Microencapsulation of Essential Oils

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In this work thyme and coriander oil were encapsulated using complex coacervation microencapsulation technique.The influence of various microencapsulation parameters on encapsulation efficiency was investigated. The release characteristic of the essential oils from microcapsule was studied.

Keywords: essential oils, collagen hydrolysate, microencapsulation, coacervation

Currently, the use of synthetic chemicals to control insects and arthropods raises several concerns related to environment and human health. Therefore,there is a growing interest to replace synthetic chemicals with natural products with bioactive properties, like good efficacy, and are environmentally friendly [1].Among those chemicals, essential oils from plants belonging to several species have been extensively tested to assess their repellent properties as a valuable natural resource [2].Essential oils, isolated from a large number of plants, have been found to have insect repellents properties against various haemato-phagous arthropods, some of them being the basis of commercial repellent formulations [3].

Essential oils from plants within the Lamiaceae family at several concentrations were evaluated for their repellent and deterrent properties against *Thripstabaci* Lindeman (Thysanoptera: Thripidae), the onion thrips. Rosemary (*Rosmarinus officinalis* L.) essential oil repelled onion thrips females at 10% concentration. Marjoram (*Origanummajorana* L.) and mint (*Mentha arvensis* L.) oil both at 0.1% and 1% concentrations significantly deterred the egg-laying activity of thrips females on treated leaf discs. Lavender (*Lavandulaangustifolia* L.) oil at 1% and sage (*Salvia officinalis* L.) oil at 0.1% concentration affected oviposition [4].

Thirteen essential oils (*Apiumgraveolens, Citrus sinensis, Eucalyptus globulus, Juniperusoxycedrus, Laurus nobilis, Lavandulahybrida, Mentha microphylla, Mentha viridis, Ocimumbasilicum, Origanum vulgare, Pistaciaterebinthus, Rosmarinus officinalis,* and *Thujaorientalis*) were tested in their vapour form against *Acanthoscelidesobtectus*. The tests revealed that most of them have a repellent action, reduce fecundity, decrease egg hatchability, increase neonate larval mortality and adversely influence offspring emergence [5].

Despite of their high potential, essential oils are unstable and fragile volatile compounds. Consequently, they could be degraded easily (by oxidation, volatilization, heating, light) if they are not protected from external factors. Such protection could provide a controlled release, thus increasing their action duration [6]. A widely used technology to increase the essential oils stability is microencapsulation, which role is to protect the active compound creating a barrier that avoids chemical reactions and/or enables the controlled release of their contents at a specific moment, or over prolonged periods of time [7]. The purposes of this study were:*i*) to encapsulate thyme and coriander oil using complex coacervation microencapsulation technique; *ii*) to investigate the influence of various microencapsulation parameters on encapsulation efficiency and *iii*) to study the release characteristic of essential oils from the containing microcapsules.

Experimental part

Materials

The essential oils were purchased from Naturela, hydrolyzed collagen was provided by National Research and Development Institute for Textile and Leather - INCDTP-ICPI, while Glutaraldehida 50% was procured from Scharlab SL Spain. Besides these, the non-ionic emulsifiers used were from internal production.

Equipment

UV: Spectra Suite UV-VIs spectrophotometer (Ocean Optics, USA) was used for recording all scan and absorbance measurements in 1cm quartz cells.

SEM: Themorphological study of the microcapsules was carried out using a scanning electron microscopy (SEM) with a scanning electronicmicroscope FEI QUANTA 200 (FEI Company, USA) at an accelerating voltage of 30 kV, and a distance about 7 - 8mm.

TGA: Thermogravimetric analyses (TGA) of the microcapsules were done with a TGA/SDTA 851 (MettlerToledo, Switzerland) in the temperature range 20-600°C, at a heating rate of 10°C/min, under nitrogen flow.

*GC-MS:*The distribution of essential oils (thyme and coriander oil) was acquired using GC-MS/MS TRIPLE QUAD (Agilent 7890 A) with DB-WAX capillary column (30 m length, 0.25 mm internal diameter, 0.25μm film thickness) and helium as carrier gas at 1mL/min. The oven temperature was initially set at 70°C increasing to 230°C with 4°C per min and hold time of 5 min. The GC injector and MS ion source temperatures were 250 and 150°C, respectively. The transfer line temperature was 280°C. The MS detector was operated in EI mode at 70 eV, with a m/z scanning range of 50-450.

Microencapsulation procedure

Aqueous dispersions containing hydrolyzed collagen in distilled water at various concentrations were prepared. The essential oil was emulsified by gradual introduction into the collagen dispersion, stirred at 800 rpm with a mechanical turbine-type stirrer and maintaining at 10-18°C.

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Table 1
ESSENTIAL OILS COMPOSITION

No.	Compound name	Oil composition, % mass	
		Thyme	Coriander
1	β-cymene	8.13	5.77
2	Limonene	13.67	7.78
3	α-pinene	0	12.88
4	γ-terpinen	0.26	3.05
5	α-terpineol	3.84	0
6	Thymol	39.29	0
7	Isothymol	31.53	0
8	d-camphor	0	4.62
9	β-linalool	0	61.85
10	Other	3.28	4.05

Various hydrolyzed collagen / oil ratios were used. The emulsion quality was improved by emulsification in the ultrasonic field at different irradiation times using a T 420 ELMA Transsonic T 420 ultrasonic bath. The coacervated phase was obtained by the gradual addition of an anionic polyelectrolytes solution containing sodium tripoly-phosphate and sodium alkylbenzene sulphonate, in the oil emulsion stirred at 800 rpm, followed by cooling to 5°C. The complex coacervation process has been improved by ultrasonic field irradiation. The resulting microcapsules were crosslinked by the slow addition of a glutaraldehyde 50% with or without addition of methanol under intense stirring at 800 rpm, the temperature of the mixture rising from 5°C from the exothermicity of the reaction. The crosslinking process was improved by ultrasonic irradiation during treatment with glutaraldehyde and a short time afterwards. The reaction mixture was stirred for a certain period of time to complete the crosslinking reaction. The microcapsules were separated and washed with 0.1% sodium alkylbenzene sulphonate solution using an Hettich ROTINA 420R centrifuge. The aqueous phase was collected for the purpose of determining by distillation of the amount ofessential oil not encapsulated. The wet microcapsules were frozen at -20° C and dried using a Martin Christ ALPHA 1-2 PLUS Freeze Drying Equipment, or they have been used as such in the preparation of foliar fertilizer compositions.

Determination of encapsulation efficiency

The encapsulation efficiency (EE) was determined using the following equation:

$$EE = m_1/m_2 * 100, \quad (1)$$

where, m_1 =the amount of essential oil contained in the microcapsules; m_2 = the total amount of essential oil used.

The amount of essential oil contained in the microcapsules (m_1) was determined using eq. (2).

$$m_1 = m_2 - m_3 \quad (2)$$

where, m_3 = the amount of the essential oil from aqueous phase collected after microcapsules filtration, by solvent extraction.

All experiments were carried out in triplicate and results presented arethe average values.

Oil release studies

The oil release studies were done using the method described by MajiT.K.et al., [8] with some modifications. A known quantity of microcapsules was placed into a known volume of 0.2% sodium alkylbenzene sulphonate solution. The solution was magnetically stirredat a constant rate

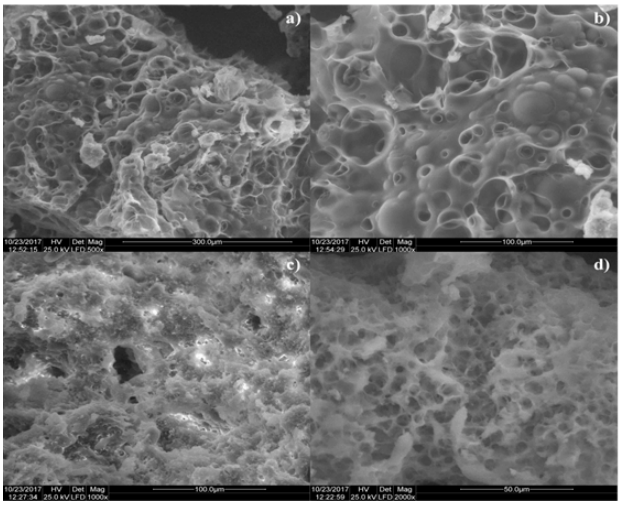

Fig.1. SEM imagines of a) surfaceand b) internal surface of thyme oil microcapsules and respectively c) surface and d) internal surface of coriander oil microcapsules

and the temperature throughout was maintained at 30°C. An aliquot (5mL) was removed at appropriate time intervals, filtered and assayed spectrophotometrically at 275 nm for the determinationof cumulative amount of oil release up to a time t. Each determination was carried out in triplicate. To maintain a constant volume, 5mL of 0.2% sodium alkylbenzene sulphonate solution was returned to the container. All experiments were carried out in triplicate and results presented arethe average values.

Results and discussions

Chemical composition of collagen hydrolysate and essential oils

The chemical composition of collagen hydrolysate was found to be the following: dry substance:34.5%, total nitrogen:14.75% and ash:5.49%. The *p*H value around 8.95 is similar to literature data.The essential oils composition is presented in table1.

Characterization of synthetized microcapsules

Scanning electron microscope (SEM) analysis

Microcapsules morphology was analysed through SEM technique (fig. 1). The images obtained through electron microscopy reveals for both samples a rough surface of microcapsules with some visible pinholes, cracks and pores (fig. 1 a and 1c). The sponge-like structure with a large number of micron-sized pores randomly distributedon the external surface (fig. 1b and 1d) was also reported by SutaphanitP. et al., [9]. They attributed the appearance of these pores to the water loss trapped by the collagen hydrolysate network during the sample preparation for SEM.

Thermogravimetric analysis (TGA)

Comparative thermogravimetric analysis (TGA) of synthesized essential oil microcapsules, pure essential oil (thyme and coriander) and core shell wall material (obtained using the same procedure without adding essential oil) are presented in figure 2. As can be seen in figure 2a thymeoil has a typical TG/DTG profile with only one evaporation stage starting from 110°C and finishing at 240°C with a maxim weight loss of 98.74% around 175°C. The core shell material exhibit a small weight loss, about 7% below 200°C attributed to absorbed water after which it decomposes above 300°C with a weight loss of 62.80% (fig. 2b).

TGA thermogram of thyme oil microcapsules showed two substantial weight losses (fig. 2c).The mass loss less

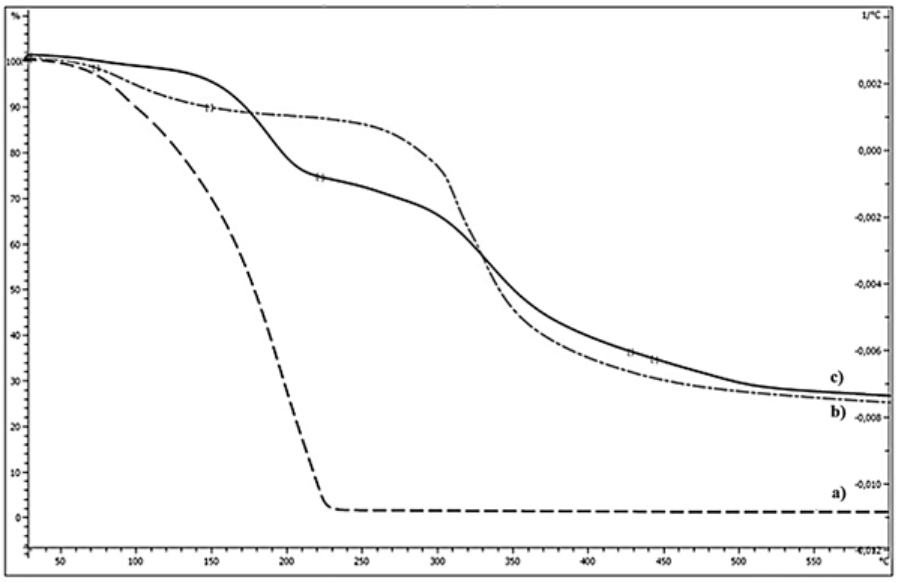

Fig. 2. Comparative TGA thermograms for a) thyme oil, b) core shell material and c) thyme oil microcapsules

than 3% recorded before 110°C indicates a small amount of adsorbed water on the microcapsule surface. The first important weight loss (approximately 25.29%) in microcapsules was observed at about 110-240°C, and it is caused by the loss of thyme oil encapsulated into the core shell.The second stage of weight loss found to be around 46.63 % was due to shell wall degradation.This clearly indicated that composition of microcapsules is about 25 % essential oil and 46 % core shell material.

The thermal properties of coriander oil microcapsules were also characterized by thermogravimetric analysis (TGA). The coriander oil microcapsules have the same behavior as thyme oil microcapsules showing two weight losses corresponding to oil release around 170°C and core shell decomposing over 300°C. The compositional analysis of coriander microcapsules based on TGA data indicates a 27% essential oil and 51% core shell content.

Encapsulation efficiency and oil release rate

The influence of microencapsulation parameters on encapsulation efficiency and oil release rate are presented in table 2 and table 3. The structure and composition of core shell wall materials of the complex coacervates strongly affect the release properties of core materials [10-11]. From the data presented in table 2, it can be observed that the encapsulation efficiency decreases from 96.28 to 88.37% when the amount of collagen hydrolysate increases. The same trend was reported for oil release rate (table 3). This outcome is consistent with the findings of other researchers[12] and was explained by the fact that the increase of collagen hydrolysate would increase the compactness in wall material and therefore cause difficulties for the volatile essential oil to be released from the microcapsules [11].

The influence of essential oil content on both the encapsulation efficiency and release rate is presented in table 2 and 3.An evaluation of the data obtained reveals that with the increase of oil content, the encapsulation efficiency decreases while the oil release rate increases. A possible explanation for the lower encapsulation efficiency (91.44%) at higher oil load (30g) might be due to the higher percentage of oil loss during encapsulation of the oil [8]. The increase in the oil release rate may be due to the decrease in microcapsules wall thickness caused by the constant concentration of collagen hydrolysed, while the concentration of the essential oil was increased [11].

The encapsulation efficiency and oil realise rate are also influence by the glutaraldehyde content. As expected, as the glutaraldehyde content increases, the encapsulation-efficiency raises while the oil rate reachesa maximum for

Table 2
EFFECT OF VARIATION OF MICROENCAPSULATION PARAMETERS ON ENCAPSULATION EFFICIENCY

Type of oil	Sample cod	Collagen hydrolysate, g	Glutaraldehyde, g	Oil, g	Efficiency, %
Thyme	T1	12	8	25	92.42 ± 1.15
	T2	12	8	30	96.28 ± 1.05
	T3	12	8	35	91.44 ± 1.83
	T4	12	6	30	79.35 ± 2.01
	T5	12	10	30	85.21 ± 1.05
	T6	8	8	30	94.22 ± 1.25
	T7	14	8	30	88.37 ± 2.12
Coriander	C1	12	8	25	94.42 ± 1.12
	C2	12	8	30	97.37 ± 2.1
	C3	12	8	40	93.44 ± 1.52
	C4	12	6	30	80.5 ± 1.14
	C5	12	10	30	87.5 ± 2.01
	C6	8	8	30	96.07 ± 1.12
	C7	14	8	30	92.52 ± 1.04

Sample cod / Time, hr	3	6	9	24	48	72	96	120
T1	6.3	18.4	21.4	35.5	37.9	40.4	42.7	43.7
T2	7.5	19.7	24.5	36.5	39.7	42.5	43.5	44.5
T3	5.9	17.9	27.7	38.2	41.2	44.2	45.2	46.5
T4	3.2	10.3	15.3	25.5	30.5	35.2	36.4	38.1
T5	2.5	7.2	10.5	20.3	28.8	31.4	33.9	35.2
T6	6.7	15.7	21.5	32.7	36.7	40.5	41.5	43
T7	5	11.4	21.5	30.1	33.9	38.5	39.9	41.1
C1	4.9	15.2	24.3	34.2	37.6	38.2	39.9	41.5
C2	6.5	20.2	30.9	37.9	41.7	42.9	44.5	44.9
C3	3.8	15.8	25.4	33.5	36.8	39.61	42.5	44.2
C4	3.8	11.5	16.5	25.4	30.1	33.2	36.5	38
C5	4.1	9.7	18.4	23.1	25.4	29.8	33.2	34.5
C6	5.2	16.7	26.9	35.2	39.1	40.2	41.5	42.2
C7	4.9	13.4	18.7	28.7	32.2	34.6	37.6	39.5

Table 3
EFFECT OF VARIATION OF MICROENCAPSULATION PARAMETERS ON OIL RELEASE RATE

8 g crosslinking, after whicht here is a decrease for higher crosslinking amount.This behaviour results from the fact that glutaraldehyde participates to the crosslinking process between the molecules of the wall membrane;more crosslinking, more compactness for the wall, which hinders the essential oil release from the microcapsules [13, 11].

Conclusions

This study presents the synthesis of thyme and coriander oil microcapsules by a complex coacervation technique. In order to obtain the best microencapsulation formulation for thyme and coriander oil, the influence of microencapsulation parameters on encapsulation efficiency and oil release rate was evaluated.The encapsulation efficiency and oil release rate depend on oil content, crosslinking and collagen hydrolysate content. The formulation containing 12g collagen hydrolysate, 8 g glutaraldehyde and 30 g essential oil was found to be the optimum condition. The microcapsules obtained under optimal condition present a sponge-like structure with a large number of micron-sized pores randomly distributed on the external surface. The rough surface of microcapsules with some visible pinholes, cracks and pores is caused by the water loss trapped by the collagen hydrolysate network during the sample preparation for SEM. The compositional analysis of thyme and coriander oil microcapsules based on TGA data indicates a 25% and respectively 27% of essential oil and 46% and respectively, 51% core shell content. The oil release rate evaluation suggest that the essential oil microcapsules continued to release their content until the 120th hour, at which time all released around 43% of their content. The synthetized essential oil microcapsules could be a viable option for producing the foliar fertilizers, bioactive for plant nutrition and growing stimulants for cereals.

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