Proceedings of Engineering & Technology (PET)
pp. 18-22

Study of Asteriscus maritimus (L.) essential oil encapsulation with almond gum -gelatin by Complex Coacervation Method

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***Abstract*— This work deals with the study of Asteriscus maritimus (L.) essential oil encapsulation by complex coacervation method with almond gum -gelatin using tannic acid as hardening agent. The effects of core material, concentration of wall material, tannic acid and Tween 80, were investigated on particle size and encapsulation efficiency.**

***Keywords*— encapsulation, Complex coacervation, essential oil, almond gum.**

I. INTRODUCTION

Nowadays, scientific advance is being used in the development of innovative products [2]. To have success in such competitive and demanding sector, the products must differentiate which can be achieved by means of using emergent technologies, such as encapsulation [3]. Encapsulation is a method designed for protection, isolation and assists in the storage and controlled release of these products. Essential oils, obtained by stream distillation of certain aromatic and medicinal plants, have long been reputed as antimicrobial and insects repellent [1]. A suitable formulation to improve Asteriscus maritimus (L.) Less. essential oil applicability as biocide has been study in this work. Complex coacervation is the technique used to encapsulate Asteriscus maritimus (L.) Less. essential oil by gelatin/ almond gum microcapsules using tannic acid as hardening agent [5].

II. MATERIALS AND METHODS

A. Chemicals and reagents

Chemicals reagents used were purchased from Sigma-Aldrich-Fluka (Saint- Quentin, France). Almond gum from P. Dulcis trees was manually collected from the regions of El maadher-Menzel bouzeine (middle of Tunisia).

B. Plant material

Aerial parts of Asteriscus maritimus (L.). Were collected from the regions of El maadher-Menzel bouzeine (middle of Tunisia) in the period of March.

Extraction method

Essential oils were extracted from air-dried and cut plants by hydrodistillation for 4 h using a Clevenger apparatus. After decantation, essential oils obtained were dried using anhydrous sodium sulfate and stored at 4 °C until use.

C. Chemical analysis

Gas chromatography/mass spectrometry (GC-MS). Analysis of essential oils was performed with GC (column, oven temperature, flow rate of the carrier gas) using a Varian Star 3400 (ENIG, Tunisia) gas chromatograph equipped with a Varian Saturn GC/MS/MS 4D capillary column HP5 MS in apolar methylsiliconate, length 30 m and internal diameter of 0.25 µm which the stationary phase consists of 5% of phenylmethylsilicone and 95% of diméthylpolyxilosane. The oven temperature is programmed at 45 ° C for 2 min, then 45 ° C to 220 ° C with a "slow rate" of 5 ° C / min and then again isothermal for 2 min at 220 ° C. The injection mode is maintenuen split mode at 250 ° C, and the injection volume is of the order of 0,5µl. The carrier gas is helium (ET) with a 1-1,2ml.min flow, split 1/60 report.

The components were identified based on the comparison of their RI (retention indices) and mass spectra with those of standards, Wiley 2001 library data (NIST 02 version 2.62) of the GC-MS system and literature data (Adams). Alkanes (C5-C24) were used as reference points in the calculation of RI.

III. ENCAPSULATION OF ESSENTIAL OIL

A. Preparation of almond gum aqueous solutions

An aqueous solution of almond gum (AL) was prepared at different concentrations (5, 7.5 and 10% (w/w)). Desired amounts of gum powder were dissolved in deionized water containing 0.02% (w/w) sodium azide and stirred overnight to ensure complete dissolution. The pH of the AL aqueous solution was adjusted to 5.0 using 0.1M HCl or 0.1M NaOH, as required [5].

B. Emulsions production

The used steps can be described briefly as follows:

Step I – Microcapsules Formation

Firstly, an emulsion of essentiel oil in water (o/w, with a ratio of 0.68 %, v/v) stabilized with 1% (w/v) of a nonaionic surfactant, and a PLA (Poly(DL–lactide)) solution in dimethylformamide (DMF) have been prepared.

Emulsification

ISSN: 2356-5608

The o/w emulsion was obtained by dispersion with an ultraturrax (IKA DI 25 Basic, yellow line) at 11,000 rpm during 70 seconds.

Coating core material

The PLA solution was added dropwise to the previously prepared o/w emulsion. The homogeneous solution of PLA in DMF, upon contact with water, promotes the precipitation of PLA around the essentiel oil core. The encapsulation process has continued under moderate stirring (100 rpm) using an impeller stirrer in a batch reactor (IKA Model LR-2.ST) during one hour at room temperature.

Step II – Microcapsules Consolidation
Hardening

The microcapsules formed were hardened by adding OCMTS (Octamethylcyclotetrasiloxane) and allowed to stand during one hour. OCMTS is a widely used hardening agent. It acts as non solvent for the PLA coacervate droplets thus promoting microcapsules solidification.

Step III – Washing and storage

After hardening, the microcapsules were decanted and sequentially washed with Pluronic® F68 solution (0.1% w/w), an ethanol solution (30% v/v), and hexane. Ethanol and hexane have the role of, respectively, removing any remaining polar and apolar compounds that weren't encapsulated. Pluronic F68, a surfactant, was added to keep the microcapsules solution stable during the washing process.
Finally, the microcapsules were freeze-dried during 40 hours and stored in powder form, or alternatively, stored directly in the Pluronic® F68 solution.

IV. CHARACTERIZATION TECHNIQUES

A. *Size distribution of microcapsules (Laser Dispersion)*

Particle size distribution of the produced microcapsules was analyzed by laser dispersion using a Laser Diffraction Particle Size Analyser LS 230 (Beckman - Coulter). The corresponding medium values in volume and number were determined.

B. *Optical microscopy and Cryogenic Scanning Electron Microscopy (Cryo- SEM)*

Microcapsules morphology was analysed by optical microscopy (Leica DM 2000 microscopy equipped with software Leica Application Suite Interactive measurement and with transmitted light mode) and by cryogenic scanning electron microscopy.

C. *Gas chromatography GC-FID/MS*

Quantification of the encapsulated essential oil was performed by gas chromatography GC/FID and the corresponding composition determined by GC/MS according to the procedure described in the paragraph 6.
The composition of esential oil was expressed in percentage values determined from GC - FID peak areas in a base without solvent. The mass of encapsulated oil has been calculated using a mass balance.
The individual components that characterize the nonencapsulated essential oil were quantified by analysing the two phases obtained after microcapsules separation by decantation (aqueous phase and microcapsules rich phase).
1 ml of the aqueous phase and 1 ml of the microcapsules surrounding solution were collected using a syringe equipped with a 0.45 µm pore size filter and thereafter analysed by GC-FID.
The mass of encapsulated oil was obtained by difference between the loaded original quantity and the nonencapsulated determined quantity. The encapsulation efficiency (percentage of essential oil present in microcapsules) was calculated based on the formula bellow.

$$\text{Encapsulation Efficiency (\%)} = \frac{m_{tot} - m_{out}}{m_{tot}} X100$$

Where m_{tot} : amount of loaded essential oil (g) and m_{out} :amount of nonencapsulated essential oil (g).

V. RESULTS AND DISCUSSION

A. *Size distribution of microcapsules (Laser Dispersion)*

The experimentally measured particle size distributions are shown in table, for the prepared microcapsules. It was observed a bimodal distribution in volume with a mean particle size of 46 µm. In number the distribution was quite narrow and unimodal, with a mean particle size around 5 µm.
The same particle size distribution measurement was quantified both relative to the total number of particles and to the total volume of particles and it was observed that 90% by number of particles have diameters smaller than 14 µm (1% > 10 µm), but this represents 18% of the particles by volume (90% > 14 µm). This means that, even a large number of microcapsules have small size. Table I show microcapsule mean particle size obtained for two replicas of the experiment (batch 1 and 2). Although the obtained distributions have a wide dispersion, the results pointed out for a good reproducibility.

TABLE I
MEAN PARTICLE SIZE IN VOLUME OF MICROCAPSULES IN TWO EXPERIMENTS.

	Particle size (µm)
1	43.78
2	55.4

B. *Optical microscopy and Cryogenic Scanning Electron Microscopy (Cryo - SEM)*

The analysis by optical microscopy had the objective to study the microcapsules morphology after the production (Figure 1 and 2). Figure 1 shows the aspect of the microcapsules in the

bright field option at different magnifications. Microcapsules have spherical shape, with different sizes.

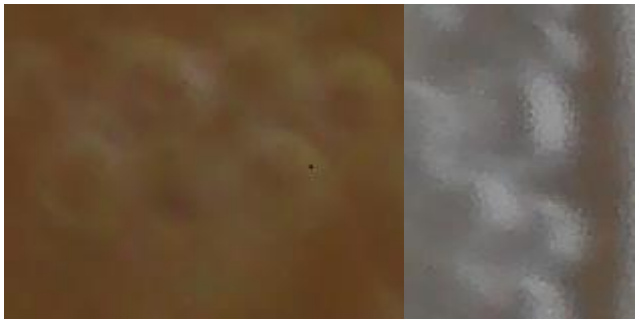

Fig. 1 Optical microscopy of microcapsules solution . Magnifications of images: 100x.

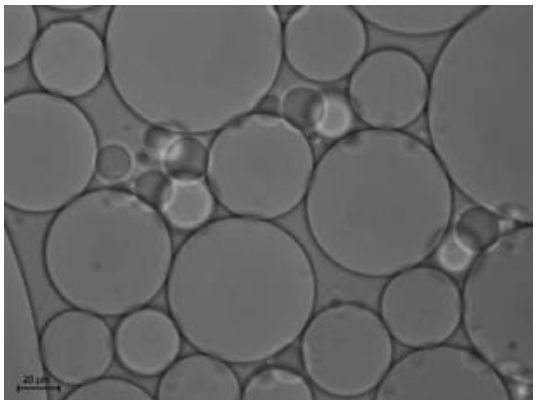

Fig. 2 Optical microscopy of microcapsules solution . Magnifications of images: 400x.

C. Gas chromatography GC-FID/MS

The major components identified from the different aerial parts of Asteriscus maritimus, their retention indices and their percentage composition are summarised in Table II. A total of 43 compounds have been identified from the different fractions studied, representing more than the 90% of the total oil for each one. The oil from the stems and leaves showed α-pinene (27.5%) and p-cymene (10.0%) as major components.
The encapsulation efficiency accounts for 55.47% of oil used in the encapsulation process. Table III shows that α-pinene and β-phellandrene have a higher percentage of encapsulation with values of 85.89% and 82.79%, respectively. On the other hand, the component with smaller percentage of encapsulation was α-phellandrene with 24.10% and fonenol with 11% .

The encapsulation efficiency can be related to the chosen type of surfactant used. Tween®20 (polyoxyethylene sorbitan monolaurate) is a non-ionic surfactant with a high value of hydrophil/lipophilic balance (HLB) (HLB of 16.7) widely used in biochemical applications. Since it contains no electrical charge, it is resistant to water hardness deactivation, thus enhancing the emulsion stability.

TABLE III
COMPOSITION OF THE ESSENTIAL OIL FROM ASTERISCUS MARITIMUS (A.M).

Compound	I	**A.m.SL**
α-pinene	**934**	27.5
α-phellandrene	1005	7.2
p-cymene	1019	10
β-phellandrene	1027	8.3
Monoterpene hydrocarbons		53.7
Oxygenated monoterpenes		0.9
Sesquiterpene hydrocarbons		8.8
Oxygenated sesquiterpenes		29.3
Total		92.7

TABLE IIIII
TOTAL, ENCAPSULATED AND NONENCAPSULATED MASSES DISCRIMINATED BY ASTERISCUS MARITIMUS OIL COMPONENT.

Component	**m_{tot} (g)**	**$m_{nonencapsulated}$ (g)**	**$m_{encapsulated}$ (g)**	**Encapsulation efficiency (%)**
α-pinene	0.78	0.11	0.67	85.89
α-phellandrene	1.12	0.85	0.27	24.10
p-cymene	1.19	0.67	0.52	43.69
β-phellandrene	0 .93	0.16	0.77	82.79
Total	4.02	1.79	2.23	**55.47**

VI. CONCLUSIONS

Microcapsules of Asteriscus maritimus, essential oil have been produced by complex coacervation using PLA dissolved in DMF. The hardening of the produced microcapsules was obtained with OCMTS. Microcapsules particle size distributions were determined by laser dispersion. It was observed a bimodal distribution in volume with a mean particle size of 46 μm. Analysis by optical microscopy and by cryogenic scanning electron microscopy has confirmed the spherical shape.
Quantification of the encapsulated Asteriscus maritimus, essential oil was calculated based on GC-FID peak areas and the mass of encapsulated A.m essential oil has been calculated

using a mass balance. The encapsulation efficiency accounts for 55.47% of oil used in the encapsulation process.

ACKNOWLEDGMENT

This work couldn't be achieved without the help and support of a great amount of people to whom I wish to express my sincere gratitude:

- Pr. Mehrez ROMDHANE my supervisor, for giving me the opportunity to do a challenging PhD research, for his guidance, his perpetual enthusiasm and his trust during these years.
- Mr. Houcine BEN DAOUED for his participation to the examination of the thesis, his trust and support.

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