

# CONCENTRATION OF COLOUR COMPONENTS IN CHILLI PEPPER AND PAPRIKA OLEORESINS WITH COLUMN CHROMATOGRAPHY AND SUPERCRITICAL FLUIDS

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Paprika (*Capsicum annum*) is one of the oldest and most important natural red colourants used as oleoresin (extract) in foodstuffs industry. Paprika differs from chilli pepper in the concentration of pungent (capsaicinoids) and colour components (carotenoids), which directly influence the quality and commercial value of a great number of food ingredients. The scope of our study was to investigate the possibility of increasing the colour intensity (CU value) of paprika oleoresin using column chromatography with different solid adsorbents at atmospheric conditions, as well as its combination with high pressure extraction of raw material and oleoresins of paprika and chilli pepper. Phase equilibria of system chilli pepper oleoresin-CO<sub>2</sub> were investigated and distribution coefficients of capsaicinoids were calculated. The results are given in terms of separation of pungent components from oleoresin.

## INTRODUCTION

Paprika and most chillies used for spice powder production are cultivators of the *Capsicum annum* species, which are utilised for their colour and flavour; the main difference between them lies in the fact that paprika is more strongly coloured but with no or only little pungency. The pungent principles are group of amides, capsaicinoids (nordihydrocapsaicin, capsaicin, dihydrocapsaicin), which are present from 0.12 to 1.69 % in chilli pepper. Capsaicin is the most abundant among these, and is used in stimulating medicines and in food preparations. Total colour capacity of capsicum raw material is given in internationally recognised units for extractable colour, ASTA (American Spice Trade Association) value. Paprika oleoresin is a liquid extract (viscous oil) with a deep red colour and typical aroma of paprika. Its colour is the most important asset from the commercial point of view and depends on the concentration of the contained pigments. Evaluation is assessed in accordance with its colour units (CU), which vary up to 350000 CU. However, chilli pepper oleoresin contains besides pigments a great deal of pungent principles (capsaicinoids), whose concentration is limited due to its usage in foodstuffs. A separation of pungent principles from chilli pepper oleoresin is therefore necessary.

In the traditional way, capsicum fruits are extracted with hot organic solvents (hexane, acetone, benzene, methilen chloride) or as an alternative, due to the lypophilic nature of carotenoids, with supercritical CO<sub>2</sub> and subcritical propane [1-6]. Designing a high-pressure extraction requires phase equilibria data on compounds of interest [7-11].

This study is divided into five different experimental set-ups. Preliminary research work was performed employing high-pressure extraction of chilli pepper with SC-CO<sub>2</sub> in order to determine optimal extraction conditions in terms of efficiency of isolating colour and pungent components. In order to concentrate the colour components in oleoresin, set of experiments was conducted using column chromatography at atmospheric conditions (different solid adsorbents) and continuous high-pressure extraction with SC CO<sub>2</sub>, as well as the combination

of both. Additionally, phase equilibria data were determined for the system chilli pepper oleoresin-CO<sub>2</sub>. On the basis of analysing samples of liquid and gaseous phase distribution coefficients of capsaicinoids were calculated.

## **MATERIALS AND METHODS**

Chilli pepper (0.211% capsaicinoids, 61 ASTA) and paprika oleoresin (76000 and 80000 CU) was donated by Raps GmbH & Co KG (Kulmbach, Germany). Chilli pepper oleoresin (15400 CU, 0.521% capsaicinoids) was supplied by Natex (Ternitz, Austria). All solvents used for analytical purposes were purchased from Merck (Germany). Carbon dioxide (2.5%) and propane (2.5) were purchased from Messer, Ruše (Slovenia) and Linde, Celje (Slovenia), respectively

### **Analytics**

Colour capacity of raw material and oleoresin was expressed in ASTA and CU value, respectively. These were determined by an UV spectrophotometric analytical method [12,13]. The HPLC method for quantifying capsaicinoids (nordihydrocapsaicin-NDC, capsaicin-C, and dihydrocapsaicin-DC) in chilli pepper samples and required reference substance was supplied by Raps GmbH & Co. KG [14].

### **Extraction of chilli pepper with CO<sub>2</sub>**

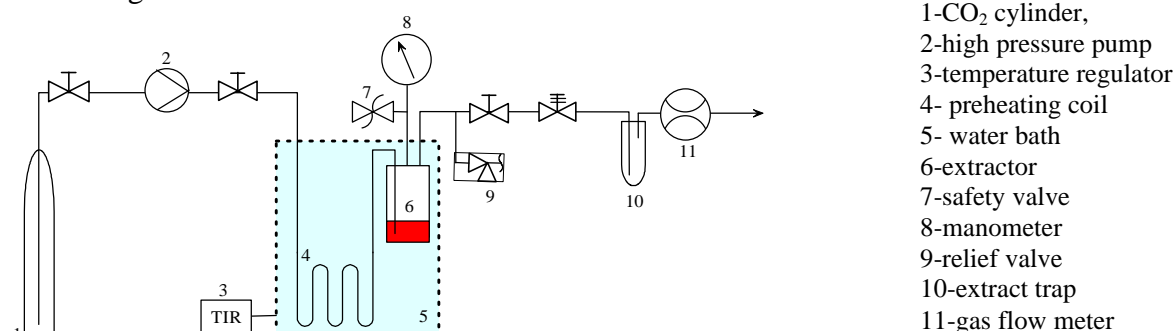
Ground chilli pepper was extracted with CO<sub>2</sub> in a laboratory scale high-pressure flow-up apparatus at temperatures of 40, 60, 80 °C and pressures 100, 200, 300, 400 bar [15]. The results showed that chilli pepper oleoresin with a medium pungency and colour intensity was obtained. The total yield of extraction was determined to be highest at 400 bar and 80 °C at 12.8%, and lowest at 100 bar and 60 °C at 0.10%. The highest CU value of obtained chilli pepper extract was 15000 CU. The yield of extraction of capsaicinoids was in the range of 90-95% when operating at conditions where the density of CO<sub>2</sub> is above 750 kg/m<sup>3</sup>. Hereby, the efficiency of extraction of colour components was above 70%. At 100 bar and 40°C, the CU value of extract was 268 CU, where least colour components were extracted. Total yield of extraction was however 8.0% and the oleoresin contained mainly aromatic components (yellow colour). The extraction efficiency of capsaicinoids was 66%. These extraction conditions were taken as optimal among investigated for further experiments of separating colour and pungent (or aromatic) components from oleoresins.

### **Concentration of paprika oleoresin with CO<sub>2</sub> and propane**

10 g of paprika oleoresin with colour intensity value of 76000 CU was placed in a 60 mL extractor and continuously extracted with CO<sub>2</sub> at 100 bar and 40°C. Liquefied CO<sub>2</sub> was passed through a preheating coil thermostated at 40°C and into the liquid phase (oleoresin), from where the upper phase was passed through a needle valve, enabling fine regulation of flow rate (approx. 0.02 kg CO<sub>2</sub>/h), and extract collected in a separator at atmospheric conditions (Figure 1). The remaining oleoresin was analysed on CU value, while the extract contained mainly aromatic compounds and was yellow to light orange in colour. The extraction yield was below 5%.

The results of continuous extraction of paprika oleoresin showed that the CU value of oleoresin was increased from 76000 to 87300 by using 1.95 g CO<sub>2</sub>/g oleoresin and up to 89000 CU when using at least 3.5 g CO<sub>2</sub> per g of paprika oleoresin. The CU value of oleoresin processed with 6.30 g CO<sub>2</sub>/g oleoresin remained at approx. 89000 CU, whereas higher amounts of CO<sub>2</sub> for extraction were not investigated. The same procedure was however repeated using propane as solvent in two extraction runs (90 bar at 40°C, and 30 bar at 30°C) and paprika oleoresin with a lower CU value (approx. 35000 CU). The flow rate of

propane could not have been maintained at such a low value. Unlike CO<sub>2</sub>, propane extracted the colour components, but however, the CU value of propane extract and remaining paprika oleoresin did not differ significantly. Further investigation using propane as solvent for increasing CU value of oleoresin still needs to be covered.



**Figure 1:** Apparatus for high-pressure extraction of paprika oleoresin

### Column chromatography of paprika oleoresin

Column chromatography of paprika oleoresin was performed in a glass column of diameter 2.5 cm, which was filled with solid adsorbent (silicagel 60, xonofill, zeolite) in methanol. Washing of adsorbent containing concentrated pigments was done with acetone. CU value of methanol and acetone fractions was measured and different amounts of solvents for elution were investigated (Table 1). The amount of methanol used was in the range from 0.65 to 2.5 L for 10 g of oleoresin, at which the CU value was increased up to approx. 237200 CU. However, the amount of acetone used for washing the adsorbed colour components from silicagel was in the range from 300 to 450 mL during all experiments. Using excessive methanol (10 L for 5 g of oleoresin) the CU value increased to approx. 254100, indicating that this is the maximum CU value that could be obtained with this column chromatography setup using paprika oleoresin with starting CU value of 76000 (80000). The total yield of oleoresin fraction obtained with acetone during the highest concentration (254100 CU) was 10%.

**Table 1:** Results of column chromatography of paprika oleoresin on different adsorbents

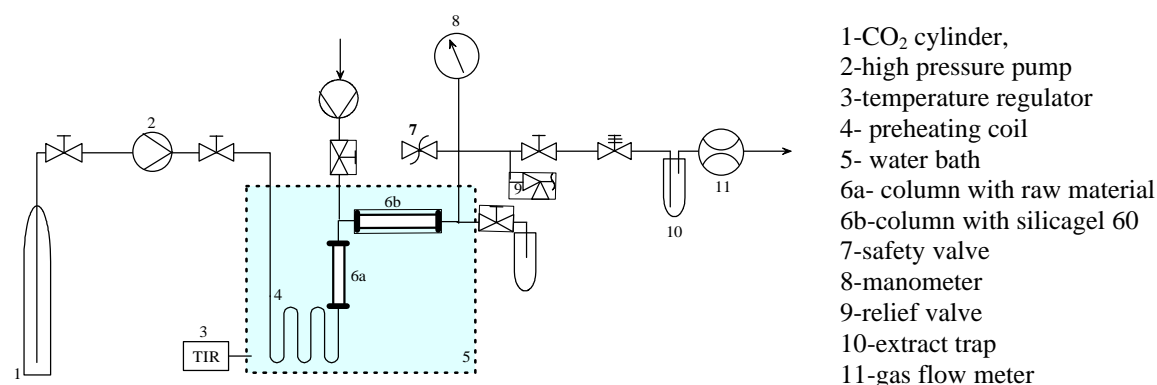
Mass of oleoresin (g)	Starting CU value	Adsorbent	Methanol fraction		Acetone fraction	
			(L)	CU	(L)	CU
5	76000	Xonofill	5.00	66000	0.40	31904
5	76000	Zeolite	10.00	34166	0.40	212395
5	76000	Silicagel 60	10.00	63492	0.30	254093
10	80000	Silicagel 60	2.50	51051	0.30	237185
10	80000	Silicagel 60	1.30	48299	0.40	141900
10	80000	Silicagel 60	1.80	49691	0.40	143273
10	80000	Silicagel 60	0.65	34808	0.45	95258

The choice of silicagel as adsorbent for colour components, in order to increase the CU value of oleoresin was the basis for further experimental combination of high-pressure extraction and column chromatography experiments.

### High-pressure extraction-column chromatography of paprika and chilli pepper

The extractor from figure was replaced with two consecutively connected high-pressure columns (Figure 3). The first column (4.6x100 mm) was filled with 1 g of raw material (paprika with 145 ASTA) and the second column (4.6x250 mm) with silica gel 60. Operating conditions were 100 bar and 40 °C. Flow rate of CO<sub>2</sub> was maintained at approx. 0,2 kg CO<sub>2</sub>/h. The extract was collected at atmospheric conditions and the released CO<sub>2</sub> measured with gas flow meter. The amount of CO<sub>2</sub> used for extraction was 500 g CO<sub>2</sub> per g of raw material.

After each extraction run, the column containing silicagel was washed with acetone by a HPLC pump. Set of experiments were also conducted with the single column filled with raw material, thus comparing the results obtained with extraction and combination of extraction-column chromatography.

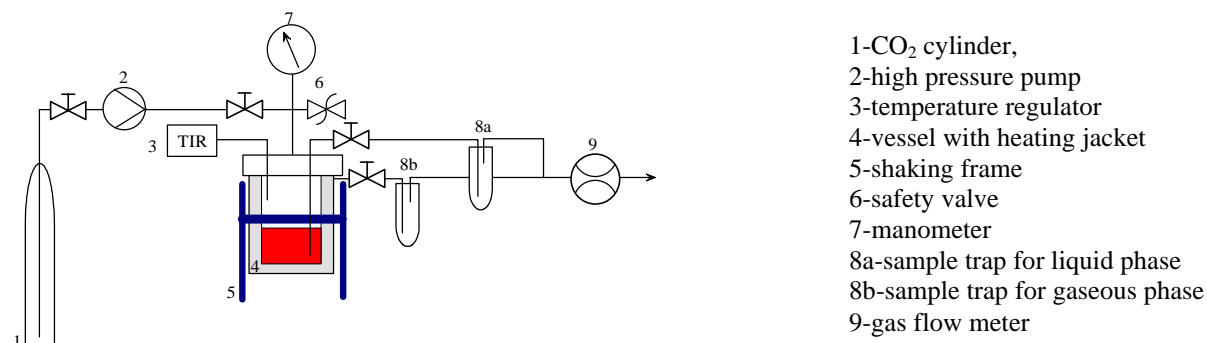


**Figure 2:** Apparatus for high-pressure extraction-column chromatography of paprika

This set of experiments showed that a combination of high-pressure extraction-column chromatography of paprika produced oleoresin with 30632 CU value in comparison with single extraction step where an oleoresin with 12125 CU value was obtained. The extraction efficiency of colour components was in the range of 90%. The extraction yield on extract mass basis was 5.2% for the single extraction step, 11.2 % (2.1 % extraction, 9.1% column chromatography) for the combination of high-pressure extraction-column chromatography. Same set of experiment was repeated using commercial chilli pepper (112 ASTA and 0.01 % capsaicinoids). It was observed that the extraction efficiency of colour components was only 2%, and oleoresins with 1396 and 3850 CU were obtained.

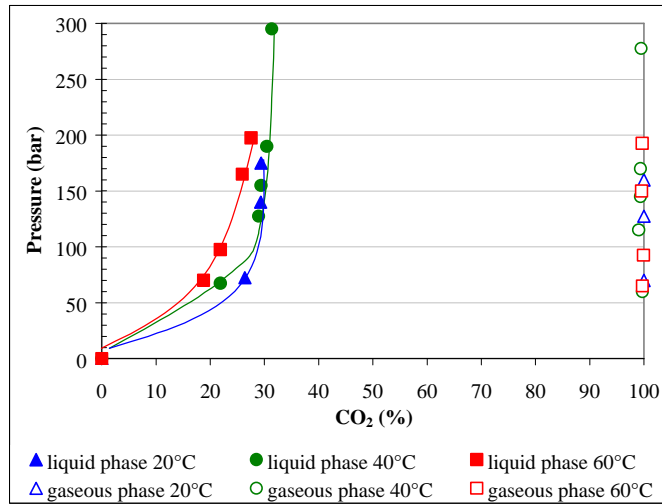
### Phase equilibria of chilli pepper oleoresin – CO<sub>2</sub>

Phase equilibria of chilli pepper oleoresin (0.521 % capsaicinoids and 15400 CU) and CO<sub>2</sub> were investigated using a static-analytic method. The apparatus is shown in figure 3 and it consists of a 0.5 L autoclave fixed onto a frame, where its content was mixed via a mechanical oscillating device. The autoclave was filled with 100 g of chilli pepper oleoresin, and samples of gaseous and liquid phase taken at temperatures 20, 40 and 60 °C and in pressure range from 50 to 300 bar. The frame was oscillated for 1 hour during which equilibrium was reached, and the frame was kept in a vertical position for 1 hour before sampling. The samples of both phases were analysed on capsaicinoids, while the liquid phase was additionally analysed on CU value. The results are shown in figure 5 and 6.



**Figure 3:** Apparatus for phase equilibria determinations of chilli pepper oleoresin-CO<sub>2</sub>

The solubility of CO<sub>2</sub> in chilli pepper oleoresin increased with pressure to approx. 30 % at 20 and 40°C whereas at 60°C to 26 % at pressures above 125 bar. The solubility of CO<sub>2</sub> in oleoresin decreased with temperature.

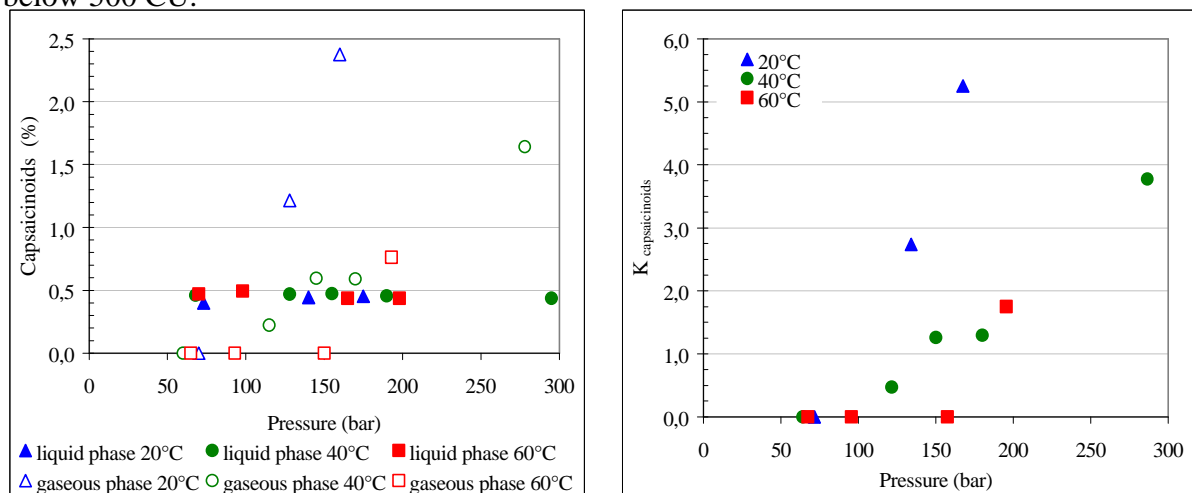


**Figure 5:** Phase equilibria of system chilli pepper oleoresin-CO<sub>2</sub>

The concentration of capsaicinoids in the liquid phase varied in the range from 0.40 to 0.49%, whereas in the gaseous phase it varied from 0 to 2.38% (Figure 6a). Comparing the concentration of capsaicinoids in liquid phase to the starting oleoresin (0.521 % capsaicinoids) it was seen that it practically remained constant around 0.45%. The distribution coefficients of capsaicinoids on a solvent free weight fraction basis were calculated as:

$$K = \frac{w_g}{w_l} \quad (1)$$

where  $w_g$  and  $w_l$  is the concentration of total capsaicinoids (%) in the gaseous and liquid phase, respectively. Hence the distribution coefficient of capsaicinoids varies from 0 to 5.25 (Figure 6b), and it has an increasing trend with pressure and decreasing trend with temperature. At 20°C, where CO<sub>2</sub> is a liquid, a selective separation of capsaicinoids from chilli pepper oleoresin can be established. The CU values of liquid phase samples varied from approx. 14600 to 19300 CU. It has to be taken into account that the standard deviation value for CU analytics was 5%, thus the CU values were in the range of experimental mistake. It was therefore determined that the increase of CU value of liquid phase samples have not occurred at employed conditions. The CU value of gaseous phase was approximated to be below 500 CU.



**Figure 6:** a) Concentration of capsaicinoids in liquid and gaseous phase samples for system chilli pepper oleoresin-CO<sub>2</sub>; b) distribution coefficient of capsaicinoids, as a function of pressure

The remaining chilli pepper oleoresin in the autoclave was analysed on the capsaicinoids in order to determine how much pungent principles were actually extracted during the phase equilibria investigation experiment. The concentration of capsaicinoids decreased by 6.5 % in overall. The CU value decreased from 15400 to 14841 CU.

## CONCLUSION

Concentration of colour components in paprika oleoresin in terms of CU value increase was also achieved using a thin film extractor [16] and countercurrent column extraction [17]. The aim of this study was to search for another way of achieving the CU value increase in capsicum oleoresins by downstream processing. It was hereby shown that a column chromatography on silicagel, as well as its combination with high-pressure extraction of raw material gives promising results. On the basis of phase equilibria data obtained in this study, it was determined that a selective extraction of capsaicinoids occurs while processing chilli pepper oleoresin with subcritical CO<sub>2</sub>. Extraction of capsicum fruits with subcritical CO<sub>2</sub> has not yet been published and it gives an interesting insight to the isolation of pungent as well as aromatic compounds.

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