

Report of the research project 2858

High-Pressure Conditioning of Natural Materials („Hochdruck-Konditionierung von Naturstoffen vor der Feststoff-Extraktion“)

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1 Introduction

Plant ingredients are commonly extracted from the natural source material through a variety of mechanical and/or thermal extraction methods. In order to enhance the efficiency of any extraction process an appropriate pretreating method is necessary. The objective of the current project is to develop a proper method for pretreatment of different plant and lichen material in order to increase efficiency of extraction of commercially valuable bioactive principles. The work is divided into a part for basic investigation of sorption effects under pressurized fluid atmosphere and a part of applying different pretreatment methods in order to enhance the yield of the extraction process on a pilot scale.

2 Objective

The work was aimed to investigate the effect of different pretreatment of plant and lichen material on the efficiency of an extraction process carried out with supercritical carbon dioxide. In order to understand the dominating mechanisms, part of this project was dedicated to investigate the sorption of carbon dioxide into different plant tissues. Further on, the effect of such a pretreatment or intermediate treatment under high fluid pressure was to be carried out for materials of different solid structures.

3 Materials and Methods

3.1. Materials

The materials used for the testing are listed in the following Table 1. Carbon dioxide used for the investigations was provided by Yara, Germany and had a quality of 99.2 %.

Table 1 Plant and lichen materials used for testing

Plant material	Part of plant	CO ₂ Solubles content [wt %]*	Application
St. John's Wort	Aerial part	5-10	Alternative medicine (natural antidepressant)
Usnea barbata lichen	Entire	~3	Alternative source of antibiotics
Oat	Seeds	~7	Slimming product (ingredient of "slim shots"), food industry
Amaranth	Seeds	~7	Source of squalene (used for cancer prevention and treatment)
Fennel	Seeds	~10	Food industry, alternative medicine

*Average values according to available literature data.

3.2. Description of pretreatment methods

Prior to extraction the solid substrate was pre-treated with different mechanical methods. Further, the so-called rapid gas decompression (RGD) was applied. The mechanical treatment was carried out in different kinds of mills. For this purpose, a cutting mill (IKA MF basic), a self-constructed flaking or roller mill and an ultracentrifugal mill (Retsch ZM 200) were used. The latter apparatus can be operated with or without a static sieve around a rotation grinding tool. In the case the mill was operated without the stator, the processing method is referred to as "impact-milling".

For the rapid gas decompression pre-treatment the solid substrate was pressurized with CO₂ to extraction conditions and held at these conditions for 1 h. Then, the pressure was reduced down to atmospheric pressure at average rates of approximately 10 MPa/min.

3.3. Sorption of carbon dioxide at elevated pressure

In order to determine eventual volume changes of the designated materials within a compressed carbon dioxide atmosphere, a high-pressure view cell is used shown in figure 1. The solid material is fixed to a sample holder and observed by means of a CCD camera.

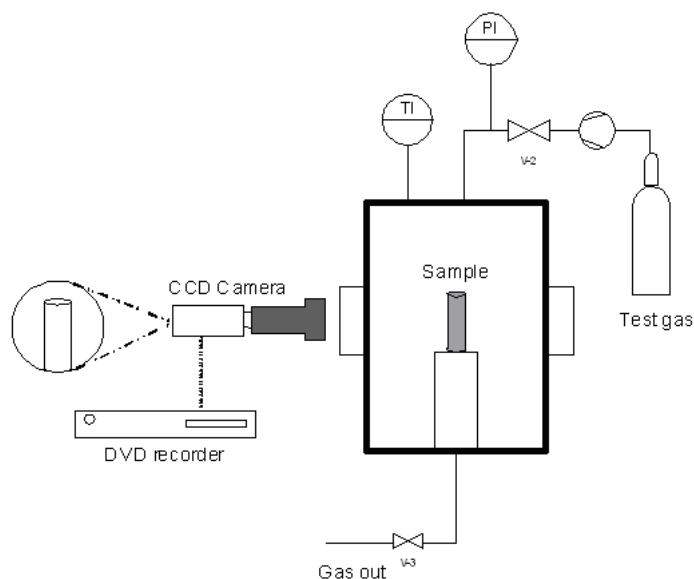


Figure 1: Setup for determining volume change of solid samples.

The amount of dissolved carbon dioxide is quantified with help of a high pressure sorption balance. The setup for these gravimetric measurements is shown in figure 2. The Material is filled into a small basket or glass containment which on its turn is attached to a permanent magnet inside an autoclave. Via a magnetic coupling the force resulting from the mass and buoyancy of the sample is transmitted to a microbalance outside the autoclave. The measurement precision of the balance is $1 \cdot 10^{-5}$ g.

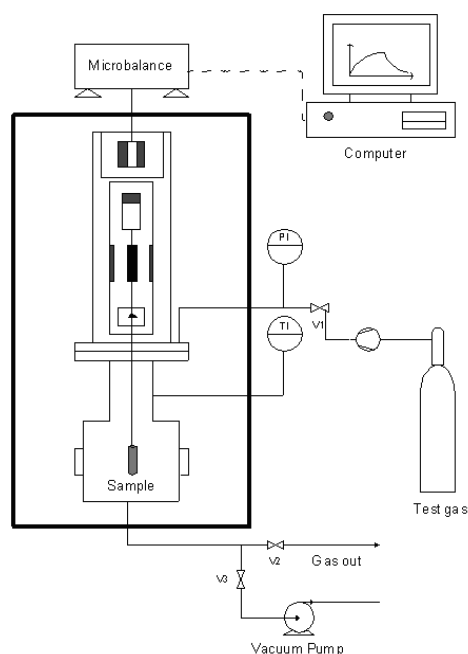


Figure 2: Setup for gravimetric determination of gas sorption.

A change in mass of the solid sample results from the gas being sorbed into the material but also from the transport of components of the plant material that are dissolved in the surrounding compressed carbon dioxide.

3.4 Extraction with supercritical CO₂

The flowsheet of the CO₂ extraction plant is shown in Fig. 3

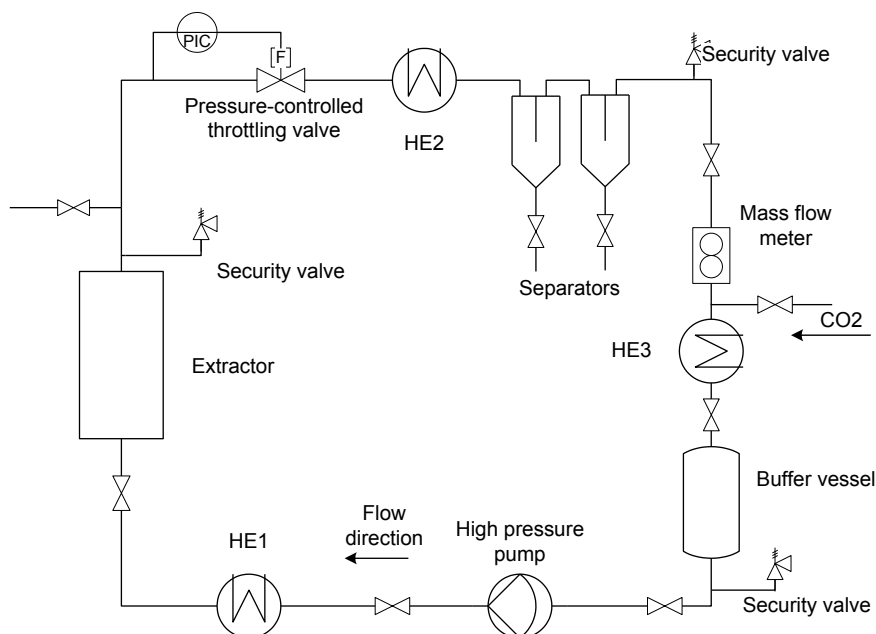


Figure 3 : Flowsheet of the CO₂ extraction plant

The CO₂ extraction process is operated semi-batch wise. The solid substrate is placed into the extractor and the compressed CO₂ is continuously flowing through the fixed bed in which the mass transfer from the solids to the solvent takes place. After expansion of the laden solvent the extract is collected in separators and discharged out of the system via valves. The amount of extract is determined gravimetrically. At the same time the total amount of CO₂ flow through the extractor is noted down from the mass flow meter.

Typical process parameters for extraction are – depending on the type of material – 12 to 50 MPa and temperatures in the range from 30 to 80 °C. Mass flow rates of CO₂ of 8 – 14 kg/h were applied. The volume of the extractor is 1.3 L (Table 2). The process conditions for the respective material were chosen according to the available literature data and our previous investigations.

Table 2 Extraction conditions

Plant material	Pretreatments	Pressure [MPa]/Temperature [°C]
Usnea barbata lichen	RM, CM, UM, RGD	30/40
Fennel	RM, UM	I fraction 90/40; II fraction 25/40

4. Results

4.1 SEM images

SEM images of St. Johns Wort before and after RGD are shown in the Fig. 4. As can be seen in the figure secretory structures can clearly be observed. However, strong effects of RGD on these structures where extractables are located in is not very evident since before and after RGD treatment seem to be similar. On the other hand, the cracks in Fig. 4b after RGD indicate that the expanding CO₂ damages the material during the decompression process.

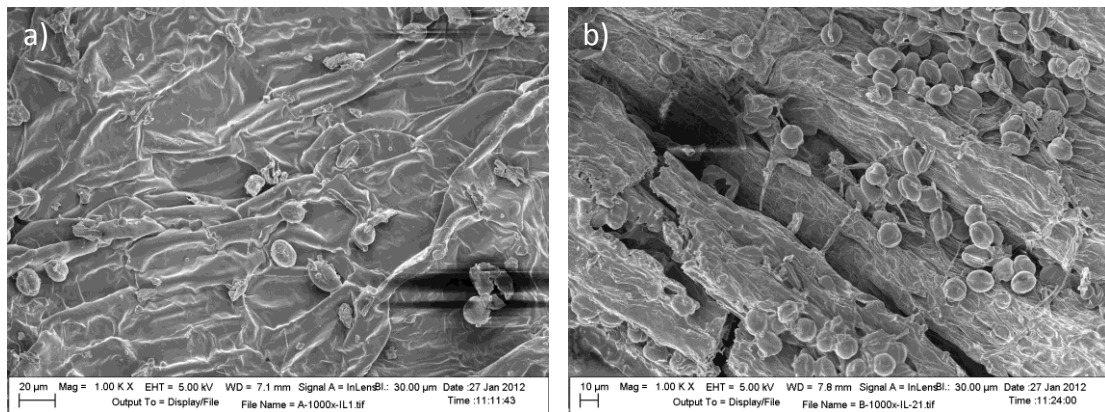


Figure 4: SEM images of St. Johns Wort before (a) and after (b) RGD (12 MPa, $T=40^{\circ}$, 1h exposure time). Magnification 1000x

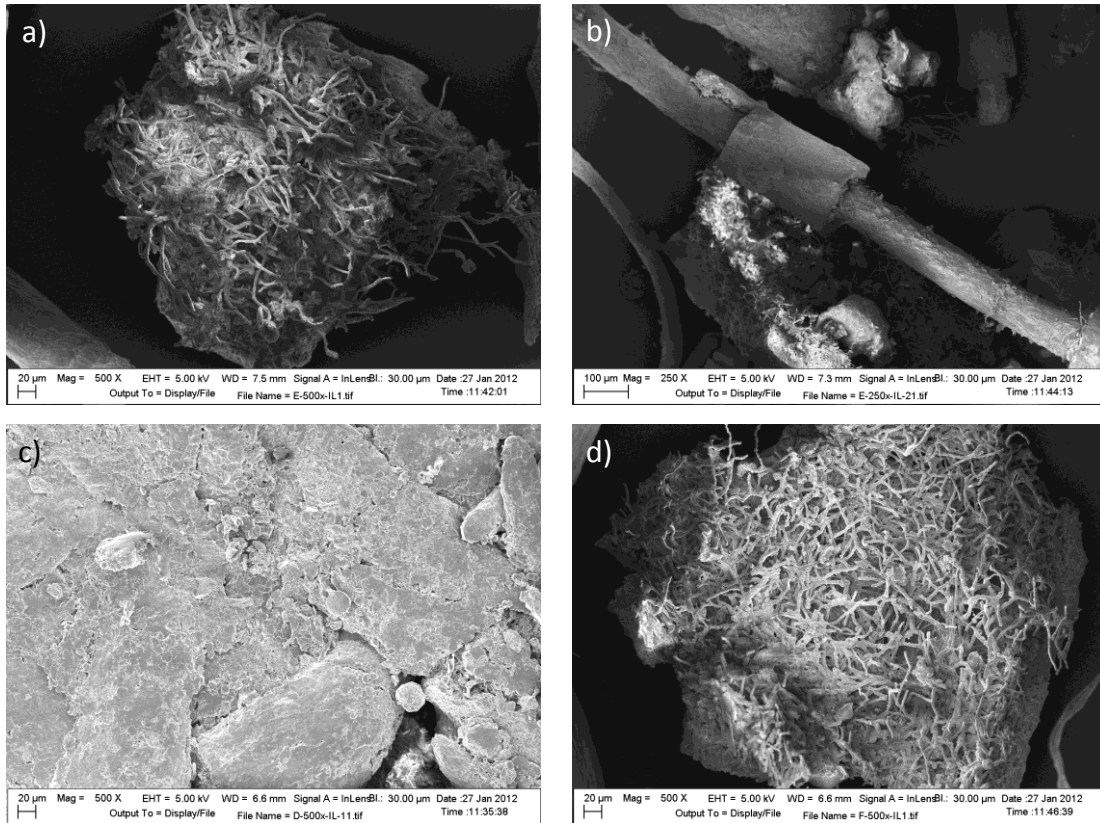


Fig. 5: SEM images of Usnea lichen. Pre-treatments: a) + b) ultracentrifugal mill, c) roller mill (flaking) d) ultracentrifugal mill + RGD (30 MPa, T=40 °C, exposure time 1h). Magnification: a,c+d) 500x, b) 250x

Unlike in the case of St. John's Wort, practically no effect of RGD on the inner structure of the material can be observed when RGD is applied to *Usnea* lichen (Fig. 5d). Flaking causes strong destruction and the physiological structure is completely ruptured (Fig. 5c).

4.2. CO₂ sorption kinetics

While the optical tests did not reveal a significant effect on the volume during and after pressurization, there was still a considerable mass uptake detected in all cases regardless whether or not the material contained large amounts of oil. Figure 6 shows the sorption and desorption in case of untreated St. John. In Figure 7 sorption in case of RGD treated St. John Wort is shown.

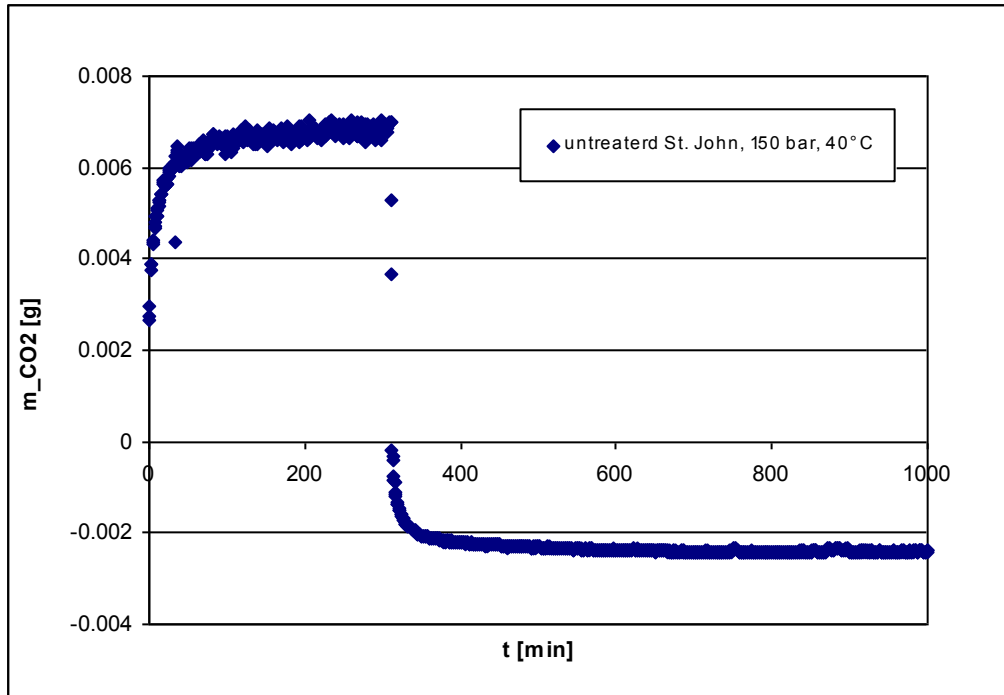


Figure 6: Sorption of CO₂ into St. Johns Wort

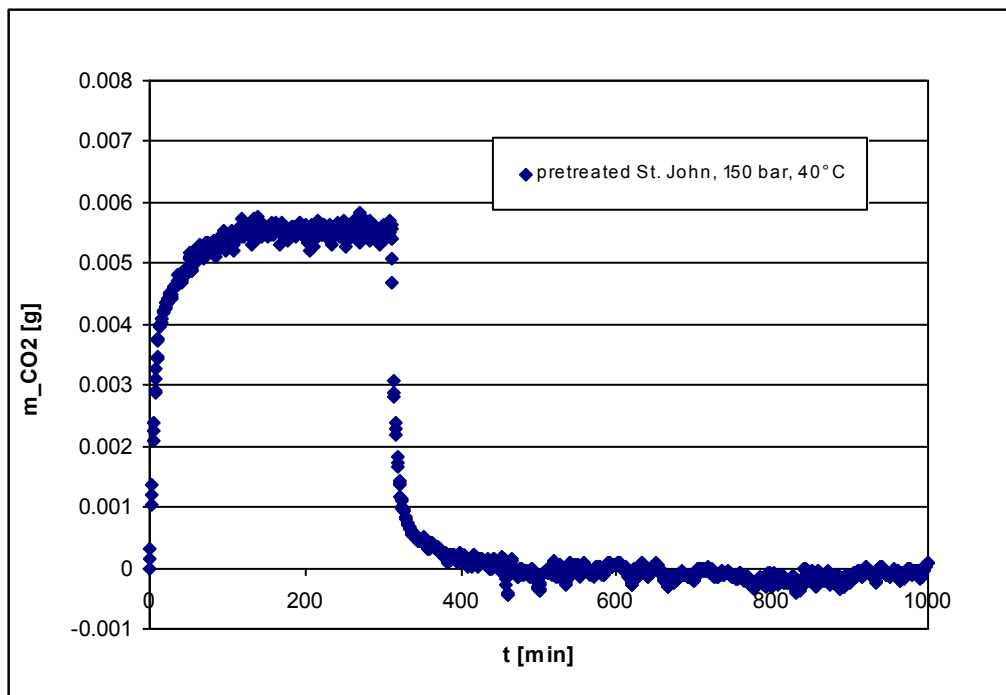


Figure 7: Sorption of CO₂ into RGD treated St. Johns Wort

The difference in mass uptake during sorption and loss of mass during desorption in Figure 6 results from the extraction of CO₂-solubles during the sorption period. In Figure 7 on the other hand, the mass after desorption remains the same as before the process. The reason for this may be that extraction of compounds by CO₂ occurs to a

smaller extent in the case of the RGD treated material that had been partially extracted during the RGD treatment prior to the sorption experiment.

The absolute amount of sorbed gas is very similar in both cases revealing that the gas is adsorbed by the solid matrix rather than absorbed by components with an affinity to carbon dioxide.

Table 3: Representative data on sorption of carbon dioxide in different solid matrices.

Material	T [° C]	P [MPa]	CO ₂ loading from sorption [wt. %]	CO ₂ loading from desorption [wt. %]
Sunflower Seeds	60	15	2.42	4.07
		25.5	3.78	10.0
Rapeseed	60	15	1.33	4.35
		26	1.59	5.57
Dried tomato	23	5	0.37	0.61
St. Johns Wort	40	15	15.40	20.88
St. Johns Wort (RGD treated)	40	15	11.36	11.32

These results of the sorption experiments together with the improved extraction kinetics of St. Johns Wort observed in literature indicate that possibly an adsorption of CO₂ to the plant matrix could be the reason for the efficiency of RGD in case of St. John's Wort.

4.3. Kinetics of the supercritical CO₂ extraction

In this project, RGD was tested for *Usnea* lichen and fennel. The resulting extraction kinetics are shown in Figs. 8 and 9. As can be seen, flaking showed by far the best results with respect to the final yield as well as the extraction rate for the tested materials.

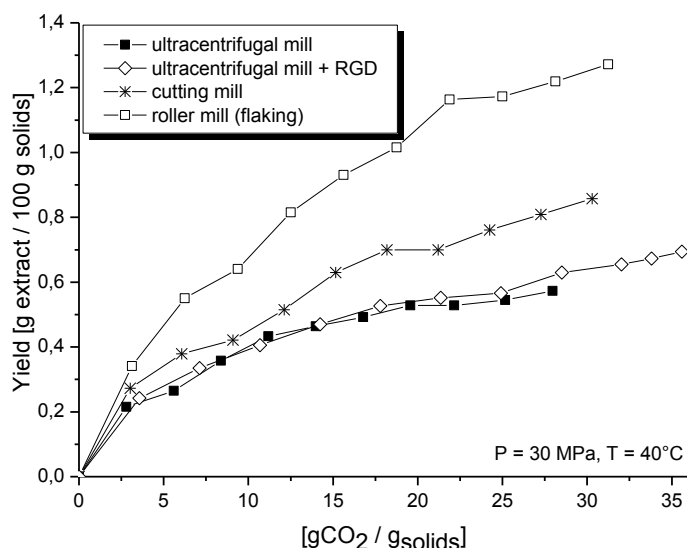


Fig. 8: Effect of pre-treatment on scCO₂ extraction of *Usnea* lichen ($P=30$ MPa, $T=40$ °C)

It was shown in literature that RGD treatment has the strongest effect in the case that prior mechanical pre-treatment was unefficient (*F. Meyer et al., Chemical Engineering and Processing 56 (2012)37-45*). For this reason, RGD was tested with lichen that was processed with the ultracentrifugal mill. However, RGD did not improve the extraction kinetics of *Usnea* lichen significantly.

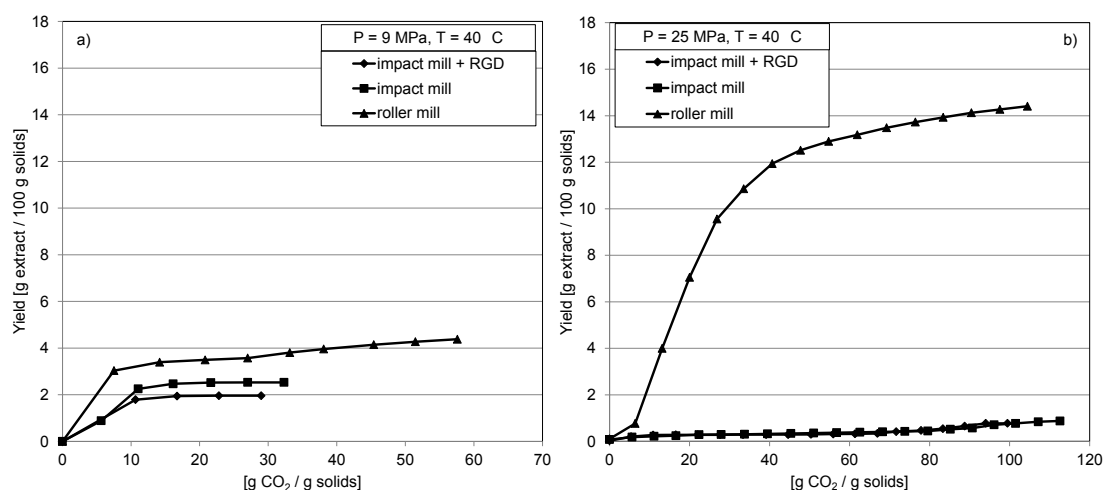


Fig. 9: Effect of pre-treatment on scCO₂ extraction of bitter fennel. a) extraction of essential oil fraction ($P = 9$ MPa, $T = 40$ °C). b) extraction of fatty oil fraction ($P = 25$ MPa, $T = 40$ °C).

The effect of pre-treatment methods on extraction of bitter fennel is presented in Fig. 9. The extraction was carried out as in two stages. First, the essential oil

fraction was extracted at a pressure of 9 MPa and a temperature of 40 °C (Fig. 9a). Then, an increased pressure of 25 MPa was applied at the same temperature in order to extract the fatty oils contained in fennel seeds (Fig. 9b). As can be seen, flaking is the most efficient pre-treatment method for extraction of both factions whereas impact-milling leads to lower yields. This fact is most evident for the extraction of the fatty oils. Thus, it seems that extraction of essential oils from fennel requires only a mild pretreatment whereas for extraction of the fatty oil fraction a stronger pre-treatment method such as flaking is recommended. This may be explained by the fact that in fennel essential oils are located in secretory ducts located as canals at the surface of the seeds whereas fatty oils are present in oil cells in the particle center. With respect to RGD treatment, a positive effect on the extraction was observed neither for the essential oil fraction nor for the extraction of fatty oils. This behavior is comparable to the extraction of fatty oils from rapeseed where RGD did not lead to an improved extraction behavior (*F. Meyer et al., Chemical Engineering and Processing 56 (2012)37-45*).

Conclusions

No positive effect of RGD on extraction yield has been observed for the extraction of *Usnea* lichen and fennel seeds. Besides RGD, different types of milling were tested as conventional mechanical pre-treatments. The best results regarding total extraction yield were observed when flaking pretreatment was applied for all tested materials. The additional sorption results of this project indicate that adsorption may play an important role for RGD treatment since remarkably high CO₂ loadings were observed St. Johns Wort for which a strong effect of RGD on the extraction is reported in literature.