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SUPERCRITICAL FLUID EXTRACTION AND MICROWAVE-ASSISTED HYDRO-DISTILLATION OF NUTRACEUTICALS FROM SPICES AND MEDICINAL PLANTS

THESIS BOOKLET

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1. INTRODUCTION AND OBJECTIVES

In the last decade, the demand for natural additives has increased, so the food industry the use large amounts of spice and aromatic plants (as flavours, pigments, antioxidants, nutritional supplements, etc.), often in concentrations higher than naturally and therefore a concentration process is needed (to obtain extract, distillate or pressed juice). The quality standards of foodstuffs are rigorous, an outstanding important aspect is the lack of organic solvent traces in the products. This practically may achieve by two (non-mechanical) separation methods: supercritical fluid extraction (almost exclusively with carbon dioxide solvent) or water vapour distillation. The most important advantages of supercritical fluid solvents are the "tunability" of the solvent power with the operating parameters (pressure and temperature), as well as high penetration ability due by high diffusion coefficient, low viscosity and near zero surface tension. The low critical temperature (31.1 °C) of the carbon dioxide makes it particularly suitable to be supercritical fluid solvent ($SCCO_2$) for the gentle removal of heat-sensitive components, and its moderate critical pressure (7.38 MPa) require relatively low technological challenge to reach supercritical fluid status. Another great advantage is their non-flammability and will be completely removed from the extract, since it is evaporated at atmospheric pressure and is even completely harmless if traces (chemical or physical bonded) residues remain in the product. Being greenhouse gas is considered as disadvantage, but this is technologically irrelevant as it operates in a closed cycle. The effects of accidental release are negligible as pollutant (compared to power-plant emissions) and can only pose a local threat. Economically, the carbon dioxide is the second cheapest solvent (after water) and is available in large quantities, in food grade purity (being by-product of fermentation industry).

The $SCCO_2$ it almost meets all requirements for ideal solvents. Only one disadvantage is that dissolve only apolar or mild polar components, but on the one hand this may considered also an advantage (selectivity), on the other hand this behaviour may partially counterbalanced by using polar modifiers as co-solvents.

The research work, which represents the basis of the dissertation, is grouped into three main themes. In the first period of my research, I examined various aspects of supercritical fluid extraction and its practical applications in the production of vegetable extracts. Subsequently, I was interested in development and testing of original microwave-assisted hydro-distillation equipment. In the third part, two important problems, closely related to the $SCCO_2$ extraction were discussed, with the tools of theoretical modelling.

The first aim of my doctoral research was to examine the effect of the operation parameters (pressure and temperature) of supercritical fluid extraction on the yield of apolar components from a given plant sample. The characteristic quantity for extraction efficiency is the yield, and for estimation of the optimum value of the operation parameters the method of experimental design and variance analysis was applied. To demonstrate the efficiency of the method, I compare the yield and composition of both the supercritical and by Soxhlet-apparatus (using *n*-hexane solvent) obtained extracts.

In case of the plant material contains volatile and fatty oils both, another target was to separate this component groups efficiently in a two-separator equipped supercritical fluid extraction pilot plant, varying the pressure and temperature in the separators. Further, new objective occurs, namely the construction and testing of a self-developed laboratory microwave-assisted hydro-distillation apparatus, since during the previous research, the conventional hydro-distillation experiments revealed their limitations, and I considered a requirement for process intensification. For testing, for model plant the fennel, was chosen, since has a high content of volatile oil with well known components, and are therefore highly suitable for carrying out comparative tests. Since a technologically important specification of the hydro-distillation is the operation velocity, a main target was to modify the equipment in such way to be able for kinetic measurements as well.

After the equipment testing, it was obvious to use for obtaining of the volatile components from a traditional medicinal plant and spice (caraway). This part of the study was focused on revealing of the influence of the drying method, the grinding time, the harvesting

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location and the extracting method (microwave pre-treatment, thermal and microwave-assisted hydro-distillation, and $SCCO_2$ extraction) on the yield and the carvone-limonene ratio of the two main components of the extracted essential oil.

During the study of supercritical fluid extraction, two other important issues emerged, leading to two further fundamental objectives.

The $SCCO_2$ is poor solvent for polar compounds, and the use of polar modifiers (co-solvents) are needed, when occurs the problem of predictability of the solvating power. I propose to find a fast and simple calculation ("shortcut") method of the solubility parameters of solvent mixture in supercritical fluid state. The goal was reduced to find an engineering approach to predict the variation of the Hansen solubility parameters in function of pressure and temperature.

The final theoretical objective was to find an explanation of the antimicrobial effect of $SCCO_2$, based on molecular biological evidence. I attempt to realize a more comprehensive and more general model in comparison with current partial mechanisms, as it has become clear from the literature data that this topic is not fully clarified. By knowing a realistic biological mechanism, the pasteurization technologies with supercritical fluids may become more effective, and the model may help us to better understanding their potential and limits, as well as their safety.

2. EXPERIMENTAL METHODS

2.1 Extraction methods of bioactive compounds from plant materials

During my research work, the bioactive substances were excreted primarily by $SCCO_2$ with the supercritical fluid extraction pilot-size apparatus shown in figure 1.

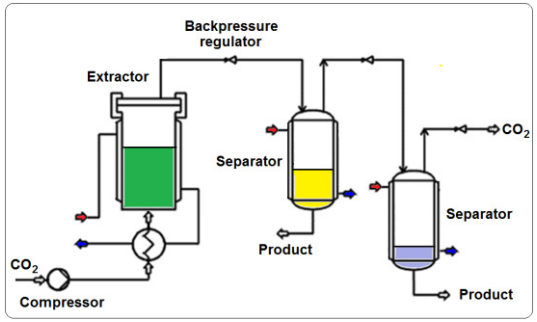

Figure 1. Scheme of supercritical fluid extraction pilot plant.

The used solvent was carbon dioxide in food grade purity. The liquid CO_2 stored in the metal tube is compressed by a high-pressure diaphragm pump at the desired pressure (p_{max} = 60 MPa) trough a preheater, where is heated above its critical temperature (T_{crit} = 31.1 °C) and then reach the set temperature in the extractor. In the extractor body the solvent flow through the preloaded plant particle bed, dissolving and extracting the soluble components. In the separators, after stepwise pressure reductions the compounds will precipitate gradually and separated do by solubility difference (fractional extraction). The unit can also be operated with a single separator, when the fractionation is not necessary. In addition, with a high-pressure piston pump polar modifier can be added to the $SCCO_2$ flow. To obtain volatile components from plant material, I used a self-developed and built-in microwave-heated hydro-distillation experimental laboratory device (figure 2.). The suspension of the grinded plant material is filled into the flask and the device will assembled. Then the microwave oven and the rotor will be alimented, and the flow of cooling water will be started.

6. POSSIBLE PRACTICAL APPLICATIONS

The use of supercritical carbon dioxide as a solvent for the extraction of bioactive substances from plant material has now become an important industrial practice. In my doctoral thesis I expound a working experimental and data analysis protocol to optimize the operational parameters of a supercritical extraction pilot plant equipped with two separators with the purpose of achieving a fractionated separation of several compound type from a given plant matrix. The developed microwave-assisted hydro-distillation laboratory equipment is suitable for obtaining the volatile oil from plant material, the distillation is faster than in conventional equipment. Moreover, the apparatus is suitable for hydro-distillation kinetics measurements.

The new short-cut method for the estimation of the Hansen parameters provide a tool in the obtaining of ,,designer" supercritical fluid mixture solvents and may contribute for simpler predictability of processes in supercritical fluid media, as extraction, chromatography and particle formulation. The importance of the method is that hyphenated with the experimental design, will be an effective tool of optimization of supercritical processes, that can lead to increased cost-efficiency of the processes. By transforming the Hansen square into a ternary diagram, more information can be applied to a graph and thus visually can be monitored by the change in solubility of a supercritical fluid mixture of solvents depending on the composition and the operational parameters. By transforming the Hansen space into a ternary diagram, more information can be represented on a single graph. Moreover, this representation provides a visual monitoring of the trend of changes of solvating power of a supercritical solvent mixture with composition and the operational parameters.

The model for the antimicrobial mechanism of the $SCCO_2$ may promote the industrial introduction of supercritical sterilization as the lack of a complete understanding of the destruction mechanisms increases the security risks, and is a major obstacle for its acceptance as an innovative non-thermal processing method. The model may have indirect effects on the development of new antibiotics by designing a more effective attack of selective targets on ribosome.

4. I developed a "short cut" calculation method based on a surface fitting to quickly estimate the Hansen parameters of supercritical fluid solvents mixture. The novelty of this is the calculation method for the Hansen parameters of pure components. I have found that the supercritical solvent does not occupy only a point in Hansen-space, but a surface , which depend from both, temperature and pressure. In the case of supercritical solvent mixtures it degenerates into a spatial domain ("cloud"). In the conventional extraction range and in the polar modifier concentration, the Hansen diagram was represented for the CO_2-ethanol mixtures [12-14,20].
5. For explanation of the bactericide effect of the $SCCO_2$ was developed a completely new theoretical model, based on molecular biological evidences. The basis of the proposed mechanism is considered the inhibition of the initiation step of bacterial protein synthesis. I have assumed with reason the addition of $SCCO_2$ to the amino group of methionine, with the formation of carbamoyl-methionine. Structural analogy was observed between a very active initiating factor 2 inhibitor molecule (N-formyl-L-thyroxin) and the proposed cMet-$tRNS^{fMet}$ [3,15]. On the *P. aeruginosa* IF2 C-2 domain, the molecules fMet-$tRNS^{fMet}$, cMet-$tRNS^{fMet}$ and N-formyl-L-thyroxin molecules were docked *in silico*. I found that the binding energy of cMet-$tRNS^{fMet}$ is more than twice than the binding energy of the initiator fMet-$tRNS^{fMet}$ molecule [19]. I developed the inhibition scenario in detail, described its possible consequences, and I made a proposal for the experimental method to prove the hypothesis. The model does not contradict the latest literature data either.

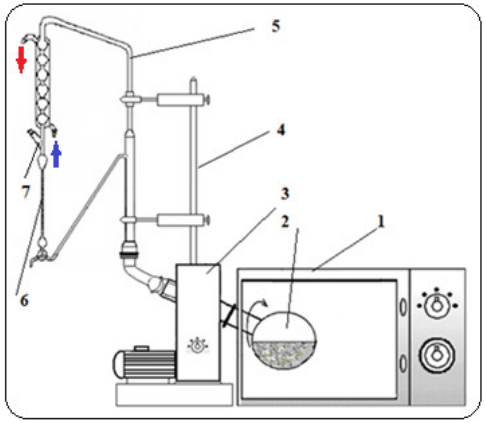

Figure 2. The scheme of the microwave-assisted hydro-distillation apparatus:1- microwave oven, 2- spherical flask, 3- rotating evaporator, 4- supporting rack, 5- Clevenger-head, 6- scaled tube, 7- safety valve / snap stopper.

3. RESULTS

3.1. Results of the extraction of okra seeds

The aim of the research was to study the extraction of fatty oils and fat soluble valuable bioactive compounds (β-sitosterol, α-,γ-tocopherols) from okra seeds by supercritical fluid extraction.
The effect of pressure and temperature on the yield of supercritical extract was determined by 3^2 complete factorial experimental design, at the centre of the experiment with three repetitions. The maximum pressure was 45 MPa (this was the safety pressure limit of the equipment), the lower pressure value was 15 MPa. The upper temperature level (60 °C) was chosen to avoid the thermal decomposition of the heat-sensitive components, while the lower level (40 °C) was slightly above the critical temperature of the carbon dioxide. The yield of the extract was the extent of the efficiency. From the Pareto chart (figure 3a) may found that the linear and quadratic terms of pressure and the interaction between pressure and temperature were the most important effects, the latter is in fact related to the density. Other terms have been found to be statistically

significant but less important. Figure 3b represents the response surface which shows both the sensitivity and the optimal operating conditions.

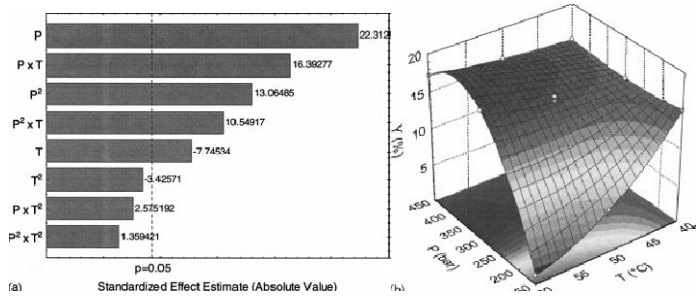

Figure 3. The influence of extraction parameters (pressure (*P*) and temperature (*T*)) on oil yield (*Y*):(a) Pareto diagram, (b) the response surface of oil yield

The effect of the operation parameters on the total sterol content of the oil samples was determined similarly. Here, the main effects are the combined effect of pressure, pressure and temperature, which has the greatest influence on sterol recovery. In the examined range, the highest yield was achieved by p=45 MPa pressure and t=60 °C.
The response surface of oil extraction and the sterol recovery show great similarity, which, also means that the extraction of oil and the sterols are not independent. About 100 g kg^{-1} of the total sterols were free *β*-sitosterol.The composition of the extracts is shown in table 1.

Table 1. The yield of the components obtained by differen extraction methodes

	SFE (450 bar, 50 °C)	**Soxhlet (*n*-hexane)**	**Soxhlet (ethanol)**
Oil yield(g·kg^{-1} d.m.)[a]	159.6 (4.8)	163.1 (5.6)	207.4 (9.4)
***β*-sitosterol conc. (g·kg^{-1})[b]**	15.0	12.3	12.9
***β*-sitosterol (g·kg^{-1} d.m.)[b]**	2.39	2.01	2.68
***α*-tocopherol conc. (mg·kg^{-1})[b]**	930	780	620
***α*-tocopherol (mg.kg^{-1} d.m.)[b]**	148	127	129
***γ*-tocopherol conc. (g·kg^{-1})[b]**	2.55	2.33	2.38
***γ*-tocopherol (mg.kg^{-1} d.m.)[b]**	407	380	494

[a] Triplicate; (in paranthesis are the value of standard deviation) [b] average value of two measurements

constituents of the essential oil of the caraway are carvone and limonene. The carvone/limonene ratio was similar in distilled essential oil, hexane extract, and in the product of second separator. The carvone/limonene ratio in the oil from first separator it was slightly lower and in the ethanolic extract was significantly higher compared to the essential oil obtained by hydro-distillation. I demonstrate, that in case of ground plant material with high fatty- and essential oil simultaneously, an optimized supercritical fluid extraction process is suitable for realising the extraction coupled with fractional separation [7]. In case of supercritical extraction, the increase in pressure increases the proportion of the volatile component (limonene) in the extract. Irrespective of the applied pressure, the limonene content of the drained samples in batch experiments decreases with the increase of purging time. This reflects that the extracted volatile component desorbed easier from the plant matrix in comparison with the more polar, and heavier carvone [5].

3. I have developed and construct a microwave-assisted hydro-distillation laboratory device that differs from the similar apparatus. By modifying the Clevenger-head, it was possible to measuring the kinetics of the distillation process. I compared first the kinetics of essential oil obtaining from fennel with thermal (HD) and microwave-assisted hydro-distillation (MWHD). I have proved that for both methods may attribute the same kinetic model. The increased velocity of the process in this type of apparatus is partially due by shortening of the initial heating. This is the result intensification of the heat transfer, caused by the convection resulting from the rotation. The device is suitable for quicker laboratory hydro-distillation as it is capable of obtaining essential oil from the given plant matrix in the quit same quantity and quality as in the classical apparatus, but in a much shorter time [4,6,9-11,17,18].

4. THE CLAIMS OF THE PhD RESEARCH

1. I extracted first from okra seeds, by supercritical extraction pilot plant with CO_2 solvent the fatty seed oil and valuable fat-soluble bioactive components (β-sitosterol, α-, γ-tocoferol) and optimized the process for fatty oil as well as valuable lipophilic bioactive compounds. By a comparative study, I proved that $SCCO_2$ is an appropriate solvent for extracting valuable components because of the composition and amount of the such obtained extract is similar to the *n*-hexane extract, but the remaining grate does not contain solvent residue therefore does not require further treatment. The adopted experimental and data analysis method is suitable for optimizing the parameters of pilot-size supercritical process. The effect of pressure and temperature on the yield of supercritical extract, both for the fatty oil and the fat-soluble bioactive compounds, was determined by 3^2 complete factorial experimental design. I demonstrate, that that the linear and quadratic terms of pressure and the interaction between pressure and temperature were the most important effects, the latter is in fact related to the density. The response surface of oil extraction and the sterol recovery show great similarity, which, also means that the extraction of oil and the sterols are not independent, so the coextraction phenomena occure. I demonstrate, that using the response surface methodology, we are able to determine the optimal process parameters, and from the slope of the surface the sensitivity of the proces, too. [3,8,16].
2. I realised first the hyphenated extraction and separation of the caraway oil in two fraction (fatty- and volatile oil) by supercritical extraction pilot plant equipped with two separator. Using experimental design, response surface method and variance analysis I determined the optimal parameters of separators operation, to give the best separation. Significant effect on separation has the pressure and the temperature of the first separator. The main

I also examined the fatty acid composition of fatty oil extracted with different solvents, and the results are summarized in the table 2.

Table 2. The fatty acid composition of the okra seed oil

	Extraction method		
Fatty acid	**SFE**	***n*-hexane**	**ethanol**
Palmitic acid (C_{16})	31.6	32.6	29.9
Stearic acid (C_{18})	3.4	2.8	3.2
Oleic acid ($C_{18:1}$)	17.2	16.1	17.4
Linolic acid ($C_{18:2}$)	46.0	47.4	47.5
Linoleic acid ($C_{18:3}$)	1.3	1.2	1.3
Arachidonic acid (C_{20})	0.5	-	0.2
Behenic acid (C_{22})	-	-	0.2
Erucic acid ($C_{22:1}$)	-	-	0.3
Unsaturated/saturated rate	1.82	1.83	1.98

3.2. Results of the extraction of caraway seed

3.2.1. SFE fractionation of the caraway seed

In this section, beside the high yield extraction, I studied the possibility of separating the fatty and volatile oil components from caraway using $SCCO_2$ solvent extraction. This method is called supercritical fractionation. The extraction was performed on the optimal parameters (p_E=450 bar, T_E=50 °C) determined during the preliminary experiments. The optimal separator parameters were determined by the method of experimental design. Varying the pressure and temperature of the two separators shown in figure 1., the composition of the separated extracts in the both separators was examined. By data analysis it can be stated that in the second separator accumulate a higher proportion of essential oil while in the first separator the non-volatile fraction is separated. The fatty oil with highest purity (0.7-0.8% essential oil content) was obtained at separator parameters of T_{S1} = 21-31 °C and p_{S1} = 8-9 MPa. Significant factors for separation were the pressure and temperature in the first separator.

The composition of the essential oil obtained by various extraction methods is shown in table 3. From the supercritical obtained oils the extracts of experiment 11 (separator parameters: p_{S1} = 40 bar, T_{S1}= 7-8 ° C) and 18 (separator parameters: p_{S1} = 80 bar, T_{S1}= 30 ° C) was examined.

Table nr. 3. The composition of essential oils obtained from caraway

t_R (min)	Hydro-dist (%)	Soxhlet (*n*-hexane) (%)	Soxhlet (ethanol) (%)	SFE (%)		Volatile component
				Run 11.	Run 18.	
				Sep.-1	Sep.-1	
4.22	15.21	13.48	7.82	14.87	10.64	limonene
6.25	0.82					unknown
6.69	0.55					unknown
7.22	83.42	80.28	80.81	65.17	52.45	carvone
	5.50	6.00	10.30	4.40	4.90	C/L rate

The results demonstrated that the main constituents of the essential oil of the caraway are carvone and limonene. By each of the examined extraction methods the two terpenes can be obtained with different concentrations. The carvone/limonene ratio was similar in distilled essential oil, hexane extract, and in the product of second separator. The ratio in the oil from first separator it was slightly lower and in the ethanolic extract was significantly higher compared to the essential oil obtained by hydro-distillation. The typical fatty acid composition of the oil obtained in the first separator is shown in the table 4. It can be stated that the main components are oleic acid and petroselinic acid together. In addition, linoleic acid and palmitic acid are present in larger quantities. The chromatographic separation of petroselinic and oleic acid cannot be achieved during the analysis.

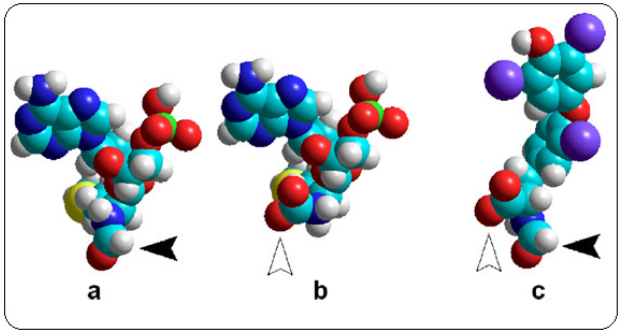

Figure 10. Structural similarities between the:
(a) fMet-AMP, (b) cMet-AMP and (c) N-formyl-L-thyroxine (The black arrows mark the a formyl-, the white ones the carboxyl-groups).

By computational molecular modelling on the IF2 C-2 domain of *P. aeruginosa* the following molecule was docked: fMet-tRNS^{fMet}, a cMet-tRNS^{fMet} (figure 11.), as well as the N-formyl-L-thyroxine.

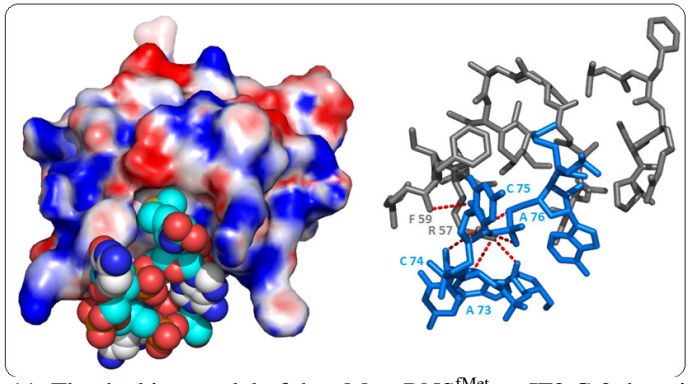

Figure 11. The docking model of the cMet-tRNS^{fMet} on IF2 C-2 domain of *Pseudomonas aeruginosa*.

The strongest binding is formed with the proven initiation inhibitor molecule, N-formyl-L-thyroxine (ΔE=-18.71 kJ/mol), the weakest with the natural initiator, fMet-tRNS^{fMet} (ΔE=-6.99 kJ/ mole). The binding strength with the hypothetical, cMet-tRNS^{fMet} has an intermediate value (ΔE=-14.94 kJ/mol) but this is is more than twice as strong as the natural initiator binding strength.

3.5. The molecular biological model of the antimicrobial mechanism of $SCCO_2$

The supercritical carbon dioxide ($SCCO_2$) has germicidal (bacterial and sporicide) effects. Although this effect has already been used in industrial pasteurization processes, the antibacterial mechanism was still unclear at the molecular level. The developed molecular biology-based hypothesis can explain the phenomenon.

The preambulum of the model is that the $SCCO_2$ react competitively to Met-tRNS^{fMet} so that the rate formation and amount of fMet-tRNS^{fMet} will decrease during irreversible substrate loss. fMet-tRNS^{fMet} has a key role in prokaryotic protein synthesis, since it is almost exclusively the only aminoacyl-tRNA initiator. The resulting carbamoyl-methionyl-tRNA (cMet-tRNS^{fMet}), which is likely to be stable under pressure and high CO_2 concentration, may stabilize by forming a molecular complex with the GTP-IF2 form of the translational initiator factor 2. Due to the strong polar binding between the C-2 protein domain and the modified aminoacyl-tRNA initiator, the latter will be unable to dissociate from the 70S ribosomal complex. In addition, the resulting inactive 70S ribosomal pre-initiation complex does not degrade after GTP hydrolysis, therefore protein synthesis is inhibited. The destruction of the microbial cell may be caused by the inhibition of protein synthesis and the significant energy loss.

Indirect evidence may be that in a high-throughput screening of 5000 compounds possessing IF2-inhibitory activity the most effective compound proved to be for N-formyl-L-thyroxine. Using Hyperchem 6.01 molecular modeling software, I compared the molecular structure of fMet-AMP, cMet-AMP and N-formyl-L-thyroxine (figure 10.). The the comparison is justified, as it is proved that the minimum ligand for IF2 C-2 domain recognition is the fMet-AMP.

Table 4. The fatty acid composition of the supercritical extract from caraway

Main components	t_R (min)	SFE (Sep.-1) (%)	Fatty acid
C_{14}	13.2	1.8	Miristic acid
C_{16}	17.2	14	Palmitic acid
$C_{16:1}$	17.6	1.1	Palmitoleic acid
C_{18}	20.9	13.7	Stearic acid
$C_{18:1}$	21.3	47	Oleic+petroselinic acid
$C_{18:2}$	22.1	21.8	Linolic acid

The Rosin-Rammler-Sperling-Bennett (RRSB) distribution function was used to describe the particle size distribution. On the measured values of the sieve residue the RRSB function was fitted. The figure 4. shows that the residual curve fit well to the measurement points. It is important to mention that the particle size analysis was performed by sieving the extracted meal, because the high oil content of the seeds causes, that the freshly grinded grains sticks together.

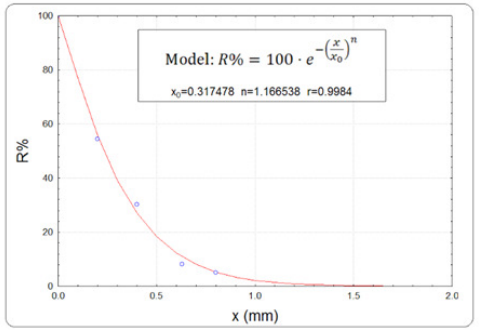

Figure 4. The fitted values of the particle size distribution parameters.

The calculated particle size distribution parameters were appropriate for extraction. In the case of small particles, excessive caking may occurs, the inside of the formed granules may remain untreated, while in the case of larger particle size - due to the morphological inhomogeneity of the seeds (seed shell and core) - the extraction of certain components will be obstructed.

3.2.2. The comparison of the caraway essential oil obtained by hydro-distillation and supercritical fluid extraction

The influence of extraction methods on the composition of the obtained caraway essential oil is presented on figure 5. With both, by hydro-distillation (HD) and by microwave-assisted hydro-distillation (MWHD) methods results an almost identical carvone/limonene ratio. The microwave pre-treatment increases the proportion of the less volatile component (carvone), but also results a drastic decrease of essential oil yield. The essential oil loss is due probably due by the insufficient cooling.

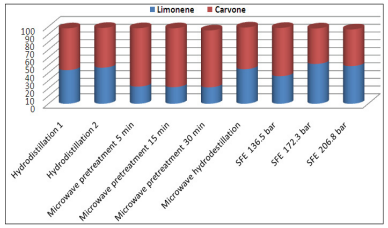

Figure 5. The influence of extraction method on carvone/limonene ratio.

In the case of supercritical extraction, the increase in pressure increases the proportion of the volatile component (limonene) in the extract. Irrespective of the applied pressure, the limonene content of the drained samples of the batch experiments decreases with the increase in purging time. This reflects that the extracted volatile component desorbed easier from the plant matrix in comparison with the more polar carvone, with higher molar mass (figure 6.).

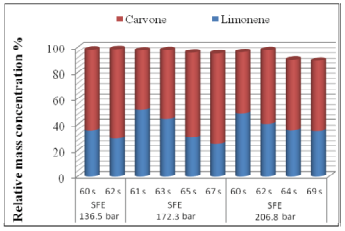

Figure 6. The influence of pressure and the purging time on carvone/limonene ratio (SFE-obtained essential oil).

In the Hansen-space the supercritical solvent is represented as a surface (dependent of temperature and pressure). In the case of supercritical solvent mixtures this degenerates into a spatial domain ("cloud"). In the conventional extraction range (P=8-60 MPa, T=275-350 K) and co-solvent concentrations ($\Phi_{ethanol}$ = 0-0.2) the Hansen diagram is shown on figure 9.

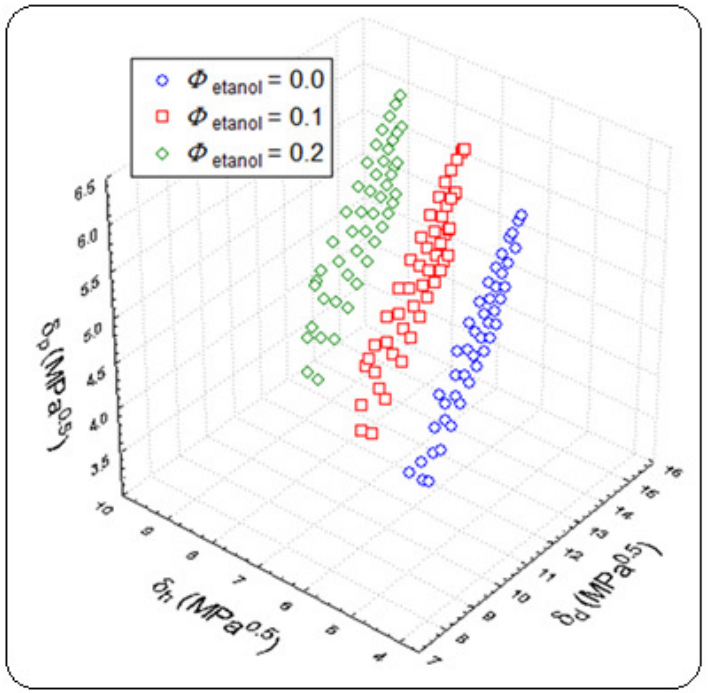

Figure 9. The Hansen-diagram for the CO_2-ethanol mixtures for the technologically relevant domain.

For the CO_2-ethanol mixtures the total solubility parameter (Hildebrand) was obtained in conformity with the formula (8):

$$\delta^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \qquad (8)$$

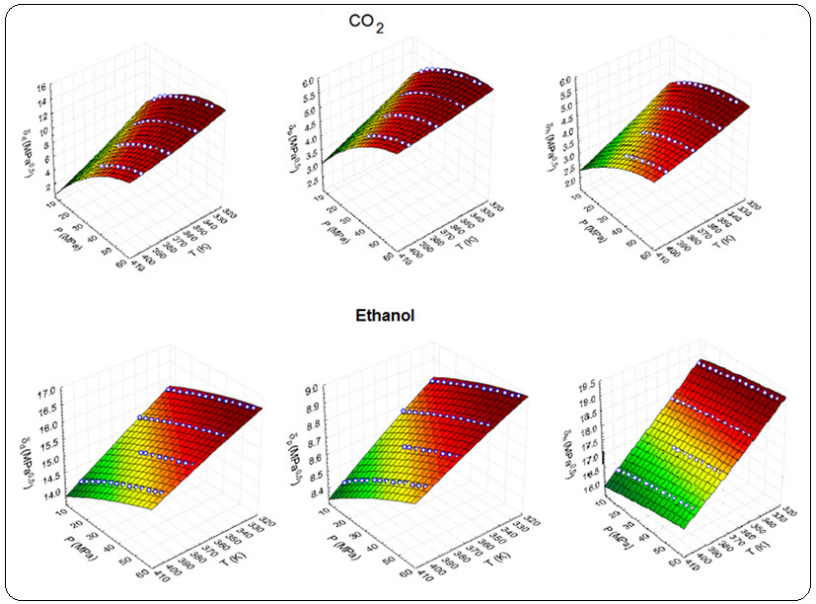

Figure 8. The Hansen solubility parameters for pure CO_2 and ethanol.

Knowing of the fitting parameters for the pure components, the Hansen parameters of the mixtures can be calculated as the weighted average of the components (7):

$$\delta_{j,mix} = \sum_{i=1}^{n} \Phi_i \delta_{j,i} \qquad (7)$$

where: Φ_i- the volumetric fraction of the i^{th} component; $\delta_{i,j}$- the j^{th} Hansen-parameter of the i^{th} component; (j=d, p, h); $\delta_{j,mix}$- the j^{th} Hansen-parameter of the mixture.

Generally, a compound is soluble in a solvent if, in the Hansen-space (δ_p = f (δ_d, δ_h)) the solvent is located in the so-called Hansen-ellipsoid of the component. In rough approximation it means, that the Hansen-distance of solvent and component is less than d = 4 $MPa^{0.5}$.

In case of hydro-distillation, although the boiling point of the limonene is lower than the boiling point of the carvone, the water solubility of the latter is higher due to the higher polarity of the carvone. The boiling water penetrates into the plant matrix, dissolves certain ingredients, including essential oil components, then re-diffuse to the main volume of the liquid, carrying the dissolved components, this phenomenon is called hydrodiffusion.

3.3. Results of the extraction of fennel seeds

I extracted and compared the essential oils from two varieties of fennel. The amount of var. *vulgare* essential oil was nearly six times greater compared with the amount obtained from the var *dulce*. In case of var. *vulgare*, the MWHD method give an amount 1.86% lower than the HD method, but the operation time decreased less than a tenth, and the specific water consumption decreased to twenty time (table 5.). The HD method was not optimized, but corresponds to the Pharmacopoeia method, so it is not excluded that the major detected difference may be exaggerated.

Table 5. Quantity of the essential oil obtained from fennel (var. *vulgare*)

Plant material	Extraction method	Amount (g)	Water volume (ml)	Time (min)	Ess. oil volume (ml)	Ess. oil content (ml/100g)
var. *vulgare**	MWHD	20	20	15	0.98	5.28
	HD	10	200	180	0.50	5.38

*dry material content of the raw plant material 92.8%

The extracts were obtained by three different procedures from var. *dulce* (table 6). I found that the yield of essential oil obtained with conventional hydro-distillation (HD) is about 5.55% higher than that obtained by the microwave-assisted hydro-distillation process (MWHD), but the operating time of MWHD is only 25% of the HD's operating time. The liquid obtained by supercritical extraction (SCF) has a yellowish colour, more viscous substance, in comparison with the essential oil, containing other components, the amount of extract

is much higher (10.4 g/100 g dry matter), the amount of essential oil cannot be determined.

Table 6. Quantity of the essential oil from fennel (var. *dulce*)

Plant material	Obtaining methode	Probe (g)	Water (ml)	Time (min)	Extract quatity	Ess. oil content (ml/100g)**
var. *dulce*	MWHD	20	100	15	0.17 ml	0.861
	HD	20	100	60	0.18 ml	0.911
	SFE[#]	0.460	-	25	173 mg	n

** dry material content of the raw plant material 98.7%, [#] p = 10 MPa, T = 50 °C

During the SC extraction, when the pressure and temperature are decreasing, the *trans*-anethole content of the extract is increasing, even when the solvent reach the liquid state (near-critical), although the total yield decreasing. The composition of the essential oils obtained by the two hydro-distillation method (MWHD and HD) differ only slightly (the two main component are *trans*-anethole and methyl-chavicol). The modified Clevenger apparatus is suitable for examination of the distillation process kinetic (figure 7.).

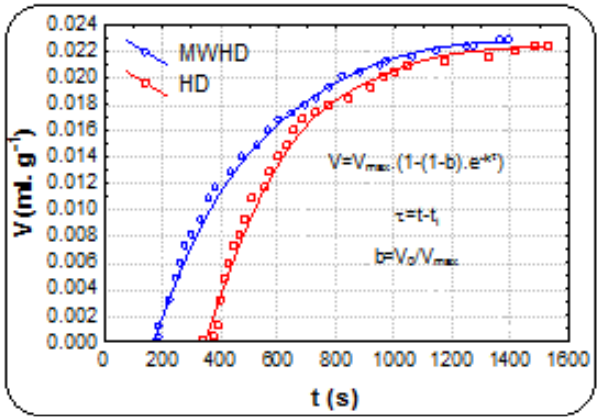

Figure 7. The comparison of the essential oil obtaining kinetics for HD and MWHD

The fitted kinetic functions are as follows (1,2):

$$V_{HD} = 0.0212 \cdot (1 - 0.9165 \cdot e^{-0.0034 \cdot \tau}) \text{ (ml/g)} \quad (1)$$

$$V_{MWHD} = 0.0228 \cdot (1 - 0.8993 \cdot e^{-0.0034 \cdot \tau}) \text{ (ml/g)} \quad (2)$$

In the kinetic equations (1) and (2) τ=0 represent the time when the first drop of essential oil appears in the Clevenger separator head. The microwave-assisted hydro-distillation (MWHD) is an accelerated laboratory technique for obtaining essential oil from plant matrix, and is a faster one compared with the classical, thermal heated hydro-distillation (HD). The acceleration of MWHD partially may attributable to the shortening of the initial heating time.

3.4. Determination of Hansen solubility parameters of the supercritical solven mixtures CO_2-polar modifiers

The Hansen parameter of pure solvents in supercritical state can be calculated by the formulas below (3-5):

$$\delta_d = \delta_{d,ref} \cdot \left(\frac{V_{ref}}{V}\right)^{1,25} \quad (3)$$

$$\delta_p = \delta_{p,ref} \cdot \left(\frac{V_{ref}}{V}\right)^{0,5} \quad (4)$$

$$\delta_h = \delta_{h,ref} \cdot \exp\left[0.00132 \cdot (T_{ref} - T) + ln\left(\frac{V_{ref}}{V}\right)^{0.5}\right] \quad (5)$$

Using the reference values and the molar volumes of the pure components (CO_2 and ethanol) the obtained results can be represented in function of pressure and temperature. By fitting a secondary order surface on the such obtained Hansen parameters in conformity with the formula 6., the fitting parameters (a-f) will be obtained.

$$\delta = a + bT + cP + dT^2 + ePT + fP^2 \quad (6)$$

where: T-temperature (K); P-pressure (MPa); a..f-fitting parameters. The obtained Hansen parameters, for both components and the fitted second order surfaces are represented on figure 8., in function of the operation parameters (temperature and pressure).