Studii şi Cercetări Ştiinţifice 2013, **14** (3), pp. 155 – 162
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

MICROENCAPSULATION OF CORIANDER OIL USING COMPLEX COACERVATION METHOD

Cristian Dima[1], Romică Crețu[2], Petru Alexe[1], Ştefan Dima[2*]

[1] "Dunărea de Jos" University of Galati, Faculty of Food Science and Engineering, Domnească Street, 47, RO-800008, Galati, Romania
[2] "Dunărea de Jos" University of Galati, Faculty of Science and Environment, Domnească Street, 47, RO-800008, Galati, Romania

*Corresponding author: dimastefan@yahoo.com

Received: February, 22, 2013
Accepted: June, 28, 2013

Abstract: In the present study, the gelatin/gum Arabic/chitosan microcapsules encapsulating coriander oil were prepared by complex coacervation using glutaric aldehide and transglutaminase as hardening agents. The effects of wall materials, core/wall ratio, particle size, cross-linking agents, dispersing medium and temperature on the release of coacervate microcapsule were investigated. The antioxidative properties and antibacterial activity of free and microencapsulated coriander oil were examined. In the case of DPPH assay, the IC_{50} values of coriander oil free and coacervat microcapsule was comparables. Addition of chitosan in wall materials did not interfere with the antioxidant activity of coriander oil, but it improves the antibacterial activity of system.
Coriander oil extraction by hydrodistillation revealed the influence of particle size on extraction yield. It increases with the decreasing of particle size resulting in maximum efficiency 0.836% for particles of 500 μm, 0.753% for those of 630 μm, and 0.704% for the size of 710 μm.

Keywords: *antibacterial activity, antioxidative properties, encapsulated bioactives, DPPH assay, flavor ingredient*

INTRODUCTION

Coriander oil is used in the food industry as a flavor ingredient in the majority of the food categories, including alcoholic beverage, candy, pickles, meat sauce and seasonings. It has been recognized as GRAS for use in food by FDA and FEMA and is approved for use by the Council of Europe.
In the recent past, there has been a rising interest in producing functional foods containing encapsulated bioactive. Microencapsulation is an inclusion technique for entrapping a bioactive compound, such as: probiotic bacteria, enzymes, vitamins, flavor oil, etc. into a polymeric matrix that may be coated by one or more semipermeable polymers.
There are many microencapsulation techniques. Some are based on physical and mechanical processes; others are based on chemical changes faced by encapsulated material [1]. Microencapsulation by coacervation method also belongs to the last group.
Microencapsulation by coacervation method is based on phase separation of one or more biopolymers from an initial solution and the decantation in their original form of coacervate on the surface of active substance droplets in the non-polar emulsion in a dispersion medium [2]. Microcapsules produced by coacervation method are insoluble in water, temperature and mechanical shock resistant also has high retention efficiency and a good release of the active substance.
Morphological structure of microcapsules prepared by coacervation method depends on the following factors: time and method of emulsification, nature and concentration of biopolymers used as encapsulated material, particle size, dispersion medium properties and the degree of crosslinking [3].
Emulsification by slow mixing at 1000-1500 rpm, leading to mononuclear microcapsules, while a high mixing speed, 2000-2500 rpm, poly nuclear microcapsules are obtained [4]. Concentration of hydrocolloids and their use in the mixture influence the morphological structure of microcapsules and provide controlled release of active substances [5].
The purpose of the present study is to study the influence of natural hydrocolloids and reticular agents on the retention and release of coriander oil in microcapsules produced by complex coacervation method. The oil was obtained by hydrodistillation of coriander seeds crushed and selected according to their size. Also, the antioxidant and antibacterial properties of the free and microencapsulated coriander oil were determined.

MATERIAL AND METHODS

Materials

Seeds of coriander (*Coriandrum Sativum L*) from Romanian production (Supremia group SRL), gelatin type A (Bloom strength: 105, viscosity: 1.85 mPa·s, 6.5 % to 50 ºC, Biochem LTD), chitosan (degree of deacetylation: 78-82 %, moisture content: below 10 %, Fluka), glutaric aldehyde 25 % (Sigma Chem. Ltd), transglutaminase, enzymatic activity: 60 U/g (Fluka), acetic acid, ultrapure water (Milli Q), petroleum ether, ethyl acetate (Sigma).

Coriander oil extraction

The coriander seeds were ground and obtained particles were selected by sieving, yielding fractions with size: 500 µm, 630 µm and 710 µm. The coriander oil by the hydrodistillation method was extracted [6].
100 g of sample is weighed and put into the still proper without using a basket. Then, 1000 mL of distilled water was added into the flask. The cover was then closed and heated for 3 hours. The condensate was channeled out in a separating funnel and allowed to settle for 10 minutes before the coriander oil was collected. The coriander oil collected was weighed, analyzed for its refractive index, antioxidant and antibacterial activities and stored in air tight glass container.

Preparation of coacervate microcapsules

Oil microcapsules were prepared using the method applied by Dong and collaborators [7] with various modifications. Separately, 2.5% (w/w) gelatin solution and 2.5% (w/w) solutions of biopolymers: Arabic gum (AG) and chitosan (Chi) in different mass ratios (AG:Chi = 1:0, 2:1, 1:1) were prepared. Equal volumes of gelatin solution and solutions of hydrocolloids were mixed and different amounts of coriander (5, 10, 20, 40 g) were added. The mixture was homogenized by ultrasonic for 2 minutes at 60 % amplitude. To obtained emulsion the ultrapure water heated at a temperature of 40 ºC was added and the pH value was adjusted to 4.5 using 10 % acetic acid solution. The obtained mixture at a speed of 400 rpm was homogenized. After 10 minutes of homogenization the pH was readjusted to 6.0, the mixture was cooled on ice bath (15 ºC) and the reticular agent (glutaric aldehyde / transglutaminase) was added. The obtained microcapsules were kept out of cool for 12 hours and then they were filtered and kept in desiccator until dry, at room temperature.

Determination of release profile of coacervate microcapsules

Five samples containing 5 mg of dried microcapsules suspended in 95 mL of buffer *p*H = 6.4 were kept at a temperature of 10 ºC. At various time intervals (1, 5, 10, 15 days) the released coriander oil was extracted, in petroleum ether, with a separating funnel. The total amount of encapsulated oil was extracted with petroleum ether using a Soxhlet device. Cumulative amount of oil released was determined by the relation:

$$Q\% = \left(1 - \frac{m_i}{m_0}\right) \cdot 100 \quad (1)$$

where m_i is the mass of oil released, and m_0 is the total mass of encapsulated oil.

Determination of antioxidant capacity by DPPH method

Antioxidant capacity of coriander oil by DPPH method was determined. In summary, by dissolving 23.5 mg of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 100 mL of methanol a solution was prepared. This stock solution was then diluted 1:10 with methanol. Both solutions at 4 °C until use were stored. Different amounts of extract (0.5 mL, 1 mL, 1.5 mL) were tested. Coriander oil volumes were added to a dilute solution of DPPH corresponding to a final volume of reaction of 5 mL. After 4 hours at 30 °C, the

absorbance at 516 nm using a Jenway 6300 spectrophotometer was read. DPPH-methanol solution was used as a control.
The antiradical activity (AA) was determined by the formula:

$$AA\% = 100 - \left[\frac{\left(Abs_{sample} - Abs_{empty\ sample} \right)}{Abs_{control}} \cdot 100 \right] \quad (2)$$

where: empty sample – 1 mL methanol + V mL coriander oil; control sample – 1 mL sol. DPPH + 2.5 mL methanol.

The concentration of DPPH remained in the reaction was calculated from the following calibration curve: Y = 0.0247X - 0.0029 with linear regression (r = 0.999). The percentage of remaining DPPH against the extract concentration was then calculated to give the amount of antioxidant needed to reduce the initial DPPH concentration with 50 % or IC_{50}. A lower IC_{50} value corresponds to a higher antioxidant capacity.

Antibacterial activity determination

Culture of *Escherichia coli* was cultivated in Mueller-Hinton culture medium agar and was thermostatically controlled at 37 °C with stirring at 150 rpm for 24 hours. Isolated colonies, which were inoculated into 50 mL Mueller Hinton and then thermostats for 24 hours at 37 °C with stirring at 150 rpm were selected. These latter cultures as inoculums for the antimicrobial activity evaluation were used. To determine the antibacterial activity of coriander oil the diffusimetric method has been used.
With a sterile pipette is inserted into each plate 1 cm^3 culture suspension of *Escherichia coli*. Then in each Petri plate were poured in thin film 15 cm^3 of culture medium (MMA Growth Agar - agar malt extract), and cooled to 45 ± 5 °C. The culture is distributed evenly by rotating them horizontally, and then is left to stand until solidification occurs. It then cuts each 3 wells in each plate of 0.5 cm in diameter in which dispense 0.25 mL of analyzed samples. The plates are placed at thermostat for 3 days, and then are observed the presence of inhibition zones around wells.

RESULTS AND DISSCUSSION

Coriander oil extraction

Coriander oil extraction by hydrodistillation revealed the influence of particle size on extraction yield. It increases with the decreasing of particle size resulting in maximum efficiency 0.836 % for particles of 500 µm, 0.753 % for those of 630 µm, and 0.704 % for the size of 710 µm. These results can be explained by the increase of the contact surface with the solvent, the decrease of particle size favors the extraction process.
Similar results were obtained analysis on the content of fat, moisture and refractive index (Table 1).

Table 1. *Characteristics of coriander oil according to particle size*

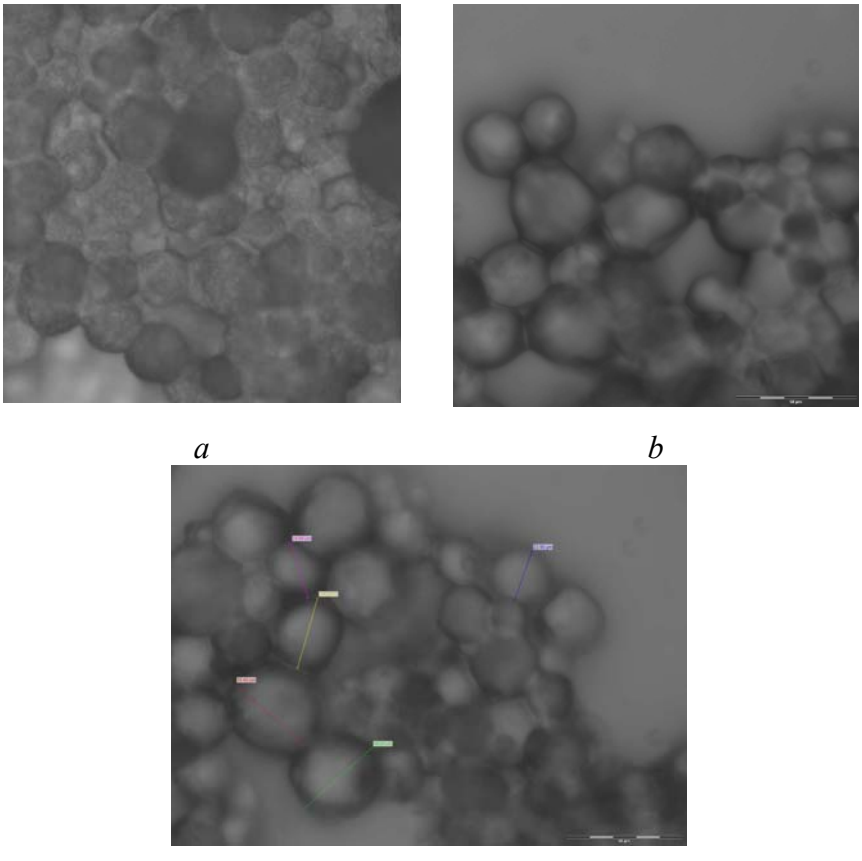
Property	500 µm	630 µm	710 µm
Extraction efficiency (%)	0.836	0.753	0.704
Fatty substances (%)	23	20	18
Humidity (%)	11	10.20	9.63
Refractive index	1.4875	1.4783	1.4768

Morphology of the microcapsules and release coriander oil

Macroscopically the coriander oil microcapsules are a reddish yellow powder with encapsulated flavor oil. The structure of microcapsules obtained by complex coacervation method was determined by light microscopy using the Olympus 420 microscope.

Images in Figure 1 show that the microcapsules composition varies by hydrocolloids. When using only Arabic gum (Figure 1a) coacervate of irregular shape with a high polydispersity are obtained.

The presence of chitosan results in a rounding and stiffening of the microcapsules' walls. Also, the average size of microcapsules increased with the increase of chitosan concentration, from 15 µm to 25 µm.



a *b*

c

Figure 1. *Light microscopic photograph of coriander oil microcapsules by coacervation with different hydrocolloids weight ratio*
(a) AG; (b) AG:Chi=2:1; (c) AG:Chi=1:1 (x40)

The nature of reticular agent does not substantially alter the shape and size of microcapsules.
Encapsulation efficiency varies depending on the composition of wall material and the amount of coriander oil. Thus, when using AG: Chi = 2:1 mixture, with a quantity of 20 mL of coriander oil was obtained an encapsulation efficiency of 82.64 %. When using only AG, encapsulation efficiency is 79.18 %, and using larger amounts of chitosan, reduces the encapsulation efficiency to 65.73 %. This is due to complex structures formed between macromolecular chains of Arabic gum and chitosan that has a negative influence on the formation of coacervate [8].
Oil release profile of microcapsules depends on the encapsulated material composition and the nature of reticular agent.
According to Figure 2, the best release occurs in microcapsules prepared with Arabic gum which is crosslinking with glutaric aldehyde. Coriander oil quantity decreases with the use of chitosan. This is because the encapsulated material becomes more compact in the interaction of the carboxylate and ammonium groups of Arabic gum and respectively chitosan. Also the reticular agent has available more amino groups to participate in condensation reactions which result in a more rigid and less permeable microcapsule wall.

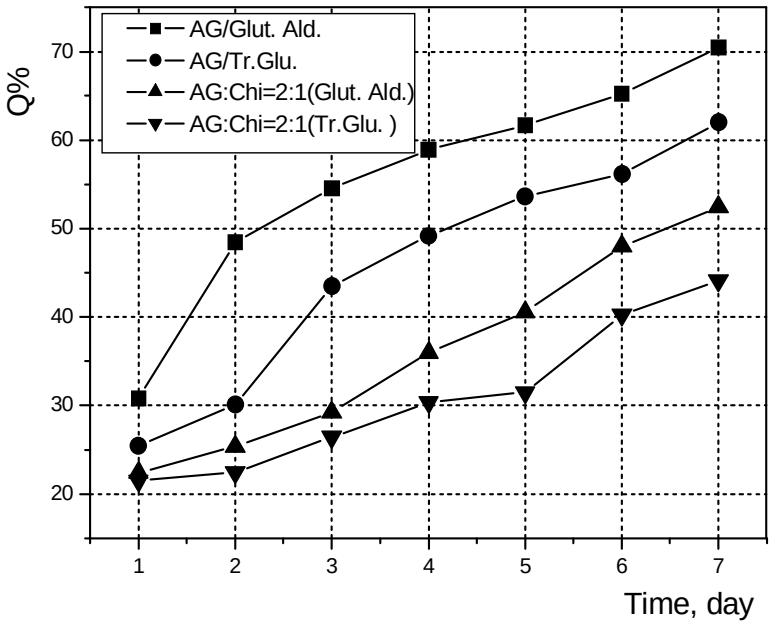


Figure 2. *Release profile of coriander oil from microcapsules*

Antioxidant and antibacterial activity

The antioxidant activity determined by DPPH coriander oil is 83.7 %. IC_{50} value is 350 ± 10 $mg \cdot mL^{-1}$, similar to results obtained by other researchers [9]. This activity is due to the presence of flavonoids, phenolic acids and terpenoids in the coriander oil composition [10].
Antibacterial activity of free and encapsulated coriander oil was tested against a strain of *Escherichia coli*. Diameter values of diffusion was found that free coriander oil

shows comparable antimicrobial activity (d = 4.6 mm) with a suspension of gum Arabic microcapsules containing coriander oil (d = 4.8 mm). Microcapsule samples were taken after 3 days of incubation in phosphate buffer solution, *p*H = 6.4. Spectacular results were obtained from microcapsules obtained with a mixture of AG:Chi = 2:1, the diffusion areas are clearly defined with a diameter of 11.8 mm. These results are due to the chitosan which show a strong antibacterial activity [11].

CONCLUSIONS

This study shows that the hydrodistillation extraction efficiency of *Coriandrum Sativum L* seeds is influenced by degree of grinding achieving maximum efficiency in samples containing particles of 500 μm. The obtained oil shows good antioxidant activity determined by DPPH method and good antibacterial activity against *Escherichia coli*. Microencapsulation of coriander oil by complex coacervation method was achieved using as encapsulation material gelatin, Arabic gum and chitosan. The results showed that the prepared microcapsules are resistant to temperature, provides encapsulation efficiency above 80 %. The release rate of oil from microcapsules depends on the composition material and the nature of reticular agent. The best results were obtained with a mixture of Arabic gum and chitosan in a mass ratio of 2:1 using glutaric aldehyde as reticular agent. The complex coacervation method can be used for encapsulation of flavour compounds with applications in food industry.

REFERENCES

1. Dima, St.: Bazele microîncapsulării in: *Microîncapsularea pentru sisteme alimentare* (Editors: Florea, T., Dima, St., Costin, G.M.), Editura Academica, Galati, **2009**, 36-44;
2. Gouin, S.: Microencapsulation: industrial appraisal of existing technologies and trends, *Trends in Food Science and Technology*, **2004**, **15** (7-8), 330-347;
3. Xiao, J.X., Yu, H.Y., Yang, J.: Microencapsulation of sweet orange oil by complex coacervation with soybean protein isolate/gum Arabic, *Food Chemistry*, **2010**, **125** (4), 1267-1272;
4. Yeo, Y., Bellas, E., Firestone, W., Langer, R., Kohane, D.S.: Complex coacervates for thermally sensitive controlled release of flavor compounds, *Journal of Agricultural and Food Chemistry*, **2005**, **53** (19), 7518-7525;
5. Burdock, A.G., Carabin, I.G.: Safety assessment of coriander (*Coriandrum sativum L*) essential oil as a food ingredient, *Food and Chemical Toxicology*, **2009**, **47** (1), 22-34;
6. Kadam, M.L., Hashmi, S.I., Kale, R.V.: Studies on extraction of ginger oil and its microencapsulation, *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **2011**, **10** (6), 2382-2390;
7. Dong, Z., Ma, Y., Hayat, K., Jia, C., Xia, S., Zhang, X.: Morphology and release profile of microcapsules encapsulating peppermint oil by complex coacervation, *Journal of Food Engineering*, **2011**, **104** (3), 455-460;
8. Turgeon, S.L., Schmitt, C., Sanchez, C.: Protein-polysaccharide complexes and coacervates, *Current Opinion in Colloid and Interface Science*, **2007**, **12** (4-5), 166-178;
9. Wangensteen, H., Samuelsen, A.B., Malterud, K.E.: Antioxidant activity in extracts from coriander, *Food Chemistry*, **2004**, **88** (2), 293-297;
10. Msaada, K., Hosni, K., Ben Taarit, M., Chahed, T., Kchouk, M.E., Marzouk, B.: Changes on essential oil composition of coriander (*Coriandrum sativum L.*) fruits during three stages of maturity, *Food Chemistry*, **2007**, **102**, 1131-1134;

11. Berger, J., Reist, M., Meyer, J.M., Felt, O., Peppas, N.A., Gurny, R.: Structure and interactions in covalently and ionically cosslinked chitosan hydrogels for biomedical application, *European Journal of Pharmaceutics and Biopharmaceutics*, **2004**, **57** (1), 19-34.