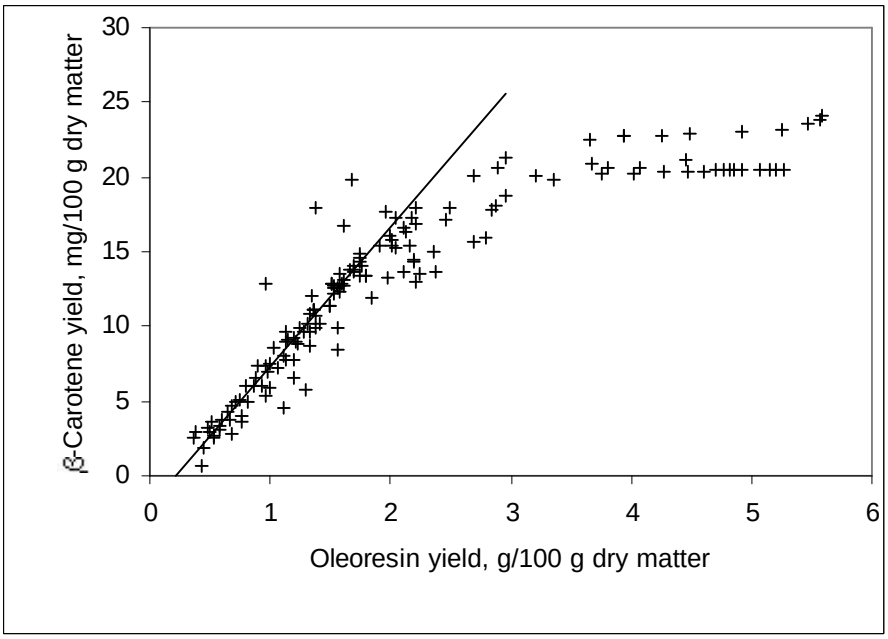

Figure 4 : Corelation of b**-carotene and oleoresin yields from stinging nettle leaves.**
(+) Experimental data, 20-28 MPa, 25-60 °C, 0-7.1 wt.% ethanol in CO_2; (——) fitted curve of the slope 9.3 mg/g.

A similar synchronisation was observed in the extraction of β-sitosterol and oleoresin from stinging nettle roots. β-Sitosterol content in the roots was 62.5 mg/100 g dry mass. The relationship between the extraction yield of oleoresin, e_o, g/100 g dry mass, and the extraction yield of β-sitosterol, e_s, g/100 g dry mass, was

$$e_o = 5.3 f_e\, e_s + 0.18, \qquad (2)$$

where the increase in oleoresin yield in the presence of ethanol is expressed by the factor f_e that increases from $f_e = 1$ for pure CO_2 to $f_e = 2$ for 9.4 wt.% of ethanol in CO_2. This time, the main part of oleoresin contains, beside the component that interacts with β-sitosterol, an inert easily soluble component whose content in the root is 0.18 g/100 g dry mass.

EXTRACTION OF MINOR POLAR COMPOUNDS

The examined polar compounds were lutein and chlorophylls a and b from the leaves and scopoletin from the roots of stinging nettle. The extraction of any of them was not synchronised with the extraction of oleoresin; the transition from the faster extraction of free

solute to the slower extraction of the solute interacting with matrix occurred much later for polar compounds than for oleoresin. Besides, the equilibrium relationship was, except for lutein, of a more complicated form than direct proportionality according to equation (1). The solubility of free solute measured in the first part of extraction curve varied between 1.2 and 11.2 mg/kg for lutein, 0.2 and 10.9 mg/kg for chlorophyll a, 0.2 and 16.4 mg/kg for chlorophyll b, and 2 and 6.5 g/kg for scopoletin. The lowest solubility values relate to pure CO_2; with ethanol modifier the solubility steeply increases (for lutein, which content in the leaves was 39 mg/100 g dry mass, see Figure 5). Thus, the percentage of polar compounds in the extract could easily be controlled by changing the ethanol concentration in CO_2, and also by changes in extraction temperature and pressure.

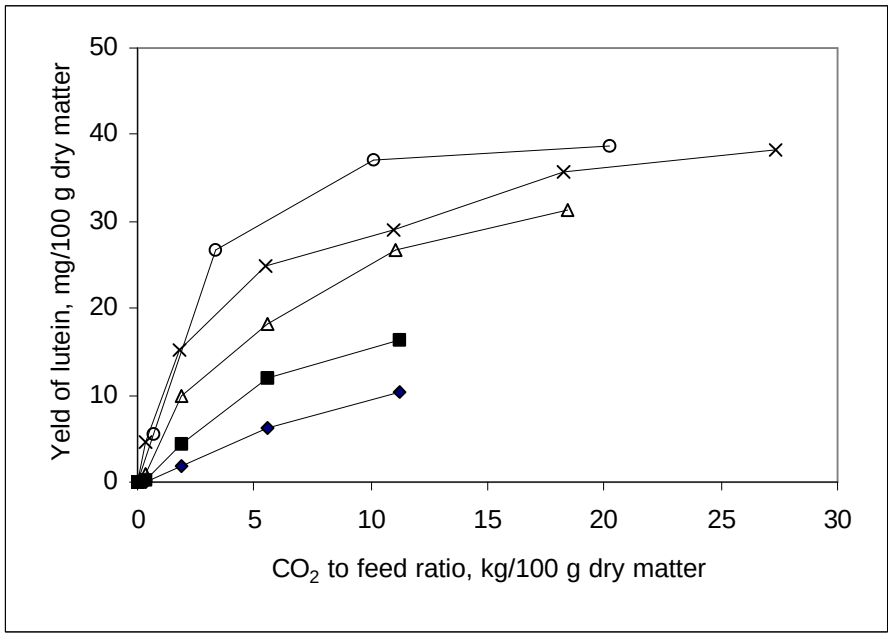

Figure 5 : Effect of the solvent modifier on the extraction of lutein at 40 ºC and 28 MPa.
Ethanol conc. in CO_2: (◆) 0 wt.%; (v) 3.0 wt.%; (△) 4.4 wt.%; (5) 5.7 wt.%; (μ) 7.1 wt.%.

ACKNOWLEDGEMENTS

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