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NON-CONVENTIONAL SUPERCRITICAL FLUIDS AS POTENTIAL SOLVENTS IN EXTRACTION PROCESSES

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Abstract

In the scientific literature the contributions about supercritical fluid phase equilibrium are discussing mostly the systems with conventional supercritical fluids, especially supercritical carbon dioxide, however also investigation of non-conventional supercritical fluids should be taken into consideration. Argon is already widely applied as a protective atmosphere for certain foodstuffs, notably fruits and vegetables, especially when deterioration of food should be slowed down. The inert behavior makes argon a good candidate for a solvent in extraction processes.

Prior to extraction experiments, solubility data of pure vanillin and ortho ethylvanillin as two most interesting compounds of vanilla pods were measured in argon at temperatures of 40 ^{0}C, 60 ^{0}C and 80 ^{0}C and at pressures up to 500 bars. The content of vanillin and ortho ethylvanillin in extracts from vanilla pods was determined after extraction with supercritical fluids; argon as a non-conventional solvent and CO2 as a well-introduced supercritical fluid at temperatures of 25 ^{0}C, 40 ^{0}C and 60 ^{0}C and at pressures of 150 bars and 300 bars. Extractions using organic solvents (conventional and Soxhlet extraction with methanol and ethanol) were performed to compare the quality of products obtained by both traditional and sustainable ways. Obtained extracts were dissolved in methanol and absorbance was measured at a wavelength of 228.5 nm (vanillin) and at a wavelength of 270 nm (OEV).

Maximal solubility of vanillin in argon is observed at a temperature of 40 ^{0}C and a pressure of 438 bars, approx. 0.015 g/g. For OEV highest solubility in argon was measured at pressures above 300 bars and at lower temperatures; about 0.5 g/g. UV analysis show high content of vanillin in extracts, obtained by extraction with supercritical fluids (up to 88 wt.% for vanillin and 76 wt.% for OEV) and 70 wt.% of vanilin and 55 wt.% of OEV in those obtained with Soxhlet or conventional extraction.

Therefore it can be concluded that supercritical fluids are way more selective solvents for vanillin's than conventional ones.

Key words: *Argon, Extraction, Vanillin, o-ethylvanilin.*

1. Introduction

In the scientific literature the contributions about supercritical fluid phase equilibrium are discussing mostly the systems with conventional supercritical fluids [1], especially supercritical carbon dioxide. However, also investigation of non-conventional supercritical fluids should be taken into consideration. Argon is chemically inert under most conditions and its critical point (T_c = -122.46 ^{0}C, P_c = 48.63 bar) can be accessed easily [2]. It is already widely applied as a protective atmosphere for certain foodstuffs, notably fruits and vegetables, especially when deterioration of food should be slowed down.

Its inert nature is taken into advantage also in medicine and laboratory analyses for quality control, in electronics as a carrier gas for reactive molecules and as an inert gas to protect semiconductors against impurities. It is also used to create an inert protective atmosphere between the liquid metal and surrounding air in metallurgy and welding. Since it doesn't react with the filament, even at high temperatures, argon is used as inert atmosphere in incandescent light bulb, to create blue light in Neon-type lamps and also as filler for windows with double glazing [3].

Though there are many compounds present in the extracts of vanilla, the compound vanillin (4-hydroxy-3-methoxybenzaldehyde) is primarily responsible for the characteristic flavor and smell of vanilla [4]. Also, the vanilla plant is a source of catechins (also known as polyphenols), which exhibit antioxidant activity and serve as anti-inflammatory agents. Extract is used primarily as a fragrance and flavoring agent.

Increased demand for vanilla and the relative shortage of supply of natural extracts have encouraged the development of new sources of vanillin, which are mainly biotechnologically oriented [5, 6]. Ethyl vanillin is used as an alternative to vanillin and possesses a flavour and odor approximately three times as intense as vanillin.

SCF technologies have been extensively researched for selective isolation of antioxidants from natural material since the mild conditions avoid oxidation and/or degradation of labile compounds [7, 8]. As already mentioned, argon has high potentials for applications in food industry. The inert behavior makes argon a good candidate for a solvent in extraction processes. Phase equilibrium for the system of vanillin and *o*-ethylvanilin in argon has therefore been investigated in present work. Furthermore, extractions of vanilla pods with CO_2 and argon were performed.

2. Materials and Methods

2.1 Materials

- Vanillin (99%, Cat. No. V110-4), 12,080-4),
- *o*-ethylvanilin (97%, Cat. No. 16,098-9),
- argon of purity 99.998 vol% ; MESSER (MG-Ruše, SI),
- CO_2 of purity 99.9 vol%; MESSER (MG-Ruše, SI)
- vanilla pods (Vanilla planifolia), Central America (7.9 wt % moisture content).

2.1.1 Preparation of materials

2.1.1.1 Particle size distribution

The pods of vanilla were ground and sieve analysis of ground material was carried out to determine the particle size distribution. Experiments were carried out on a laboratory scale, in small quantities and the heating due to grinding the raw material was minimal.

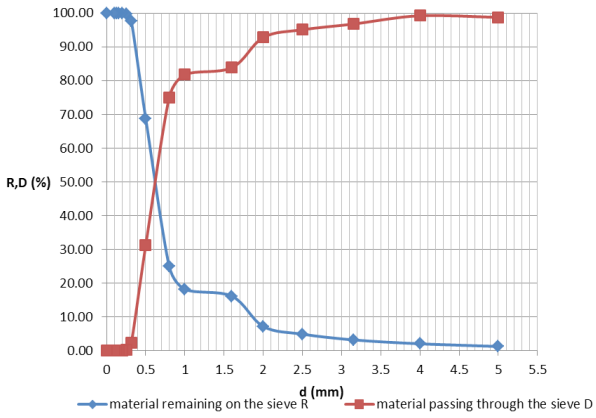


Figure 1. Sieve analysis: cumulative size analysis

The curve of cumulative size analysis (Figure 1) clearly indicates that medium particle size was 0.62 mm.

2.1.1.2 Analysis of water content in plant material

Measurements were performed gravimetrically by means of Mettler Toledo HB43-S Compact Halogen Moisture Analyzer.

2 g of plant material were dried for 120 minutes at 105 ^{0}C until constant mass. The content of water was calculated as:

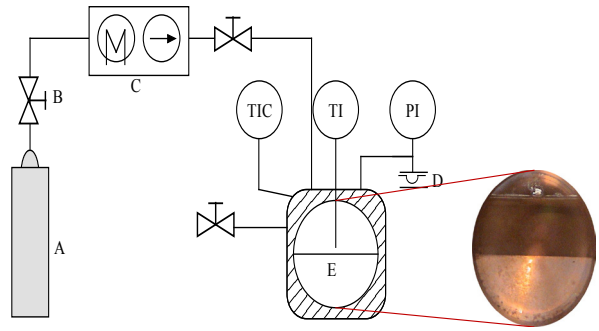
Content of water (%) = (mass plant material - mass residue)/mass plant material) x 100

Water content in material was 7.9%.

2.2 Methods

2.2.1 High pressure view cell – Solubility measurements

Prior to extraction experiments solubility measurements were performed for the pure vanillin and *o*-ethylvanilin in argon using a high pressure view cell, supplied by SITEC (Sieber Engineering AG, Zurich, Switzerland), presented in Figure 2.



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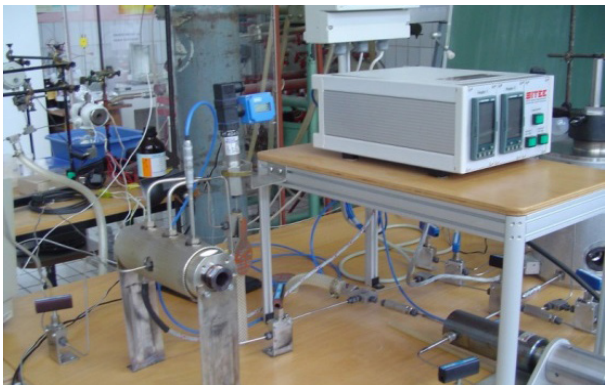

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Figure 2. High pressure view cell: flow sheet (a) and photo (b) of the apparatus

The volume of the cell was 74 mL (or 37 mL); volume can be decreased by half. Maximum working pressure was 1000 bar, maximum working temperature was 500 ºC.

A static-analytic method was used for measuring solubilities of vanillin and *o*-ethylvanilin in argon. A detailed description of the experimental procedure is presented in literature [5].

2.2.2 High pressure extraction unit apparatus

Extraction experiments were performed on extraction unit presented in Figure 3. The plant is operating up to pressure 400 bar and temperature 100 ^{0}C.

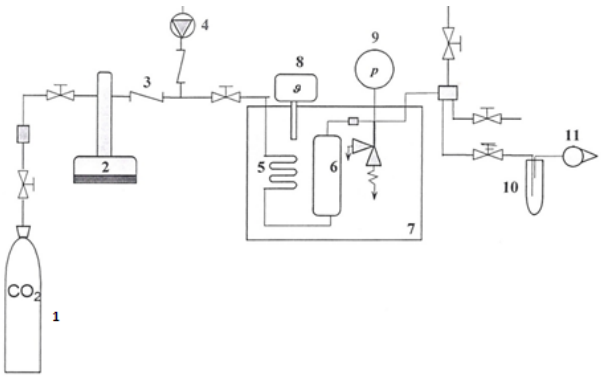

Figure 3. High pressure extraction apparatus 1 - gas cylinder; 2 - high pressure pump; 3 - one-way valve; 4 - HPLC pump; 5 - heating coil; 6 - autoclave; 7 - thermo bath; 8 - temperature indicator and regulator; 9 - manometer; 10 - sampling trap; 11 - rothameter

2.2.3 Extraction experiments

- SC extraction

Extractions of milled vanilla pods were performed with argon and additionally with CO_2 for a comparison. Extractions were performed at temperatures of 25 ^{0}C, 40 ^{0}C and 60 ^{0}C and pressures from 150 bars up to 300 bars. Extracts were collected through the sampling valve in a tube with a trap. The tube was immersed into a ethylene glycol–water bath at 268 K. Extractions using organic solvents (conventional and Soxhlet extraction with methanol and ethanol) were also performed. A detailed description of the method is given by Hadolin [9].

- Conventional extraction

Conventional and Soxhlet extraction with methanol and ethanol were performed.

2.2.5 Soxhlet:

An apparatus, equipped with a condenser was used. 20.2245 g of milled material was put into the flask which was slowly filled with warm solvent (180 mL of MeOH). The solvent was heated to reflux. After 120 min of mixing using a magnetic stirrer at a temperature of 60 ^{0}C the solution was separated by filtration and the solvent was removed by means of a rotary evaporator, yielding the extracted compound, which was later dried and weighed. The samples were stored in a dark and cool place.

2.2.6 Conventional extraction

Two different solvents, MeOH and EtOH were used to perform conventional extraction experiments. The ratio between milled material and solvent was 1:10 (20 g of material and 200 ml of solvent). After 120 min. of mixing using a magnetic stirrer at a temperature 70 ^{0}C the solution was separated by filtration. The solvent was evaporated and the extract was dried and weighed. The samples were stored in a dark and cool place.

3. Results and Discussion

3.1 Solubility data (Thermodynamics)

The knowledge of solubility of extracted substance in the supercritical solvent is essential for the economy of extraction process. The highest possible loading of SC solvent should be achieved in extraction step of the processes, while in separation step of the process the solubility of solute in solvent should be the lowest [8].

Solubility data of pure vanillin and o-ethylvanilin in argon at temperatures of 40 ^{0}C, 60 ^{0}C and 80 ^{0}C and at pressures up to 500 bars were measured. System in the cell could be observed during measurements through the sapphire window of the cell (Figure 4). Maximal solubility of vanillin has been obtained at a temperature of 40 ^{0}C and a pressure of 438 bars, approx. 0.015 g/g of gas.

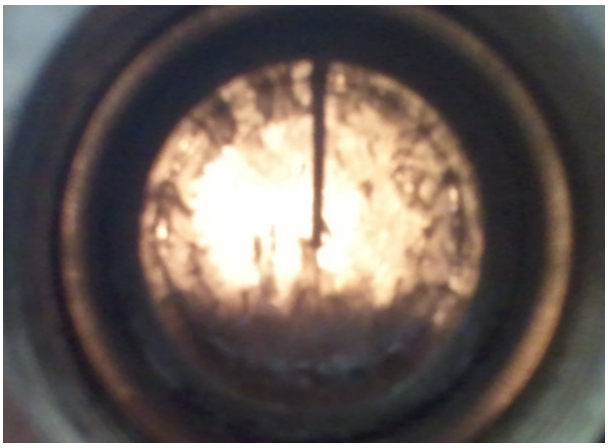

Figure 4. Phase equilibrium for the system of vanillin in argon at a pressure of 150 bars and at a temperature of 40 ^{0}C

Solubility is higher in case of lower temperatures and also a high impact of pressure is noted up to about 200 bars. With additional increase of pressure only a slight impact on solubility is observed. In comparison to solubility of the substance in CO_2 solubility is higher in argon at lower temperatures and pressures up to about 200 bars. Again, an increase of pressure does not exhibit a noticeable impact on solubility.

For *o*-ethylvanilin in argon highest solubility was measured at pressures above 300 bars and at lower temperatures; about 0.5 g/g of gas. In comparison, solubility of *o*-ethylvanilin in CO_2 is lower, approx. 0.18 g/g of gas at a temperature of 60 ^{0}C and a pressure of 300 bars (Figure 5).

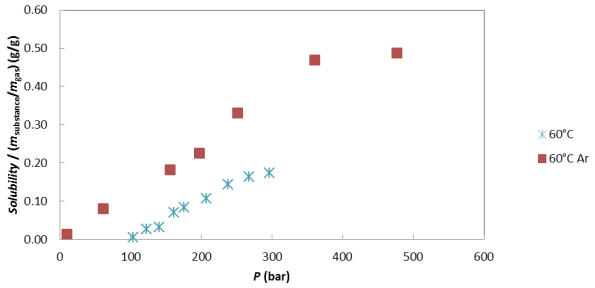

Figure 5. Solubility of o-ethylvanilin in argon and in CO_2

A comparison of the solubility data of pure vanillin and *o*-ethylvanilin in argon and in CO_2 was done and it can be concluded that solubility is higher in argon at lower temperatures and pressures up to about 200 bars.

3.2 Extraction experiments

3.2.1 SCF extraction

An impact of temperature is observed in case of the kinetic curves obtained at a constant pressure of 300 bars (Figure 6). The highest yield for SCF extraction with CO_2 was observed at 25 ^{0}C and 300 bars (5.7%). Overall, higher yields are observed in case of higher pressure. When applying argon as solvent it was determined that yield increases with increasing pressure, but decreases with an increase of temperature.

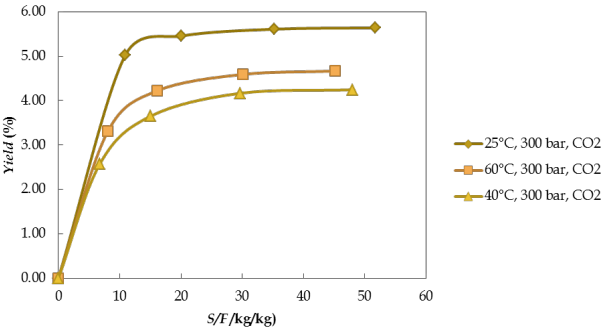

Figure 6. Kinetics of semi-continuous extraction of vanila pods with CO_2 at temperatures of 25 ^{0}C; 40 ^{0}C and 60 ^{0}C at a pressure of 300 bars

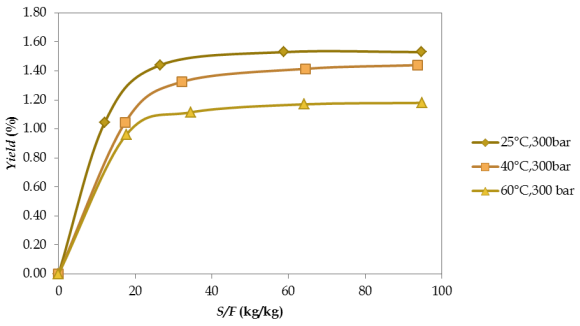

Figure 7. Kinetics of semi-continuous extraction of vanila pods with argon at temperatures of 25 ^{0}C; 40 ^{0}C and 60 ^{0}C at a pressure of 300 bars

Comparing extraction yields obtained with both solvents, yields are higher in case of CO_2. The highest yield in case of argon is observed at 25 ^{0}C and 300 bars (approx. 1.53 %) as presented in Figure 7, where impact of temperature on extraction kinetics is demonstrated.

3.2.2 Soxhlet

Extraction time was app. 20 hrs and 46 cycles were performed. The yield was app. 51.88%. An experiment under similar conditions was performed with EtOH as a solvent and the yield was app. 32.86%. Extraction time was shorter, app. 7 hrs and only 15 cycles were performed (Figure 8).

3.2.3 Conventional extraction

Higher yield is observed in case of extraction applying MeOH (approx. 39%) than in case when EtOH was applied (30.5% - 32%). However, extraction yields in case of Soxhlet extraction are somewhat higher (Figure 8).

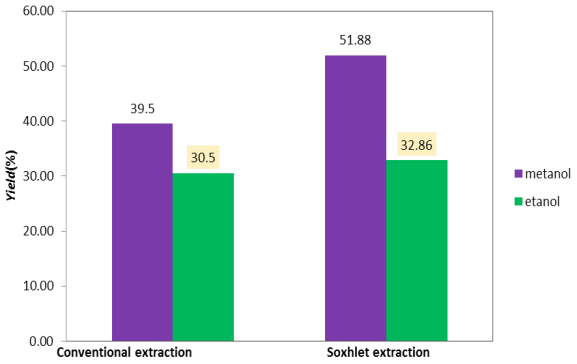

Figure 8. Extraction yields in case of conventionally obtained extracts

4. Conclusions

- The main disadvantage using conventional solvents is that the extracts contain residues of organic solvents which cannot be easily removed. The legal limitations of solvent residues and solvents (for products used in human applications) and easy isolation/fractionation of special components from total extracts in combination with different formulation and sterilization processes will increase the use of dense gases for extraction applications [10].

- The possible limitation of SCF technologies could be in high investment costs, but the legal restrictions on solvents and solvent residues, fractionation of highly valuable compounds from total extracts in combination with formulation processes will lead to an increase in the use of gasses under high pressure for extraction applications [11].

- Maximal solubility of vanillin in argon is observed at a temperature of 40 ^{0}C and a pressure of 438 bars,

approx. 0.015 g/g of gas. For *o*-ethylvanilin the highest solubility in argon was measured at pressures above 300 bar and at lower temperatures; about 0.5 g/g of gas.

- UV spectrophotometric analysis of extracts indicate high content of vanillin in extracts, obtained by extraction with supercritical fluids either with CO_2 or argon (up to 88 wt.% of vanillin), while extracts obtained with Soxhlet or conventional extraction contained app. 54 wt.% of vanillin.

- Therefore it can be concluded that supercritical fluids are way more selective solvents for vanillin's than conventional ones. Phase equilibrium of Ethyl vanillin was investigated due to the fact that is used as an alternative to vanillin and possesses a flavour and odor approximately three times as intense as vanillin. Comparing solubilities of vanillin and *o*-ethylvanilin in argon it can be summarized that argon is a suitable processing media also for artificial vanillins.

- As vanilla extracts contain several compounds, approximately 200 substances main compounds are 4-hydroxy-benzaldehyde, vanillic acid and 4-hydroxybenzoic acid in addition to vanillin, a raw preliminary evaluation of obtained extracts is presented in present work.

5. References

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