Thesis for the PhD degree

Recovery of Active Ingredients from Plants by Supercritical Fluid Extraction

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INTRODUCTION AND AIMS

Food, pharmaceutical and cosmetic industries require more and more ingredients of natural origin. Supercritical fluid extraction (SFE) provides an unique possibility for recovery valuable plant extracts in comparison with traditional separation methods (steam distillation, solvent extraction with ethylene glycol, alcohol, hexane). Solubilities and selectivities in supercritical fluids can modify easily by extraction pressure and/or temperature. Only the desired components are dissolved from starting material at suitable pressure and temperature parameters. The solubiltiy can be also improved by adding entrainers (alcohol, water, acetone, hexane) into supercritical solvent. Solvent separated from the extract can be recycled. There is not any trace of solvent in the extracts obtained by supercritical fluid extraction, while the main problem by solvent extraction is the organic solvent residue in products. SFE is carried out at near room temperature so thermolabile constituents can be extracted without any damage and decomposition, and extracts preserve natural composition of plants in contrast to a boiling operation. Nowadays supercritical fluid extraction is economical mainly in production of valuable materials (unsaturated fatty acids, coffeine, etc), and in combination several oparation steps (extraction and purification of vegetable oils). SFE extracts are of high quality and expensive basic materials of pharmaceutical and cosmetic preparations. In general CO_2 is used as solvent in supercritical fluid extraction because it is environmentally safe, non-toxic, clear, odourless and non-flammable.

The aims of this dissertation are to show the applicability of supercritical fluid extraction for recovery of different kinds of products from plant materials, to give a method to map the effects of pressure and temperature on the extraction yield and yield of a selected active ingredient, and to describe the extraction curves by means of appropriate mathematical models.

EXPERIMENTAL MATERIALS AND METHODS

Plant materials were purchased from market and from theirs cultivation places.

CO_2 of 95-96 w/w% purity was used as supercritical solvent. Extractions were carried out in pilot-scale and small-production plants. Suitable ranges of operation parameters (pressure, temperature) for extractions were determined. The effects of the extraction parameters on the yield and the yield of a selected active ingredient were examined by 3^2 full factorial designed experiments.

Plant ingredients were separated by gradual increasing of extraction pressure or fractionation by using two separators connected in series. Adding ethyl alcohol into CO_2 the effects of the entrainer on the solubility of oil in CO_2 and extraction rates were examined.

Traditional separation methods (steam distillation, solvent extraction) were performed to compare those to supercritical fluid extraction. Ethyl alcohol and hexane of analytical grade were used for solvent extractions.

Moisture content and characteristic distributions of particle sizes of plant samples were determined.

Identification and quantitative determination of active components present in the products were carried out. Volatile constituents in supercritical extracts and essential oils were analysed by GC and GC-MS methods. Non-volatile components were examined by TLC-densitometry, MPLC, HPLC and GC-MS techniques. Fatty oils were analyzed by SFC, HPLC and GC. Emulsifying, foaming and absorption properties of meals remaining after extraction of vegetable oil and those of protein isolates were determined.

Results obtained by pilot-scale apparatus were used to design extractions in small-production one. Considerable amounts of extracts were produced by both units for further application experiments.

SUMMARIES OF THE NEW SCIENTIFIC RESULTS

1. Pale-yellow, essential oil rich extract with oily feature was obtained at low extraction pressure (100 bar) from freshly harvested finely cut flowering tops of the clary sage (*Salvia sclarea* L.). Appearance of oily CO_2 product differed from that of essential oil. There were relevant differences in compositions of two products: i) The ratio of lynalil acetate to linalool was more higher (appr. 13 times) in CO_2 extracts than in distilled oil, therefore this product is of good quality. ii) Only CO_2 product was found to contain sclareol. iii) The concentration of α-terpineol was higher in distilled oil. The yield obtained by SFE (0.19 g extract/100 g fresh material) was comparable to that of obtained by steam distillaton (0.11 ml oil/100 g fresh material) [2].

2. Ingredients of greek sage (*Salvia triloba* L.) were separated by stepwise increasing of extraction pressure, and by fractionation using two separators connected in series. The quality and quantity of extract were determined by the extraction pressure to a great extent. The most volatile constituents were dissolved at low pressure, but a part of less volatile ones became soluable besides volatile components by rising the pressure and those amounts increased considerably in the extracts. Yield obtained by fractional extraction (6.74 g extract/100 g dried material) was compared to those obtained by other separating methods (steam distillation: 1.98 ml oil/100 g dried material, extraction with hexane: 6.87 g extract/100 g dried material). The composition of volatile concentrate obtained in the first step of gradual SFE (I) was very similar to that of distilled oil (II). Main components were 1,8-cineol (I: 33.1%, II: 38.9%), camphor (I: 8.1%, II: 8.4%), α-terpineol (I: 3.7%, II: 4.9%), α-pinene (I: 4.3%, II: 5.9%), β-pinene (I: 3.2%, II: 4.4%) in both products. The amount and the composition of the product collected in the second separator were determined by the pressure of the first separator[3].

3. The specific mass of CO_2 used and the extraction yield depended on the quality of the marigold (*Calendula officinalis* L.) raw material. The effects of the extraction parameters (pressure, temperature) on the yield and on the yield of selected active ingredient (faradiol) were determined by 3^2 full factorial designed experiments. Linear effect of temperature was found to be significant, linear effect of pressure was close to the usual significancy level, the quadratic effects of pressure and temperature, and the interaction between pressure and temperature were not significant on the basis of statistical assessment. The largest extraction yield and faradiol yield were obtained at the same extraction parameters (P_E = 450 bar, T_E = 60 °C). Fractional separation was carried out at constant extraction pressure. Yield obtained by SFE (5.27 g extract/100 g dried material) was comparable to that of obtained by hexane extraction (6.37 g extract/100 g

dried material), while alcohol extraction resulted a considerably higher yield (42.39 g extract/100 g dried material) due to its polar character. There were not relevant differences in the composition of the essential oil and the volatile constituents containing SFE product. Amounts of valuable faradiol (5.30 - 5.50 g/100 g extract) in the extracts obtained at different extraction parameters were slightly different. The amount of the faradiol monoester measured in CO_2 oils was two order of magnitude higher (12.00 g/100 g extract) than that in the alcoholic extract (0.10 g/100 g extract). Marigold was also extracted in a small-production plant [11].

4. Using feverfew (*Tanacetum parthenium* L.) in the supercritical fluid extractions a 3^2 full factorial design was performed to map the effects of pressure and temperature on the extraction yields and - related to that - parthenolide yields. Linear and quadratic effects of pressure, the interaction between linear effects of pressure and temperature were found to be considerably significant, linear effect of temperature and the interaction between quadratic effects of temperature and pressure were close to the usual sigficancy level on the basis of statistical assessment. The largest extraction yield (5.22 g extract/100 g dried material) and parthenolid yield (0.50 g/100 g extract) were obtained at the same SFE conditions (P_E = 400 bar, T_E = 60 °C). Different kinds of extracts were fractionated by two separators connected in series at constant extraction pressure. Yield obtained by SFE was comparable to that obtained by hexane extraction (4.29 g extract/100 g dried material), while alcohol extraction resulted many times higher yield (24.02 g extract/100 g dried material). The composition of the distilled feverfew oil was compared to that of CO_2 extract containing the volatile compounds. Camphor and chrysanthenyl acetate were the major compounds in both products [4,12].

5. Laboratory experiments with corn germ (*Zea Mays* L.) were carried out at constant pressure with supercritical CO_2 and CO_2 + alcohol solvent mixture (alcohol ranging from 0 to 10 % by weight in CO_2). The rates of extractions and specific solvent consumptions were exerted considerably by increasing the alcohol content of mixture (using 10 w/w% alcohol the specific solvent consumption reduced to tenth of the pure CO_2 consumption). Due to the increasing alcohol concentrations in CO_2 the extraction time was decreased significantly because of higher solubility of the oil. The solubility of phospholipides is very poor in pure CO_2 (0.026 g/100 g oil), but it improved considerably by increasing the alcohol concentration (0.756 g/100 g oil). Yield obtained by SFE (50.10 g oil/100 g dried material) was similar to that obtained by hexane extraction (50.13 g oil/100 g dried material). Oils produced by SFE were special delicious and of good

quality. The effects of the amount of entrainer on the composition of oils as well as on the nutritive value and functional properties of extracted meals and protein isolates were examined. There were not large differences among the values characterizing the emulsifying properties of isolates examined. It seems that there is an optimum content of lipids for emulsion formation, while the emulsion stability decreased with oil content. The capabilities for the foam formation of germ protein were good, and adding alcohol into CO_2 foaming characteristics of extracted meals improved. The water absorption of extracted meals was considerably high. Due to the increasing alcohol content in the CO_2 there was a slight effect on the water and oil absorption parameters of meals. The same trend was observed for the water absorption of the isolates [1, 9].

6. According to preliminary tests moisture and size of olive husk (*Olea europea* L.) samples had considerable effects on the extraction rates, yields and the specific consumptions of solvent at different extraction parameters. Three kinds of olive husk and seed samples were used for the experiments. CO_2 extractions were suitable for obtaining almost the total oil from seed samples, so hexane extraction performed after SFE resulted only low yield (0.16 – 0.64 g oil/100 g dried material), while in the cases of husk samples significant yield were obtained (13.46-18.69 g oil/100 g dried material). Large amounts of oils were produced by alcoholic extraction from all the samples. The main constituents were glycerides (monoolein, monolinolein), triglycerids (mainly trilinolein), and diglicerid (possibly dilinolein) in the oils. Extracts obtained at the beginning of the extraction contained high amount of monoolein, while continuing on the process the ratio of triglycerides was increased in the extracts. Major constituents were mainly triglycerides (triolein, glycerin-1,2-oleate-3-palmitate) in the samples, but contained a low amounts of glycerin-1,3-palmitate-2-oleate and oleic acid as well. There were not considerable differences between the successive extracts obtained from olive husk samples by SFE. The amounts of squalene and α-tocopherol increased slightly in the subsequent oil samples [18, 20].

7. Mathemathical models were used to describe the results obtained from SFE experiments.

   Applicability of model (1) was examined in the extraction of uniform material of small quantity:

   $$Y = Y_{¥} (1 - \exp(-kt)) \qquad (1)$$

   where Y is the amount of material obtained with the fluid phase during the time t (kg/kg), $Y_{¥}$ is the total amount of soluable material (yield obtained in infinite time) (kg/kg), k is

the mass transfer coefficient (1/s), t is the time of extraction (s). Model (1) was found to fit into the experimental results well in spite of a few experimental data.

Extractions of non-uniform materials were approached with model (2) of two parts:

$$Y = Y_{1¥} (1 - \exp(-k_1 t)) + Y_{2¥} (1 - \exp(-k_2 t)) \quad (2)$$

where $Y_{1¥}$ and $Y_{2¥}$ are the total soluable amounts of non-volatile and volatile constituents (kg/kg), k_1 and k_2 are the mass transfer coefficients (1/s), and Y is the total yield (kg/kg). The first part of equation (2) describes the extraction of the non-volatile rich fraction, while the second part shows that of the volatile rich one.

The model (2) is applicable for description of fractional separation well. It was found that $Y_{2¥}$ is in direct ratio, while $Y_{1¥}$ is in inverse ratio to the pressure of first separator related to extraction results of greek sage. That means the more volatile constituent are in the extract collected in second separator the larger $Y_{2¥}$. The mass transfer coefficients are in inverse ratio to the pressure of first separator.

Experimental results obtained from extractions of marigold and feverfew were described with the help of model (1). The same effects were found to be significant on $Y_{¥}$ values, which were significant on $Y_{measured}$ in both cases.

Model (1) was used to describe changing of valuable main constituents determined in marigold extracts with time. Values of $Y_{¥}$ differed from measured values of Y only slightly.

Applicability of model (3) in the extraction of large amount of uniform material (> 45%) was investigated:

$$\begin{aligned} &Y = b \cdot X \qquad ha \quad X \le Y'_1/b \\ &Y = Y'_1 + Y'_2 \left\{1 - \exp\left[-\frac{b}{2}\left(X - \frac{Y'_1}{b}\right)\right]\right\} \qquad ha \quad X > Y'_1/b \end{aligned} \quad (3)$$

where Y is the amount of extract (g oil/100 g dried material), Y'_1 is the amount of the free oil on the surface (g oil/100 g dried material), Y'_2 is the fixed oil inside the plant particle (g oil/100 g dried material), b is the solubility (g oil/kg solvent), and X is the specific solvent consumption (kg CO_2/kg dried material).

Model (1) was used in the extraction of lower amount of vegetable oil. The less humidity and smaller particle size of examined olive samples were, the larger $Y_{¥}$ and k were resulted [28].

UTILIZATION POSSIBILITIES OF CO_2 EXTRACTS

Clary sage product can be recommended for cosmetics and food industry because it is rich in volatile constituents. Extract containing essential oil obtained from greek sage can be used for pharmaceutical industry above all. Oily extracts of marigold and feverfew have excellent therapeutical values, therefore they are useable for pharmaceutical preparations. Due to non-volatile fatty, waxy components presenting in marigold and feverfew extracts they are able to fulfil multiple functions in the final products in cosmetics. Extracts of marigold have been used in the production of pharmaceutical and cosmetic products licensed for years by Gradiens Ltd. Corn germ oil and meal are valuable raw materials for food industry. Oil of good quality obtained from olive is usable for food industry.

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