ENCAPSULATION OF SENSITIVE NATURAL SUBSTANCES USING PGSS-TECHNOLOGY

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High pressure technology like PGSS (Particles from Gas Saturated Solutions) allows to generate microcapsules with properties (particle size, size distribution, morphology, etc.) which are difficult to achieve by classical methods.
Micro encapsulation of sensitive ingredients, available in liquid form, in a solid matrix has become a very attractive process in the last decades.
In this study encapsulation of soybean oil in a polymer matrix was performed as a model for the encapsulation of sensitive ingredients. The aim is to investigate the maximum concentration of oil which can be encapsulated by the PGSS process. In order to determine the morphology of the particles SEM pictures were taken. It was shown that up to 18% soybean oil can be encapsulated. Additional investigations were made for the particle size distribution and the quality of the product. Particle size distribution was measured by a laser diffraction method. The quality of the products was measured by antioxidant activity using DPPH (diphenylpicrylhydrazyl) assay. The results have shown that during the process the antioxidant activity was maintained.
The stability of oil can be extended by using spice extracts with antioxidant properties.
For the extraction of the antioxidant components Savory plant (*Satureja hortensis L.*) was selected and two different methods (supercritical fluid extraction and Soxhlet extraction with organic solvents) were investigated. Soxhlet extractions of savory with two different solvents (ethanol and pentane) were performed. Supercritical fluid extractions was carried out at 450 bar and 40°C using carbon dioxide.

Keywords: sensitive natural substances, extraction, PGSS encapsulation

INTRODUCTION

Autoxidation of lipids has been long recognized as a major deterioration process affecting both sensory and nutritional quality of food. Effective control methods against oxidation include the use of metal inactivators, minimizing the loss of natural tocopherols and exposure to air and light during the process, hydrogenation and the use of antioxidants.
Hydrogenation of polyunsaturated oils is becoming less and less attractive because of recent evidence that “trans”- isomers, which are formed during the process, may have adverse nutritional effects.

The inhibition of free radicals by antioxidants is of considerable practical importance in preserving polyunsaturated lipids from oxidative deterioration. Synthetic antioxidants have been commonly used in the US and several other countries to inhibit lipid oxidation and to retard the development of rancidity in foods. Although these synthetic antioxidants are

efficient and relatively cheap, special attention has been given to the use of natural antioxidants, because of a worldwide trend to avoid or minimize the use of synthetic food additives [1].
The encapsulation of active components in powders has become a very attractive process in the last decades, not only in food but also for the pharmaceutical industry. The main objective is to entrap a sensitive ingredient in a capsule of "wall", physically isolating the ingredient from the environment [2]. This barrier may confer protection against oxygen, water and light. Additionally micro encapsulation opens new possibilities for the controlled release of the active substances.
Using matrices like PEG 6000 the first goal of this work is to investigate the maximum concentration of a sensitive substance which can be encapsulated by the PGSS process. As a sensitive substance soybean oil was used.
Soybean oil has a triglyceride composition rich in monounsaturated (23% oleic acid), and polyunsaturated fatty acids (57% linoleic acid; 7% linolenic acid). Polyethylene glycols (PEGs) are widely used as additives for formulations and encapsulation of different pharmaceutical and food products. PEGs can be designed with tailor- made morphologies and size by supercritical spray processes [3].
In order to see if the parameters during the process had any influence on the quality, the antioxidant activity of the oil before and after the process (oil from the powder) was measured by using DPPH assay.
The second part of the work is to extract active compounds with antioxidants properties and their use for the encapsulation of the oil by the PGSS process in order to extend the stability. As a source of active components Savory plant was selected. *Satureja hortensis* L. is an annual, herbaceous plant belonging to the family *Lamiaceae*. It is known as summer savory, native to southern Europe and naturalized in parts of North America [4], and it is used in pharmaceutical and food industries (as a condiment). The main components which contribute to the antioxidant activity of savory are phenols (carvacrol and thymol), phenolic carboxylic acids (rosmarinic and caffeic) and triterpenoids (oleanolic and ursolic acids).
The quality of the extracts was evaluated by antioxidant activity using DPPH radical scavenging activity.

MATERIAL AND METHODS

Materials

For encapsulation by the PGSS process as a core materiel refined soybean oil was purchased from a Supermarket in Bochum, Germany.
As a shell material polyethylene glycol 6000 from Merck was used.
Dry savory plant (*Satureja hortensis*.L) was supplied by Rózsahegyi Ltd., Hungary.

Principle of the PGSS process

In Figure 1 the flow sheet of the PGSS pilot plant is shown. The maximum operating pressure and temperature of the used plant are 350 bar and 200°C, the maximum mass flow values are approximately 50 kg/h for shell material, 10 kg/h for core material and 150 kg/h for carbon dioxide (carbon dioxide flow is measured with Coriolis flow meter, the amounts of shell material and liquid are measured with differential weight).

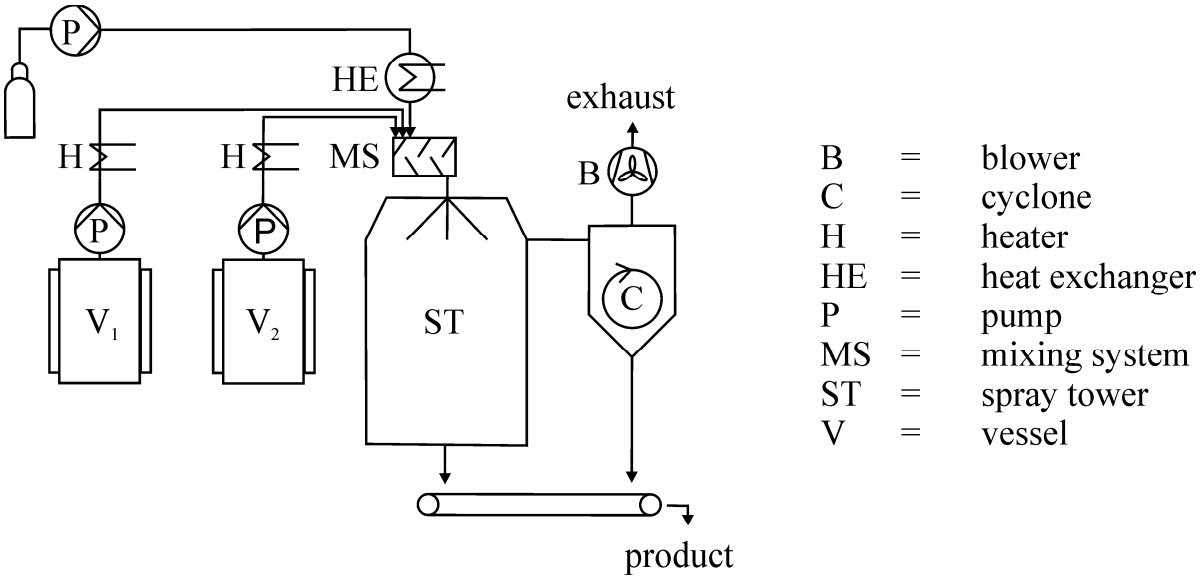


Figure 1: Flow sheet of the pilot plant

For encapsulation of liquids by the PGSS process the shell material (the material for the encapsulation) is filled and melted in vessel 1 (V1) and the core material (the substance to be encapsulated) is filled into vessel 2 (V2). By means of two high pressure pumps, both substances are dosed into a static mixer where they are continuously mixed and homogenized under high pressure (in carbon dioxide). Inside the static mixer micro droplets of the core material are dispersed in the liquefied shell material. The dispersion is expanded, to ambient pressure, through a nozzle into a spray tower (ST). By expansion two effects occur – formation of fine droplets and their cooling (Joule Thomson effect). The shell material solidifies and forms a cover around the drops of liquid, generating powderous composites.
Morphology and the size of these particles can be adjusted by the operating process parameters (pre expansion pressure and temperature, temperature in spray tower, mixing efficiency, gas to liquid ratio, nozzle geometry).

Solvent extraction

Soxhlet extractions of savory with two organic solvents with different polarity (ethanol and pentane) were carried out for 24 h.
The extracts were concentrated by rotary vacuum evaporator. The percentage yield was expressed in terms of dried weight of the plant material. The extracts thus obtained were used for the estimation of antioxidant activity.

Supercritical fluid extraction

The extraction experiments were performed with an extractor vessel (delivered by Natex Austria) with a capacity of 5 L. The extractions were performed with neat supercritical CO_2 (Linde, Repcelak).
Two extractions were carried out: the product was collected in one separator and two separators connected in series in order to separate the volatile oil from undesired compounds. A more detailed description of the equipment is given extensively elsewhere [5].
Both extractions were performed at 450 bar and 40°C. Samples around 1000 g of plant

material were weighed and put into the extraction vessel. The desired temperature and pressure were adjusted, and the CO_2 feed was started. The carbon dioxide flow rate was 7 kg/h. The accumulated product samples were collected and weighed at certain time intervals. The extractions were carried on until the amount of the last product sample decreased under 0.1 % of the raw material.

Sample preparation

The antioxidant activity was measured for the powder with 12% oil encapsulated.
Therefore 50 g powder was mixed for 1 h with 200 ml distilled water in a glass flask. To separate the oil phase from polymer-water phase 40 min of centrifugation at 11000 rpm were applied.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity of the extracts dissolved in methanol was tested by using DPPH assay.
The DPPH assay was carried out following the same method as reported elsewhere [6, 7].
Different concentrations of various extracts dissolved in methanol were added to 2.5 ml methanol solution of DPPH. After 30 min incubation period at room temperature, the absorption was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated in the following way:

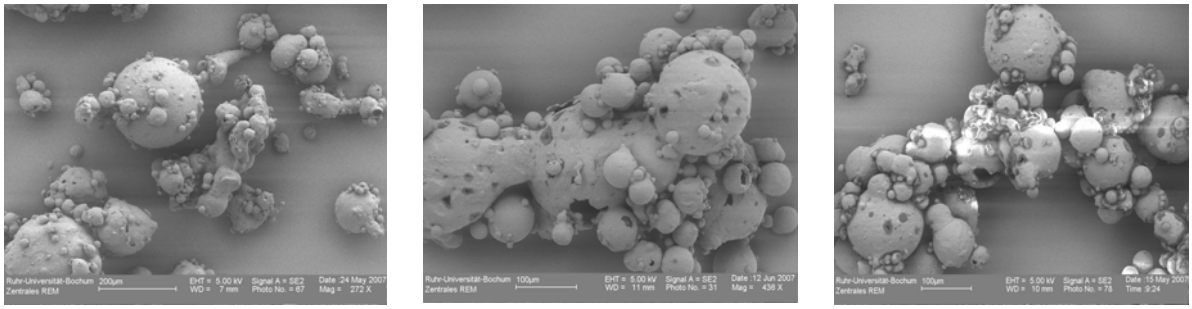
$$I\ \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100 \quad (1)$$

where: A_{blank} is the absorbance of the control reaction (containing all reagents except the test component) and A_{sample} is the absorbance of the test component.
Results were reported as IC 50%, where IC 50% was defined as the extract concentration necessary to decrease the initial DPPH concentration by 50%.

RESULTS

Encapsulation of soybean oil

PGSS experiments were performed on the system: PEG 6000/ soybean oil.
Three experiments with varying oil concentration in the obtained composite powder by using the PGSS process were carried out. The particle size distribution was determined with a Malvern Master-seizer 2000.
For the experiments PEG 6000 was heated at 80°C and used in molten form. Soybean oil was used at room temperature. The temperature in the spray tower was between 31 and 39°C.
The maximum concentration of oil which could be encapsulated was 12%. Spherical particles were obtained. By increasing the concentration of the oil to 18% on the surface of the particles small wet parts could be observed which can be only from the oil. By increasing the concentration to 28% on the surface of the particles oil droplets can be observed.
The influence of the concentration of soybean oil on the morphologies of the particles is shown in Figure 2.
Free flowing powder was obtained for the experiments with 12% concentration of oil. The powders with 18% and 28% were agglomerated.



12% 18% 28%

Figure 2: Morphology of the particles at different concentrations of soybean oil

Besides the morphology of the particles, the size distribution was determined via Laser diffraction. As can be seen in Figure 3 for the three different concentrations of oil manly two different particle sizes could be found.
The medium particle size ($d_{0.5}$) for the powder with 12% oil encapsulated is 145 µm and 70 µm for the powders with 18% and 28% oil.

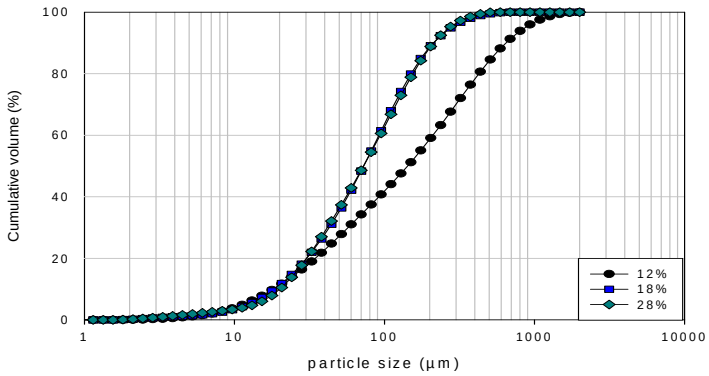


Figure 3: Cumulative size distribution for the system PEG 6000/soybean oil

The quality of the oil before and after processing

The principle of the antioxidant activity is the availability of electrons to neutralize any so-called free radicals. In this work, the antioxidant activity of soybean oil before encapsulation and of the oil after encapsulation was evaluated by using DPPH scavenging.
As is shown in Table 1 the quality of the oil during the PGSS process was maintained. The value of the percent inhibition of the free radical DPPH for the oil before the processing was 82.57 % and after encapsulation was 81.80 %.

Table 1: Characterization of the oil before and after encapsulation

Sample	**Antioxidant activity IC 50%**
soybean oil before encapsulation	82.57
soybean oil after encapsulation	81.70

Recovery percent and antioxidant activity of the extracts
The effect of polarity of the solvents on the yield and antioxidant activity of the plant material is shown in Table 2.

Table 2: Extract yield and the antioxidant activity of the extracts obtained with solvent extraction and supercritical fluid extraction.

Solvent	% Yield of extract	IC 50% (µg/ml)
Ethanol	25.36	24
Pentane	3.08	83
SFE 1	3.02	138
SFE 2	2.54	103

SFE 1 – the product was collected in one separator; SFE 2- the product was collected in two separators connected in series (the antioxidant activity was measured for the sample which was collected in the second separator)

Using the Soxhlet extraction the antioxidant activity of the extract obtained with ethanol was higher (the required concentration for the inhibition 50% was 24 µg/ml) than that of the extract obtained with pentane (the required concentration for the inhibition 50% was 83 µg/ml).
The explanation could be found in the polar character of the antioxidants. This would also explain the higher extraction yield found for the extraction with ethanol. Therefore the extract obtained with ethanol will be used for the encapsulation of the oil by the PGSS process.
In the supercritical fluid extraction the volatile oil obtained by fractionation in two stages has shown better antioxidant activity than the extract obtained in one separator, but worse than the extracts obtained with organic solvents.

CONCLUSION

The PGSS process was applied until now for many combinations of core and shell materials.
In this work the encapsulation of a sensitive substance by PGSS process was investigated. The results have shown that it is possible to encapsulate up to 12% soybean oil. By increasing the concentration of the oil small oil droplets could be observed on the surface of the particles. The parameters used during the process did not influence the quality of the products. Free flowing powder was obtained with 12% oil encapsulated.
The extract from Savory plant with ethanol has shown a higher antioxidant activity than the extracts obtained with pentane and supercritical carbon dioxide. The ethanol extract will be also used for encapsulation by PGSS process.

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