SYNTHESIS AND CHARACTERISATION OF MICROCAPSULES BASED ON NATURAL BIOPOLYMERS AND LAUREL ESSENTIAL OIL

CIPRIAN CHELARU[1], MADALINA GEORGIANA ALBU[1], DURMUS ALPASLAN KAYA[2], AURELIA MEGHEA[3], IGNAT MADALINA[1], ŞEVKET ÖZTÜRK[2], MARIA GIURGINCA[3], NALBAT SEVGI[4], MUSA TURKMEN[2], LAURENTIU DINCA[5]

[1] *INCDTP - Division Leather and Footwear Research Institute, 93 Minulescu Ion Str., 031215, Bucharest, Romania, cchelaru_cciprian@yahoo.com*

[2] *Mustafa Kemal University, Faculty of Agriculture, 31030, Antakya-Hatay, Turkey*

[3] *Politehnica University of Bucharest, 1-7 Gheorghe Polizu Str., Bucharest, Romania*

[4] *Ege University, Faculty of Engineering, Department of Leather Engineering, Erzene Mh., 35040, Izmir, Turkey*

[5] *The National Research & Development Institute For Textile and Leather, 16 Patrascanu Lucretiu Str., 030508, Bucharest, Romania*

The aim of this paper was to obtain and characterize some microcapsules based on natural polymers, collagen hydrolysate and sodium alginate and laurel essential oil. The composition of Laurel essential was determined by GC-MS. By varying different synthesis parameters, water-oil emulsion method was choosing for obtaining the liquid microcapsules. The microcapsules were dried by lyophilisation. Collagen hydrolysate and microcapsules based on polymers were characterized by FT-IR spectroscopy, optical and scanning electron microscopy and particle size.

Keywords: natural polymers, essential oil microcapsules, collagen hydrolysate, sodium alginate.

INTRODUCTION

Microcapsules obtained from natural polymer especially gelatin/collagen, sodium alginate that contain active principles like essential oils with therapeutic actions, are widely studied by researchers worldwide (Devi *et al.*, 2012; Kaya *et al.*, 2012; Kim *et al.*, 2006; Martins *et al.*, 2014; Ocak, 2012) being the top issues at this time. In this paper the water-oil type solution was the method to obtain the microcapsules and characterization methods were optical and electron microscopy, FT-IT(ATR), GC-MS, size dimension.

MATERIALS AND METHODS

Materials

Collagen hydrolysate was obtained from calf pelt by alkaline hydrolysis of at 125°C, 2 atm during 8 hours by currently used technology in Collagen Department (Sirbu *et al.*, 2009).

Sodium alginate (100,12 molecular mass) and glutaraldehyde was purchased from Sigma - Aldrich (Germany). Acetic acid was provided by Chimactive (Romania).

Laurus nobilis L essential oil was provided by the Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops (Kaya *et al.*, 2012). *Laurus nobilis L* essential oil was obtained from dried leaves that were collected from Amanos Mountain (Anatolia region) during flowering season, which was dried at room temperature. The essential oil was obtained after 100-150 g of dried leaf were subjected to steam distillation for 3-4 hours. The resulting essential oil was dried with anhydrous sodium sulfate and stored at -20°C.

Synthesis of Microcapsules

In order to obtain the microcapsules, the compositional recipes from Table 1 were used.

Table 1. Compositional recipes for syntesized microcapsules

No.	Code	Collagen hydrolyzate	Sodium alginate	Glutaraldehyde	Acetic acid	Essential Oil, ml
		g/100 ml water				
1	MC-CH 1	3	-	0.4	2.5	1
2*	MC-CH 2	3	-	0.4	2.5	1
3	MC-SALG	-	1	0.4	2.5	1
4	MC-CH-SALG	3	1	0.4	2.5	1
5	MC-SALG-CH	1	3	0.4	2.5	1

MC – MicroCapsule; CH – Collagen Hydrolysate; SALG – Sodium Alginate
* 1 hrs stirring

Initially 3% CH was solved in water and heated between 50-60°C. The solution was subjected to a fast stirring. At the desired temperature the essential oil was added, in drops and a water solution (3%) of SALG, also in drops. The pH of solution was corrected with acetic acid until pH 3.75 was achieved. After the pH correction, the solution was cooled down between 5-10°C in order to hardness the microcapsules. The obtained microcapsules were cross-linked using 0.4% glutaraldehyde. After crosslinking, the temperature was increased at 50°C and continuing stirring another 3 hrs (Kaya *et al.*, 2012).

Methods

The essential oil components were analyzed by GC/MS – Qualification and quantification was carried out by using a Finnigan-Trace GC–MS equipped with an auto sampler. One micro liter of sample volume was injected using split method with 50 split ratio. Chromatographic separations were accomplished with a Zebron ZB-5 capillary column (5% phenyl–95% dimethylpolysiloxane, 0.25mm i.d. × 60 m, film thickness 0.25 m) with injections in the split mode with 50 split ratio. Analysis was carried out using helium as the carrier gas, flow rate 1.0 mL/min. The column temperatures were programmed from 50 to 240°C at 3°C/min. The sample sizes were 2 µL. The injection port temperatures were 250°C. The ionization voltages applied were 70 eV, mass range m/z 41–400 a.m.u. The separated components identified by matching with GC–MS results of National Institute of Standards and Technology (NIST) mass spectral library data. The quantitative determination was carried out based on peak area integration.

FT-IR spectrometry analysis was performed with Jasco 4200 FT-IR spectrophotometer, equipped with an ATR - diamond sensor.

Optical microscopy analysis was performed with a Leica S8AP0 stereomicroscope for 20-160X magnification and Leica CME microscope for 40-1.000X magnification.

Electron microscopy analysis was performed using Quanta Fei 200 scanning electron microscope that has the magnification range between 1.000 X - 1000.000 X.

Particle size was determined using Malvern ZetaSizerNano equipment that has the possibility to determine dimension between 0.6 nm - 6 µm.

RESULTS AND DISCUSSION

Optical and Scanning Electron Microscopy of Collagen Hydrolysate and Sodium Alginate

Optical microscopy images show that the collagen hydrolysates have spherical form, white color and sizes of the grain vary between 8 and 25 µm (Figure 1a) and sodium alginate has granular form, translucent, white color and sizes of the grain vary between 20 µm and 400 µm (Figure 1c). Scanning electron microscopy images reveal that the collagen hydrolysate grains have spherical shapes, which confirm the observation made by optical microscopy analysis (Figure 1b) and sodium alginate grains have an irregular morphology with smaller grains on top (Figure 1d).

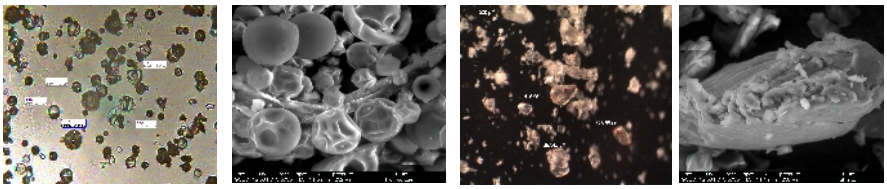


Figure 1. a) Optical microscopy image of CH (400X); b) SEM image of CH (5000X); c) Optical microscopy image of SALG (80X); d) SEM image of SALG (5000X)

Composition of Laurel Essential Oil

Table 2 presents the components of essential oil determined by GC-MS chromatography. The essential oil has 23 components and the majors ones are eucalyptol – 54.00%, alpha-terpinenyl acetate – 10.93% and sabinene – 7.96%.

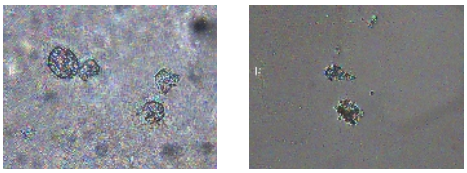
Table 2. Essential oil (*Laurus nobilis L*) components

No.	Component	Retention time	Aria (%)
1	alpha-pinene	11.33	4.68
2	Sabinene	12.86	7.96
3	2-beta-pinene	13.18	3.44
4	p-cymene	15.12	1.34
5	dl-limonene	15.28	1.22
6	1,8-Cineol (Eucalyptol)	15.52	54.10
7	gama-terpinene	16.54	0.77
8	Linalool	18.31	0.77
9	4-Terpineol	22.47	2.55
10	Beta-Fenchyl alcohol	23.16	1.52
11	Ocimenyl acetate	28.31	0.63
12	alpha-terpinenyl acetate	29.80	10.93
13	Eugenol	30.38	0.08
14	Beta-elemene	31.66	1.21
15	Methyleugenol	32.26	0.49
16	trans-caryophyllene	33.20	0.81
17	alpha-caryophyllene	34.76	0.14
18	Germacrene-D	35.80	0.22
19	Beta-Selinene	36.22	0.11
20	Beta-Bisabolene	36.42	0.26
21	Cis-alpha-Bisabolene	37.88	0.22
22	Caryophyllene oxide	40.13	0.21
23	Beta-Eudesmol	42.99	0.11

Characterisation of Microcapsules

The microcapsules obtained according with methods previously described in presented in Table 1 were characterized by optical and scanning electron microscopy, particle size and FT-IR spectroscopy.

The figure 2 shows the difference between MC-CH 1 and MC-CH 2.

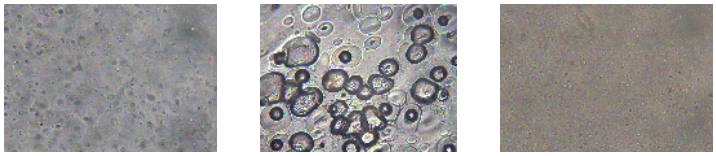


a) b)

Figure 2. Optical microscopy images of microcapsules: a) MC-CH 1 and b) MC-CH 2

Optical microscopy for MC-CH 1 – Figure 2a shows the presence of some aggregates that have different shapes and sizes. The aspect of the microcapsules indicates that they start to form a microcapsule incipient structure but are not complete. Because MC-CH 2 was obtaining in a similar condition like MC-CH 1, optical microscopy shows the presence of some aggregate in a more incipient form than MC-CH 1 - Figure 2b.

Figure 3a shows the optical microscopy image for MC-SALG but the sizes of the microcapsules are too small in order to be observed in good condition.



a) b) c)

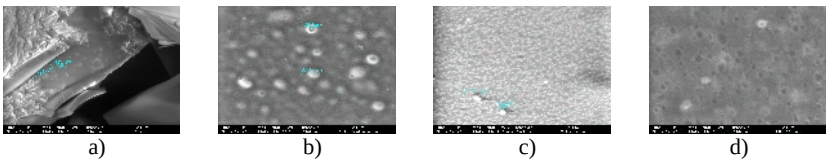
Figure 3. Optical microscopy images of microcapsules: a) MC-SALG; b) MC-CH-SALG; c) MC-SALG-CH

Optical microscopy image for MC-HC-SALG (Figure 3b) indicates the presence of microcapsules; the outer shell and also the internal component – essential oil – can be noticed more clearly.

The MC-SALG-CH microcapsules image (Figure 4c) indicates that they are too small to be seen in optical microscopy.

In order to observe them in more detail, the microcapsules solution was dried by lyophilization to transform them from liquid into solid state.

Even if the SEM of microcapsules can provide more information about their morphologies, the exact forms of the microcapsules from Figure 4 cannot be identified.



a) b) c) d)

Figure 4. SEM for microcapsules: a) MC-HC 1; b) MC-ALGS; c) MC-HC-ALGS and d) MC-ALGS-HC

In Figures 4 b, d some ovoidal shapes can be observed, but the images are not eloquent. Figures 4 a, c show some formations of microcapsules, very different even optical microscopy clearly indicate microcapsules form.

Size of Microcapsules

The microcapsules sizes are presented in Table 1.

Table 3. Size of microcapsules

No	Name	Dimension (nm)	Intensity (%)
1	MC-CH 1	6025 385.6	52.7 47.3
2	MC-CH 2	2673	100
3	MC-SALG	229 10.28	92.2 7.8
4	MC-HC-SALG	1020	100
5	MC-SALG-HC	1164	100

The size analysis of microcapsules indicates that the size distribution of microcapsules is in a large interval, from several to hundred micrometers.

FT-IR (ATR) Spectroscopy

Even if the MC-CH 1 and MC-CH 2 solutions were made by varying the stirring time, the difference between them is not so significant.

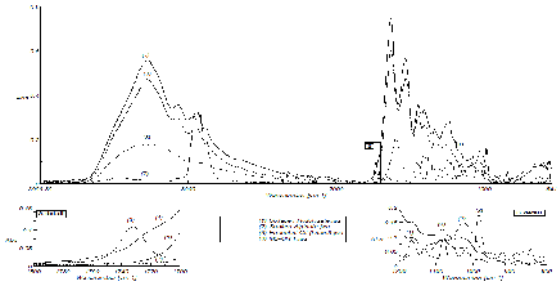


Figure 5. FT-IR (ATR) overlays spectra for collagen hydrolysate, sodium alginate, essential oil, MC-CH 1

The FT-IR (ATR) overlay spectra for collagen hydrolysate, sodium alginate, essential oil, MC-CH 1 (Figure 5) show that the existence of peak at 1732 cm^{-1} in the essential oil determine structural changes in the microcapsules structure – figure 5 (left). In figure 5 (right) is also possible to see some interaction between collagen hydrolysate and sodium alginate.

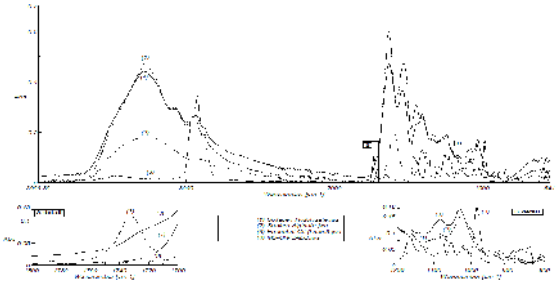


Figure 6. FT-IR (ATR) overlays spectra for collagen hydrolysate, sodium alginate, essential oil, MC-CH-SALG

FT-IR (ATR) spectrum for the MC-HC-SALG indicates major similarities with MC-CH 1 and MC-CH 2 spectra.

A more detailed analysis highlights the fact that the addition of sodium alginate in synthesis solution leads to changes of 1031 cm^{-1} peak intensity (0.0629) of collagen hydrolysate to MC-HC-SALG peak intensity (0.1686) – figure 6 right detail.

The presence of peak at 1732 cm^{-1}, in essential oil FT-IR (ATR) spectra leads to a structural change in the chemical composition of the microcapsules and can be observed in Figure 6 - left side detail.

CONCLUSION

The microcapsules obtained from collagen hydrolyzate, sodium alginate and essential oil (*Laurus nobilis L*) were obtained and characterized by FT-IR spectroscopy, optical and SEM microscopy and size particles. The results showed that the obtained microcapsules had micrometer sizes and the essential oil influenced the microcapsule structures.

Acknowledgement

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