Innovative Romanian Food Biotechnology *Vol. 9, Issue of September, 2011*

Received July 10, 2011 / Accepted August 14, 2011

RESEARCH ARTICLE

ENCAPSULATION OF GARLIC ESSENTIAL OIL BY BATCH PGSS PROCESS

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Abstract

Garlic (*Allium sativum* L.) is reach in several volatile and non-volatile compounds responsible for treatment or prevention of different diseases, but also for the characteristic pungent aroma and taste that could be reduced by encapsulation methods. In this study, a hydrophilic and biodegradable compound had been used to formulate garlic essential oil by means of the PGSS process. The particles properties (size, distribution, bulk density and morphology) were depending on the experimental conditions (pre-expansion temperature and pressure, GPR and type of mixing element). Powders with particle sizes ranging from 71.124 μm to 205.64 μm were obtained, showing an increasing of the particle size when pre-expansion temperature is increasing. The batch PGSS system has been applied and a good encapsulation efficiency of garlic oil (26.10 – 48.93%) was obtained. The optical microscope images indicated agglomeration of the particles as the quantity of oil increase, suggesting that some fraction of oil that is not encapsulated makes particles sticky.

Key words: particle from gas saturated solution, supercritical carbon dioxide, *Allium sativum* L. essential oil, PEG

Introduction

Allium sativum L. is the most common food being used for prevention and treatment of the parasitosis, digestive tract disorders, fungal infection (Iciek, 2009), anticoagulant, antihypertensive, anticancer, hepatoprotective, immune-modulation effect, diabet type 2 and cataract (Banerjee *et al.*; 2000, Kimbaris *et al.*; 2006, Lanzotti, 2006).

Essential oils are fractions obtained from plants by physical methods (steam distillation, supercritical fluid extraction) being sensitive materials under the action of oxygen, light and moderate temperatures (Martín *et al.*, 2010). Garlic essential oil is in a liquid form at room temperature, non-miscible in the water and presents a specific odour due to the organo-sulphur compounds. These aspects can be overcome by encapsulation techniques. Encapsulation methods can be classified into physicochemical methods (interfacial

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polymerization, simple and complex coacervation, molecular inclusion, liposome encapsulation, co-crystallization, gelification and high pressure technologies) and mechanical methods (spray drying, extrusion, fluidized bed coating) (Dima, 2009).

The use of supercritical fluids techniques for formulations of essential oils allows innovative formulations due to the properties of supercritical carbon dioxide: near ambient critical temperature 31.06°C and a moderate critical pressure 7.385 MPa, no contamination of the product, attractive solvent for processing heat-sensitive materials, easy to be separate from the final products (Sanders, 1993; McHugh & Krukonis, 1994; Saengcharoenrat and Guyer, 2004; Martín *et al.*, 2010; Nalawade *et al.*,2007). Particles from Gas Saturated Solutions (PGSS) process is a successful encapsulation technique based on the high solubility of supercritical carbon dioxide in molten biopolymers, oils and fats (Martín *et al*., 2010). In this research, the polyethylene glycol 6000 (PEG 6000) was used as the biodegradable shell material. As well, the influence of the PGSS operation conditions on particle size, particle distribution, morphology, bulk density and garlic oil encapsulation efficiency has been studied.

Materials and methods

Materials

Garlic oil produced by steam distillation was purchased from Silvestris & Szilas Ltd. (Kerepes, Hungary). PEG6000 (polyethylene glycol with molecular weight 6000) with the melting rage 60 - 63°C was supplied by Fluka Chemika GmbH. (Steinheim, Switzerland) and used as shell material for encapsulation of garlic oil. Carbon dioxide (96%) was provided by "Carburos metálicos" (Barcelona, Spain).

Essential garlic oil formulation by batch Particles from Gas Saturated Solutions (PGSS)

The flow sheet of the batch PGSS plant used for this research is showed in figure 1. The PGSS process produces particles by spraying of gas saturated solutions in two steps: the substance it is melted and mixed with the dense gas in a high pressure vessel (*PV*). The vessel was heated and pressurized, so CO_2 was dissolved in PEG, decreasing the melting point and viscosity of the biopolymer (Wendt *et al*., 2007). The mixture was continuous mixed using different type of mixers (*M*), during a period longer enough to reach phase equilibrium. In the second step, the biphasic mixture was expanded through a nozzle into a spray tower (*ST*). During to the expansion at atmospheric pressure, due to the Joule – Thomson effect, the dense gas cools down and the biopolymer solidifies and forms a covering layer around the essential oil droplets. The particles with small sizes are formed in a spray tower (*ST*) and CO_2 and finer particles are removed. In order to keep the pressure constant in the vessel during depressurization a piston pump which allows selecting the set-pressure, was used. Maximum operating pressure and temperature of this plant were 30 MPa and 200°C. Maximum volume flow rate of supercritical carbon dioxide pump (*P-01*) was 16.3 l/h. The experimental conditions applied for garlic oil formulation by PGSS using two types of mixers (figure 2) are presented in table 1 (4 flat mixing elements) and table 2 (3 round mixing elements), respectively.

Powders characterization

A laser diffraction method using a Dynamic Light Scattering (Malvern Mastersizer 2000 particle analyzer with accuracy ± 1% and measuring range between 2 and 2000 μm) was used for determination of the particle size and particle size distribution of the garlic oil in PEG 6000 powders. During laser diffraction measurements, particles are passed through a focused laser beam. The particles scatter light at an angle that is inversely proportional to their size. The angular intensity of the scattered light is measured by a detector. The particles size of the powders were measured dispersed in air (Scirocco 2000 dry powder feeder).The results are the average of three measurements. The following optical parameters: PEG refractive index 1.402 and air refractive index 1.000 has been applied. Morphology of particles was examined using the Olympus BX 41 with contract phase's automated microscope and equipped with epifluorescence. Bulk density of the powder important for the transport and storage purpose was determined by the tapping method (Hanu, 2010; Varona *et al*., 2010).

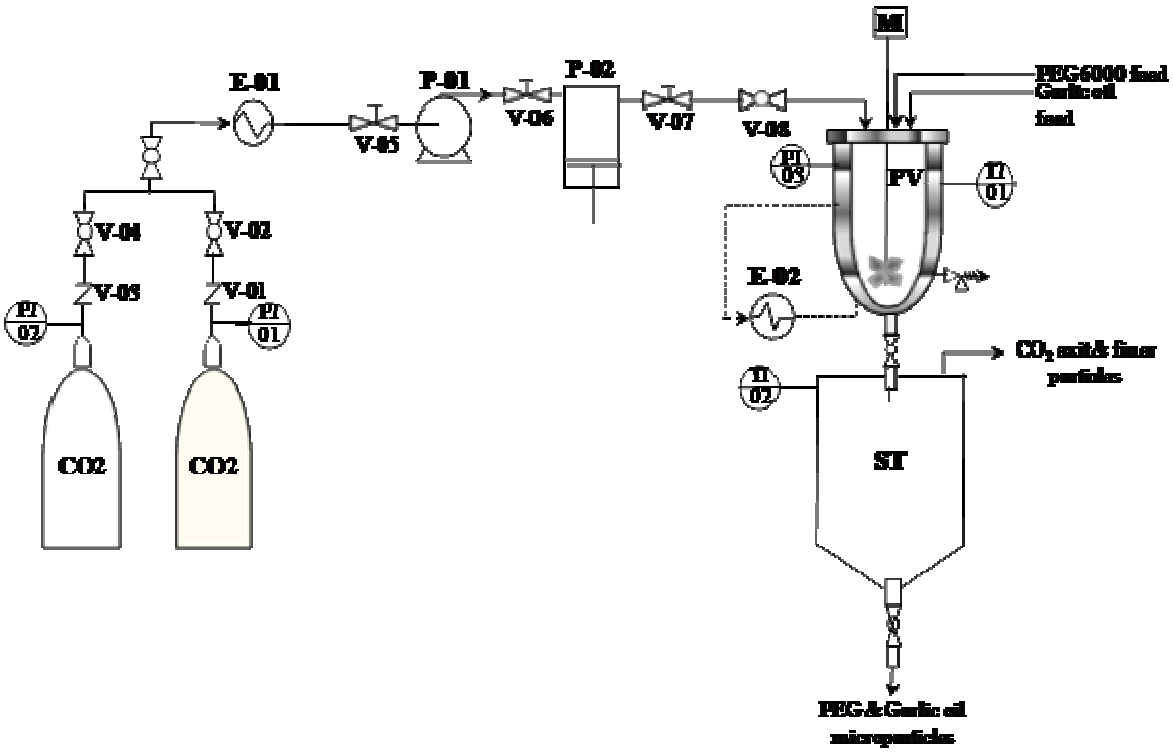
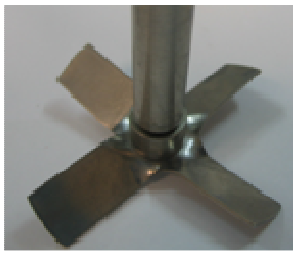


Figure 1. *Flow diagram of the batch PGSS process*

PV – pressure vessel; ST – spray tower; M – mixer; E-01 – cooling unit for CO_2; E-02 – water bath; P-01 – supercritical carbon dioxide membrane pump; P-02 – piston pump; PI – pressure indicator; TI – temperature indicator; V-01, …, V-08 – valves.



a) 4 flat mixing elements

b) 3 round mixing elements

Figure 2. *Mixers with different number of mixing elements used in PGSS experiments*

Table 1*. Experimental parameters for formulation of garlic oil by PGSS process using mixer type with 4 flat mixing elements*

Experiment	**GA1-4**	**GA2-4**	**GA3-4**	**GA4-4**	**GA5-4**	**GA6-4**	**GA7-4**
GA oil/ PEG 6000 mass ratio	0.214	0.174	0.451	0.236	0.29	0.224	0.214
$t_{before\ exp}$,°C	56.61	58.58	58.52	53.86	60.76	62.21	54.03
$p_{before\ exp}$,bar	166.61	186.01	186.063	167	196.96	201.17	189.48
GPR ($m_{CO2}/m_{PEG+Garlic\ oil}$)	129.53	110.23	109.05	171.8	99.15	97.17	134.55
t_{ST},°C	25.53	24.58	24.46	21.17	21.87	24.97	21.78

Table 2. *Experimental parameters for formulation of garlic oil by PGSS process using mixer type with 3 round mixing elements*

Experiment	**GA1-3**	**GA2-3**	**GA3-3**	**GA4-3**	**GA5-3**	**GA6-3**	**GA7-3**
GA oil/ PEG 6000 mass ratio	0.267	0.409	0.293	0.308	0.343	0.362	0.213
$t_{before\ exp}$,°C	52.07	55.44	56.32	51.28	49.36	52.95	61.05
$p_{before\ exp}$,bar	173.35	197.39	198.68	157.65	171.2	184.17	203.4
GPR ($m_{CO2}/m_{PEG+Garlic\ oil}$)	127.5118	129.0668	145.1091	148.138	152.847	126.9559	98.128
t_{ST},°C	26.1	26.43	25.16	23.91	23.57	21.89	22.41

Determination of the encapsulation efficiency of garlic oil

PEG 6000 is widely soluble in water and possesses a low vapor pressure even at high temperature. The encapsulation efficiency of garlic oil powders was determined by dissolving about 1.5 g of microcapsules in 100 mL distillated water due to the solubility of biopolymer in this solvent. The mixture was maintained in a Clevenger-type apparatus for 4 h according to Moretti *et al.* (2002) with some modifications. After the system was cooled, the encapsulated oil was determined gravimetrically.

Results and discussion

Encapsulation of garlic oil in PEG 6000 by PGSS process

Garlic oil was encapsulated in biodegradable biopolymer PEG 6000 in a batch PGSS system. The experimental conditions presented in table 1 and table 2 indicate a variation of garlic oil/PEG 6000 mass ratio, gas to product ratio (GPR), temperature and pressure before expansion, the number and shape of mixing elements. Particles produced have sizes between 50 μm and 210 μm. In figure 3 and figure 4 is presented the influence of process parameters on particles size. It can be seen that particles size increases when the pre-expansion temperature is increased (figure 3) and decreases when GPR (gas product ratio) is increased (figure 4). The highest GPR values are within the range of 97.1 to 171.8. Under these conditions, particles having sizes in the range from 71.12 μm to 205.63 μm are obtained.

In conclusion, an increase of GPR leads to a decrease of the mean particle size. The trend of the temperature can be explained considering the influence of temperature on CO_2 solubility. Since CO_2 solubility in polymers decreases with temperature, the viscosity of the molten compound is decreases due to the dissolved gas. Therefore, the saturated mixture is efficiently sprayed to form small droplets in the precipitation chamber. In the case of GPR an increase of this parameter also enhance the atomization.

The PGSS process allows decreasing the melting point of the biopolymer. As the melting point of the PEG 6000 it is around 60°C, the encapsulation of garlic oil experiments were performed to under these temperatures. At 51.28°C lower particle sizes were obtained (66.85 μm). This can be explained because the lower pre-expansion temperatures determine more intense cooling due to Joule-Thomson effect and a faster solidification of smaller particles. The distribution of the volume frequency versus particle size for the mixer with 3 round mixing elements is approximately narrow (figure 5) with minimum values for d_{01} = 12.128 μm and maximum d_{09} = 350.88 μm, respectively.

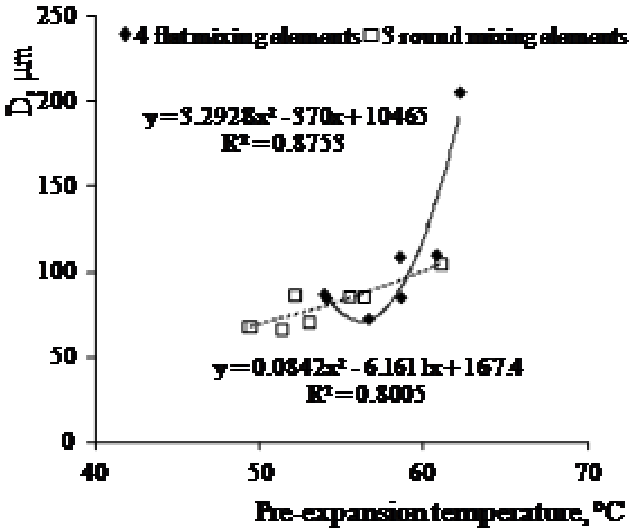


Figure 3. *Influence of the pre-expansion temperature on particles size*

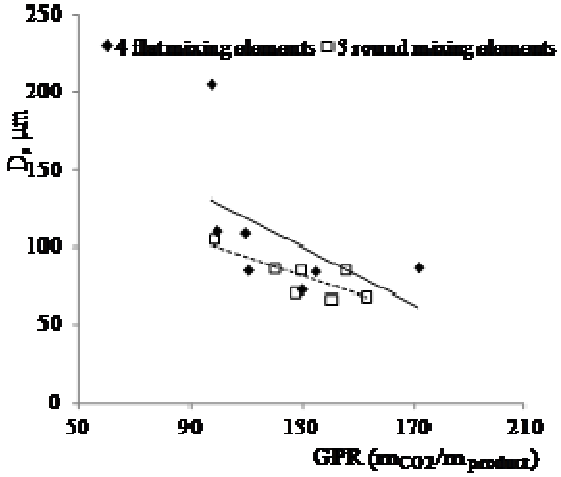


Figure 4. *Influence of the gas to product ratio on particles size*

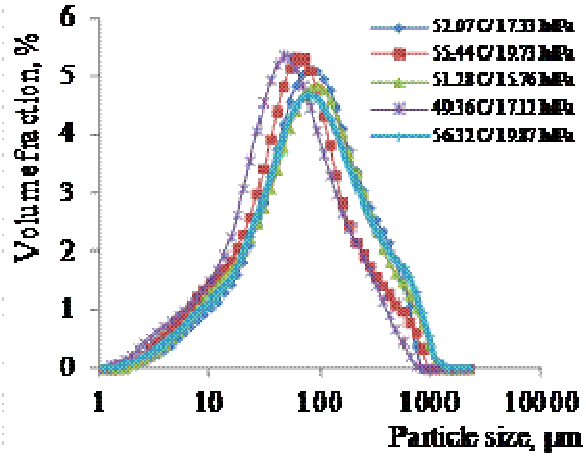


Figure 5. *Particles size distribution for powder obtained by PGSS process with 3 round mixing elements*

The encapsulation efficiency of the garlic oil in PEG 6000 varied between 30.9 – 35.3% for the mixer with 4 flat mixing elements and 26.1 – 48.9 % for 3 round mixing elements mixer type, respectively (figure 6). For the both type of mixers, encapsulation efficiency decreased when pre-expansion pressure and GPR are increased. The higher values of the pre-expansion pressure determined the increasing of the solubility of essential oils in CO_2 and completely miscibility with supercritical carbon dioxide above the critical point (Martín *et al.*, 2007). The gas amount increasing determines reduction of the encapsulation efficiency because the essential oil is extracted to the gas phase.

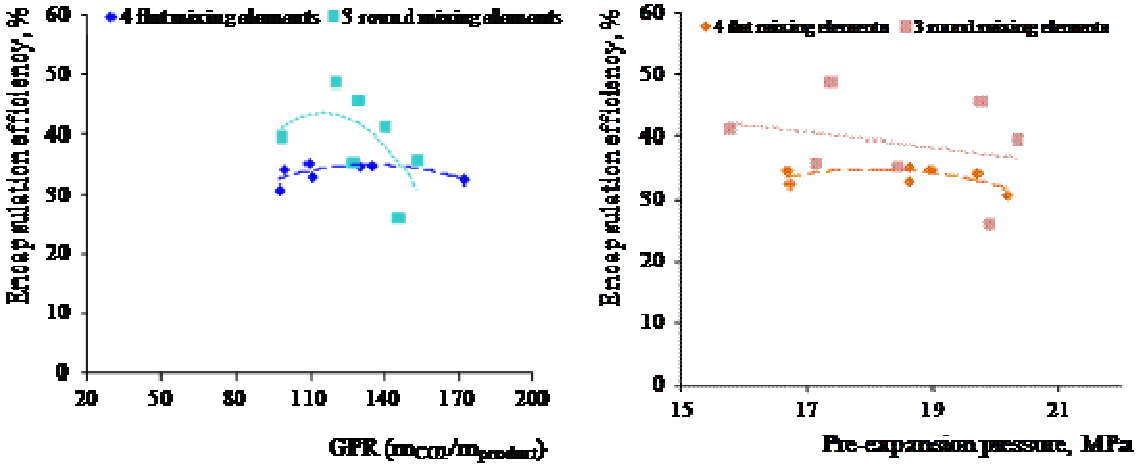
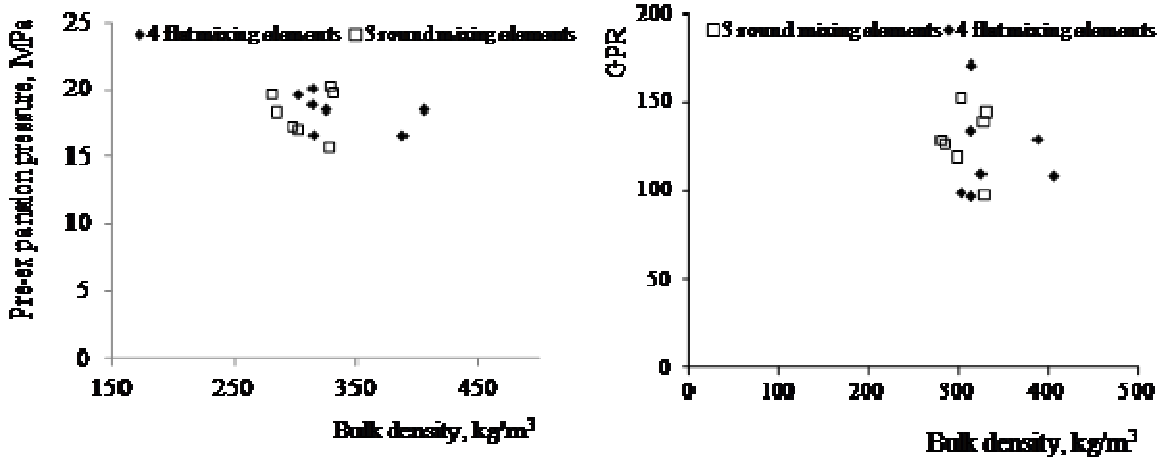


Figure 6. *Influence of the process parameters on the encapsulation efficiency*

Bulk density compared to particle size shows a similar dependence on pre-expansion pressure. An increasing pre-expansion pressure leads to a decrease of bulk density (figure 7a). The bulk density exhibits a maximal value of 404.52 kg/m^3 for a pre-expansion pressure of 186 bars for 4 flat mixing elements and 326.72 kg/m^3 at 157 bars for 3 round mixing elements, respectively. The same dependence of the bulk density on the pre-expansion pressure was reported by Wendt (2006).



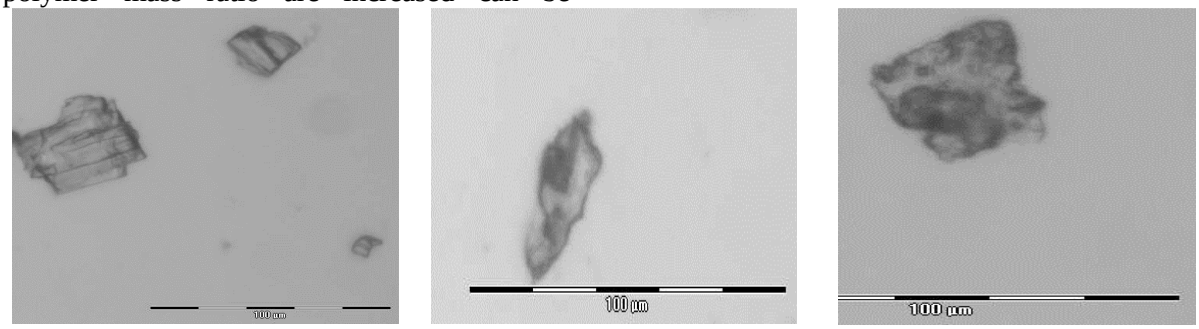
a. ***b.***

Figure 7. *Influence of pre-expansion pressure and GPR on bulk density*

The GPR influences the powders bulk density (figure 7b). A smaller GPR determine a higher bulk density value. For a minimum value about 98.12, a bulk density 327.80 kg/m^3 is obtained. The higher bulk density value 404.52 kg/m^3 occurs for approximately GPR value 100.

Morphology of the powders obtained by batch PGSS process

The influence of the process conditions on the particles morphology is presented in figure 8. In all cases amorphous particles have been obtained. When the pre-expansion pressure and garlic oil biopolymer mass ratio are increased can be observed some agglomeration of particles (figure 8 c, e, d). These images suggest that some oil is not encapsulated. This it is in accordance with decreasing of encapsulation efficiency at higher pre-expansion pressure (figure 6) that making the particles sticky.



a) PEG 6000 without essential oil

b) garlic oil /PEG 6000 = 0.409 55.44°C, 197.39 bar

c) garlic oil /PEG 6000 = 0.214 61.05°C, 203.4 bar

d) garlic oil /PEG 6000 =0.451 58.52°C, 186,06 bar

e) garlic oil/PEG 6000 = 0.174 58.58°C, 186.01 bar

Figure 8. *Microscope images (x20) of garlic oil particles loaded in PEG 6000 by batch PGSS process*

Conclusions

Particle of garlic oil loaded in PEG 6000 was obtained by PGSS process. PEG 6000 is a biodegradable biopolymer and very suitable shell material for encapsulation of essential oils. The highest encapsulation efficiency was achieved at 47 % for 197 bars when the used mixers have 3 round mixing elements. Moreover, when the pre-expansion pressure and oil – biopolymer mass ratio are increased the particles are more agglomerated and became sticky. Our results demonstrated the efficiency of the high pressure technologies for formulation powder with potential applications in food sciences.

Acknowledgments

This work has benefited from financial support through the 2010 POSDRU/89/1.5/S/52432 project, Organizing the National Interest Postdoctoral School of Applied Biotechnologies with Impact on Romanian Bioeconomy, project co-financed by the European Social Fund through the Sectoral Operational Programme Human Resources Development 2007-2013.

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