Encapsulation of essentials oils using biopolymers for their use in ecological agriculture

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A suitable formulation to improve Lavandin essential oil applicability as biocide has been study in this project. Agrochemical formulations are required to be long-term physical stable and enhance biological performance of the agrochemical. Particle encapsulation of lavandin oil had been considered as possible formulation. Two high pressure spray techniques (PGSS and PGSS drying) had been applied to encapsulate lavandin oil in a biodegradable polymer. Operating conditions were selected to reduce oil loses due to it dissolution in SC-CO_2. A comparison between the characteristics of microspheres obtained with OSA (n-octenyl succinic anhydride (OSAN)-modified starch) and PEG (polyethylene glycol) were performed. The obtained results revealed that encapsulation efficiencies of lavandin oil were higher for PEG microcapsules obtained by PGSS (14-66% of initial oil encapsulated). The morphology of these particles is spherical and presents a narrow particle size distribution, which seems to allow a controlled release of lavandin oil.

1.INTRODUCTION

Essential oils, obtained by stream distillation of certain aromatic plants, and their derivates have long been reputed as antimicrobial and insects repellent. These oils are presented as an alternative to conventional synthetic chemical pesticides, based on their reduced health risk and biodegradability [1]. Recent investigations confirm the biocide action of some essential oils (fennel, peppermint, caraway, eucalyptus, geranium and lemon). These essential oils have contact and fumigant insecticidal actions against a number of economically important insect and mite pests, as well as plant pathogens fungis. [1,2].

Lavandin oil (*lavandula hybrida*) has been selected as essential oil because of its great availability in the region of Castilla y León (Spain) and for its biocide properties. An bibliographic study on the activity of its individual components had been done. It has been demonstrated that several monoterpenes (α-terpineol, terpinen-4-ol and α-pinene) [3], linalool (30% lavandin oil) [4] and 1-8- cineole (8% lavandin oil) [5] show the biggest biocide activity.

A formulation that allows to protect the essentials oils from high temperatures, oxidation and UV light, live must be found. There are different agrochemical formulations and in this case microencapsulation has been considered as the most appropriated. The main advantages of the encapsulation are minimized evaporation, increased shelf live and controlled release, which may increase the biological efficiency. Conventional encapsulation techniques (solvent evaporation, phase separation and spray drying) require relatively high temperatures, which can be inappropriate for the stability of essentials oils and are not suited for producing microspheres with control particle size. High pressure technology allows to produce powders with properties difficult to achieve by classical methods. One promising possibility of these processes (e.g. PGSS (Particles form gas saturated solutions) or PGSS drying) is to obtain filled microparticles, solid-solid, liquid-solid.

For this application the carrier or shell material must be biodegradable and non toxic, so modified OSA-starches and Polyethylenglycol 9000 (PEG) has been considered, because they have been approved for its use in foods and medicine.

The more suitable process and polymer has been selected based on the amount of

encapsulated oil, particle size and particle morphology. Microcapsules of PEG produced by PGSS gave the higher encapsulation efficiency (66%), narrower particle size distribution and sphere like morphology.

2. MATERIALS AND METHODS

2.1. Materials

Lavandin oil used in this project was purchased from Silvestris & Szilas, which is a certified manufacturer of fragrances, essential oils and hydrosols in Hungary. This lavandin oil was produced by steam distillation.

As shell materials modified OSA starch derived from waxy maize (HI-CAP100), purchased from National Starch Group and Polyethylenglycol 9000 (PEG 9000) were used.

2.2 Preparation of feed emulsions

Water in oil emulsions were prepared by a two step process. First coating matrices suspension was prepared by dispersing the emulgent (modified OSAN-starch) in destillated water with the aid of Ultra-Turrax® UTC during 15 min at 3000rpm. Afterwards the oil was gradually dispersed in the aqueous phase to the suspension and the emulsion was agitated for 5 min at 6400 rpm.

2.3. Stability of lavandin oil emulsions in CO_2

Lavandin oil emulsion will be processes in a PGSS plant and during this process they will get in contact with supercritical CO_2. If the emulsion is not stable enough at operating conditions two phase will be formed and the encapsulation of lavandin oil in modified starch will be not possible. The stability of emulsions was tested in the temperature and pressure range of the operating conditions of the PGSS plant. The stability of lavandin oil emulsions at different CO_2 conditions was studied visually by a high-pressure view cell. This cell contains two shapphire windows and can withstand pressures of up to 450 bar. A heating jacked allows operating at constant temperature, which is measured by a thermocouple fitted in the cell. There is also a stirrer which allows an optimal mixing of the phases.

2.3. Particles from gas saturates solutions (PGSS)

The PEG lavandin oil particles were produced using the PGSS technique. In PGSS technique, PEG was used in molten form and CO_2 is dissolved in the liquids to form a gas-saturated solution. PEG and the core material (lavandin oil) are pumped through thermostated pipes and dosed into a mixing system were the two substances are intensively mixed in presence of heated CO_2 under high pressure. Thus micro droplets of the liquid are generated and are dispersed in the liquefied shell material. Upon a rapid expansion through a nozzle to ambient pressure very fine particles are produced as the gas come out of the solutions, due to the high level of supersaturation. Simultaneously the mixture is cooled down due to the Joule Thomson effect. The shell material solidifies and forms a covering layer around the liquid droplets. The particles are collected in a spray tower and a cyclone. The PGSS process takes advantage of the fact that a gas is more soluble in a liquid than the corresponding liquid is in the same gas. Therefore it uses small amounts of dense gas and allows to product fine powders under mild conditions [6]. The product characteristic may be modified by adjusting the process parameters, such as solute concentration, pre-expansion temperature and pressure, expansion temperature and pressure, and nozzle geometry [7].

2.4. PGSS drying

This technology was used to process water emulsions of lavandin oil and OSA, in order to obtain powders. In this spray drying processes a supercritical fluid facilitates the formation of extremely fine droplets which dry very fast resulting in fine powders. The liquid solution

is contacted with the supercritical gas in a static mixer and then expanded in a nozzle. The conditions in the spray tower are adjusted in a way that the solvent removed together with the gas and superheating of the mixture can be achieved.

The residual humidity in the dried powder depends on the relative humidity in the spray tower. Considerable amount of the solvent is already extracted into the gas in the static mixer. Therefore after expansion only a small amount of solvent has to be evaporated. By the combination of coextraction in the static mixer and evaporation of residual solvent the demand of CO_2 is reduced [8].

2.5. Particles characterization

Particle size and particle size distribution of the samples obtained by PGSS drying and PGSS, were measured with a laser diffraction method using the Mastersizer 2000 particle analyzer. The morphology of the particles was determined with a scanning electron microscope (SEM). The moisture content of the samples was measured by Karl Fischer volumetric titration using 870 KF Titrino plus from Metrohm.

Bulk density was determined by the tapping method. A 25 ml graduated cylinder was filled with the powder. The cylinder containing the powder was tapped on a flat surface until a constant volume of 25 ml was reached. The final weigh of the powder was recorded and bulk density was calculated by dividing the sample weigh by the volume. [9]

2.6. Analysis of ancapsulated oil

Total encapsulated oil in the PGSS powder was determined gravimetrically. Ten grams of powder were placed in an extraction thimble. The powder was extracted with hexane for 4 hours. Each extract was evaporated to dryness at 40ºC and 335mbar on a rotary evaporator. The total encapsulated oil was determinated by weighing the residue.

3. RESULTS

3.1. Stability of lavandin oil emulsions in CO_2

The stability of lavandin oil emulsions was determinate at different pressures, temperatures and lavandin oil concentrations of the emulsion. Using a solution of 250 g /L (maximum amount of OSA soluble in water), different amounts of lavandin oil were added in the ratio lavandin oil/OSA 0.2, 0.3 and 0.4 and the emulsion was formed as it is described in section 2.2. It had been considered that the emulsion is not stable when two phases appears or when it present creaming. The results shown in table 1, suggest that emulsion stability is hardly affected by pressure and slightly by temperature. For the operating conditions (around 100 bar and 100ºC) the emulsion is stable enough for all cases.

	Lav/OSA: 0,2					Lav/OSA: 0,3					Lav/OSA: 0,4				
T (ºC) /P (bar)	1	75	80	90	100	1	75	80	90	100	1	75	80	90	100
40	60	45	45	30	10	60	45	30	30	15	60	45	15	15	15
60	60	45	35	12	8	60	45	-	-	-	60	30	35	15	12
70	60	-	-	-	-	60	45	-	-	-	60	-	-	-	-
80	60	40	-	12	7	60	45	-	-	-	60	-	-	15	12

Table 1. Stability of lavandin oil emulsions in minutes for different emulsion compositions

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3.2. PGSS

The carrier component used was PEG 9000 and the core material was lavandin essential oil. To study the morphology, particle size distribution, bulk density and

encapsulated lavandin oil, experiments were carried out varying the lavandin oil/PEG ratio, gas to product ratio (GPR) and pre-expansion pressure. The temperature before expansion, temperature in the collection vessel of the final product and nozzle (1,4mm) were kept constant. The results of the experiments are reported in table 2.

	V1	V2	V3	V4	V5
Lav/PEG	0,25	0,32	0,29	0,37	0,34
$T_{before\ exp}$ (ºC)	80	84	77	82	76
$P_{after\ exp}$ (bar)	56	57	54	56	85
GPR ($m_{CO2}m_{PEG+Lav}$)	0,68	0,66	0,58	0,92	1,26
Lav/PEG	0,25	0,32	0,29	0,37	0,34
T_{tower} (ºC)	33	34	34	31	30
%Lav encapulated	33	66	44	19	14
$d_{0,5}$ (µm)	80	95	105	88	129
Bulk density (kg/m^3)	512	540	461	367	213

Table 2. Results of the PGSS experiments.

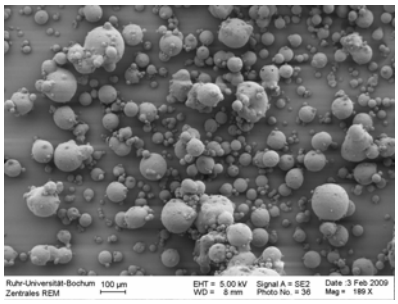


Figure 1. PEG particles produced by PGSS.

Encapsulation efficient obtained varied from 14 to 66 %. It resulted to be dependent mainly on the pre-expansion pressure decreasing until 14% when the pressure reached 85 bar. Pre-expansion temperature and GPR have no a clear influence on the encapsulation efficiency. This fact can be explained because lavandin oil solubility in CO_2 increases sharply with the pressure [10]. Particle size is hardly influence by pressure and slightly influence by GPR. Particles obtained present a narrow particle size distribution, almost perfect sphere morphology and no agglomerates. It could be observed in SEM picture presented in figure 1.

3.3. PGSS drying

The carrier component used in this case was OSA and the core material was lavandin essential oil. Both were used in the form water/oil emulsion. To study the morphology, particle size distribution, bulk density and encapsulated lavandin oil, experiments were carried out varying the lavandin oil concentration in the emulsion, gas to solute ratio (GTS) pre-expansion temperature, pre-expansion pressure and static mixer. The static mixer used in all the cases was formed for 10 elements with an internal diameter of 10mm. The results of the experiments are showed in table 3.

The efficiency of the encapsulation of lavandin oil in OSA microcapsules varies from 6 to 52 %, as shows table 3. The content of oil in the liquid homogenized lavandin oil emulsion was varied from 4 to 17 g/100g emulsion and consequently, the encapsulation efficiency varied. The static mixer is another main parameter, in fact operating without static mixed results in encapsulation efficiencies from 8 to 18 %. pre-expansion temperature hardly affects encapsulation efficiency and Pre-expansion pressure seems not to have an important effect on encapsulation efficiency.

In order to study the oil delivery, the release of encapsulated oil before 20 days was determined for all samples. All the samples were stored at 15ºC in a dry room. The obtained results suggest that oil release is related mainly to initial amount of oil in the emulsion.

The residual moisture content, measured in samples obtained, was in the range 6,7-4,12 wt%, similar to the starting material one (moisture content initial OSA 5wt%).

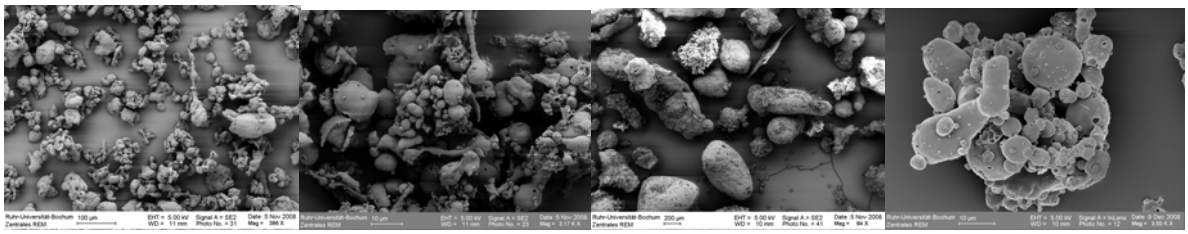
The particle size distribution of OSA particles obtained by PGSS drying exhibit a bimodal and unimodal distribution. An explanation for bimodal distribution could be the agglomeration of particles, because in these experiments due to the process parameters

applied the Joule-Thomson effect seems to be lower and the temperature in spray tower is higher. Particle size is hardly dependent of pre-expansion pressure, as show the results represented on table 3. Pre-expansion temperature and GPR have a negligible effect on encapsulation efficiency.

	V1	V2	V3	V7	V8	V4	V5	V6	V9	V10	V11
oil/starch	0,2	0,2	0,2	0,2	0,3	0,4	0,4	0,4	0,4	1,0	1,0
Pre-exp P (bar)	124,	120	100	103	104	120	121	117	105	106	90
Pre-exp T (ºC)	127	117	129	100	116	112	131	127	113	114	108
GPR	22,5	27,3	22,4	33,2	35,8	25,1	24,1	33,3	41,2	28,0	35,5
Temp. (°C)	64	66	72	68	60	71	70	72	75	73	74
CO_2 (kg/h)	77	75	75	79	79	72	73	90	91	88	77
Nozzle	1,2	1,4	1,4	1,4	1,4	1	1	1,4	1,4	1,4	1,4
Emulsion (kg/h)	3,7	3,6	3,9	2,4	2,9	4,1	3,6	2,7	2,6	3,6	2,6
Static mixer	yes	yes	no	yes	yes	yes	no	no	yes	yes	yes
encapsulate oil %	30	52	18	34	32	18	6	13	43	46	45
linalool%	40	39	32	-	-	27	9	6	-	-	-
g oil deliver/100g product in 20 days	0,02	0,61	0,28	0,35	0,87	1,74	1,62	0,90	5,56	14,55	16,07
d(0,5) um	21	25	194	49	25	29	15	40	33	46	48
Bulk density (kg/m^3)	167	337	260	175	150				156	114	181
Residual moisture %	6,71	5,88	4,78	4,12	5,75	5,02	4,72	5,15	5,49	4,57	5,26

Table 3. Results of the PGSS drying experiments.

SEM pictures of the particles (figure 2) demonstrate that it possible to obtain filled micro PEG particles with lavandin oil encapsulated. The different particles shapes obtained by PGSS drying are mainly amorphous crystal or spheres. Low pre-expansion temperatures produce many crystals and few but spheres (figure 10b,). At high temperature mainly small spheres (figures 10a, 10c, 10d) are produced. It can be seen also, that at low pre-expansion pressures there is a clear predominance of particles with spherical shape (figure 10c). The used of static mixer favours the production of non porous particles and more regular spheres (figure 8c, 8d). These results coherent with the results obtained by Rodrigues et al. [11].



a b c d

Figure 2. Example of OSA particles produced by PGSS emulsion drying: a=V1, b=V2, c=V3, d=V5.

4. CONCLUSIONS

Owing to the potential of powder in agrochemical formulations, an intensive study was performed in order to obtain stable capsules of lavandin oil using biodegradable polymers (OSAN-starch and polyethylene glycol) as carrier materials. High pressure technology (PGSS drying and PGSS) was successfully applied for the production of OSA and PEG microcapsules filled with lavandin oil respectively. Ongoing investigation bases whether and how particle size, morphology, bulk density and percentage of encapsulated oil may be influenced by operating conditions.

The effectiveness of microencapsulation is the most important characteristic features of the process. With PGSS drying the encapsulation efficiency varied from 6 to 55%. Higher encapsulation efficiency was found for the PEG microspheres produced by PGSS varying from 14 to 66 %. This result can be expected, because for PGSS no water has to be removed and operating conditions are milder comparing with PGSS drying. However, The possibility of the incomplete oil recovery also should be considered, because in both processes some oil might had been lost by evaporation, solubilised in CO_2.

Lavandin oil release from OSA microcapsules was study in order to warrant that the formulation suits its purpose. This release results almost dependent on the percentage of lavandin oil of the initial emulsion and slightly operation conditions.

The microspheres produced obtained by PGSS drying usually present two kind of particle: spherical particles together with small irregular shaped crystals. It can be concluded that PGSS drying has some limitations, because latter morphology is not appropriate to be used if a controlled release of the oil is required. In case of PGSS spherical particles without agglomerates had been obtained. The observed behaviour was justified considering the faster precipitation route performed during the SC-CO_2 processing that may prevent droplet coalescence or aggregation phenomena typically observed during solvent evaporation process.

These results suggest that, starting from an emulsion, particle morphology, particle size distribution and encapsulation efficiency of the produced particles are nor only related to the precipitation process but also related to solvent elimination process.

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