



CHITOSAN-POLYVINILPYRROLIDONE/ ELLAGIC ACID BASED MEMBRANES WITH CONTROLLED ANTIOXIDANT PROPERTIES

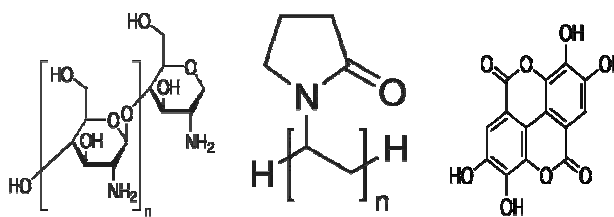
Elena NEAGU,^{a,*} Gabriela PAUN,^a Andreia ALECU^a and Gabriel Lucian RADU^b

^a Centre of Bioanalysis, National Institute for Research-Development of Biological Sciences, 296 Spl.Independentei, PO Box 17-16, 060031, Bucharest 6, tel./fax: 021-2200900, Roumania

^b Faculty of Applied Chemistry and Materials Science, Politehnica University of Bucharest, 313 Spl.Independentei, 060042, Bucharest, Roumania

Received April 12, 2013

They were characterized by Fourier transform infrared spectroscopy (FTIR). The efficiency of EA entrapment revealed values $\geq 50\%$. The released ellagic acid quantities were spectrophotometrically determined at various time intervals: 1, 2, 3, 7 days, and various pH: 3.0, 7.5, 8.0, 9.0. The best cumulative release of ellagic acid from membranes was obtained at lowest pH, pH=3. The antioxidant capacity of EA released from membranes was evaluated by inhibition percent of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The best result was found also at pH=3.0, 20% DPPH inhibition (at 48h). The properties of such membranes recommend them as biomaterial with controlled antioxidant biological activity.



INTRODUCTION

Many plants contain high quantities of polyphenols, having antioxidant, anti-diabetic, anti-viral, anti-inflammatory, anti-atherogenic and anti-cancer properties. The polyphenol compounds were described as inhibitors of cancer development and propagation; they could be used as chemopreventive agents.¹

The addition of polyphenol compounds in foods represents a research area in full development.² The encapsulation of bioactive compounds as functional ingredients in different foods and beverages is of interest, because it allows enriching them with natural antioxidants.³ The nourishing contribution of phytopigments was estimated to be approximately 1 g/day for a healthy alimentary regime.⁴ The polyphenols have low stability and

solubility,⁵ some of them having also an unpleasant taste. Such drawbacks could be overcome by encapsulation.

The literature data regarding the encapsulation of polyphenolic antioxidants are limited. Among numerous materials used for encapsulation, the researchers' attention was focused especially on the sodium alginate and chitosan, as systems of drug release, due to their very good compatibility.⁶ Chitosan has various applications in the pharmaceutical industry in the fabrication of tablets with controlled release⁷⁻⁹ or to improve the drug adsorption.¹⁰ The current techniques of polyphenols' encapsulation include: spray drying, spray cooling/chilling, extrusion, fluidized bed coating, coacervation, liposome entrapment, composite inclusion, centrifugal suspension separation, lyophilisation, co-crystallization and

* Corresponding author: lucineagu2006@yahoo.com

emulsifying, nanoencapsulation etc.¹¹⁻¹³ New materials, such as cyclodextrin, were used to encapsulate an extract of olive leaves.¹⁴ The use of polyphenols as food encapsulated ingredients should ensure their protection as well as preservation of their nutritional properties.¹⁵ Without such protection polyphenols could be exposed to unfavorable conditions inside foods: pH, enzymes and other components. Some polyphenol sources, like catechins in tea, curcumin and others, were encapsulated using methods¹⁶ like: coacervation¹⁷ entrapment in liposomes, composite inclusion, co-crystallization, nano-encapsulation,^{5,18} lyophilisation and emulsifying.¹⁶ The polyphenols of medicinal plants (raspberry, hawthorn, ivy, milfoil, nettle) were encapsulated through electrostatic extrusion into alginate-chitosan microbeads;¹⁹ gallic acid was encapsulated into starch, inulin and acetylated starch by spray-drying;²⁰ the rosmarinic acid was embedded in membranes of polygalacturonic acid cross-linked with hyaluronic acid;²¹ quercetin was included in nano-particles by nano-precipitation technique.²²

The ellagic acid represents a dimer derived from gallic acid, and it is rarely found in free form, but more often forming complexes with glycoside residues (glucose, xylose); it is found prevailing in pomegranate, persimmon, raspberry, strawberry, peach, nutmeats, and almonds. The ellagitannins are not adsorbed at human intestine level, but are hydrolyzed into ellagic acid.

The ellagic acid became attractive as food ingredient due to its benefic effects against some diseases.^{23,24} The cytostatic effects of ellagic acid were studied on more types of cancerous cells, where it exhibited anti-proliferative activity, having the ability to block the cell cycle and to induce apoptosis in several human cancerous cell lines.²³

The ellagic acid was immobilized on peptidic microtubules,²⁵ in liposomes of soybean lecithin,²⁶ on chitosan based nano-particles.²⁷

The aim of this study is to obtain a chitosan membrane with polyvinyl pyrrolidone (PVP) and ellagic acid immobilized by entrapment, the respective membrane being possible to be used to obtain biomaterial with controlled release of polyphenols with antioxidant activity.

MATERIALS AND METHODS

1. Materials

Chitosan with medium molecular weight and acetylating degree of about 75-85% was obtained

from Aldrich. Ellagic acid (HPCE) was provided from Fluka. Polyvinyl pyrrolidone (PVP), average mol wt. 360,000 provided from Fluka, 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Aldrich, phosphate buffer (PBS) from ROTH. All used chemicals were of analytical purity.

2. Apparatus

Fourier transform infrared spectroscopy (FTIR) was performed by FTIR-VAR technique with a beam incidence angle of 45°, on a Bruker TENSOR 27 instrument (Germany) using the OPUS software version 6.0. The samples were not pre-treated, as whole pieces fixed on a gold mirror and all the spectra were registered against a background of clean gold foil between 600 and 4000 cm⁻¹. The spectral resolution was 4 cm⁻¹, and the co-added scans 64, with an aperture of 6 nm.

The Thermo Scientific Evolution 260 Bio UV-Visible type spectrophotometer (USA) was used for release ellagic acid analysis from membrane (at 270 nm) and for analysis of the antioxidant activity using DPPH (at 516 nm).

a. Preparing the chitosan polyvinylpyrrolidone membranes

Chitosan (1.5% w/v) was dissolved in acetic acid (2% v/v) by continuously mixing till a homogenous solution was obtained; the polyvinylpyrrolidone (PVP) (1% v/v) and glycerin (1% v/v) were added under permanent stirring. Ratio CH/PVP was 1.5: 1. Finally, the solution was poured in Petri dishes or on glass by aid of a "doctor blade". The obtained membranes were dried in oven, at 60°C, during one hour and then were treated with sodium hydroxide (NaOH) (5% wt/v). Further, they were washed several times with distilled water to remove the NaOH and dried.

b. Entrapment of ellagic acid in the chitosan membranes

Ellagic acid was added to the chitosan solution 0.1% (w/v) the chitosan polyvinylpyrrolidone membranes with entrapped ellagic acid resulted.

Two types of membranes were obtained: chitosan, polyvinylpyrrolidone – CH –PVP - and chitosan, polyvinylpyrrolidone and ellagic acid entrapped – CH-PVP/EA.

c. Swelling methods

To determine the swelling degree of the obtained chitosan-polyvinylpyrrolidone membranes

(without ellagic acid – CH-PVP), certain size (25–30 mg) fragments were introduced into phosphate buffer of pH: 3.0, 7.5, 8.0 and 9.0, at room temperature. At 10 minutes intervals the fragments were taken out, dried and weighed, being recorded the dependence between the swelling degree and time interval (t).

The swelling degree was calculated by reference of absorbed water to the dried membrane weight, according to the formula:²⁸

$$\% \text{ swelling} = [(w_t - w_0) / w_0] \times 100$$

where: w_0 = dried membrane weight;

w_t = membrane weight at t moment (after immersion in aqueous medium)

d. In vitro release studies

The ellagic acid release from the chitosan-polyvinylpyrrolidone membranes (CH-PVP/EA) was determined with a spectrophotometer, reading

$$EE = \frac{\text{used total amount of EA} - \text{supernatant EA amount}}{\text{total EA amount}} \times 100$$

f. Antioxidant activity: DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging assay

The free radical scavenging activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the modified method of previous studies.^{29,30} Briefly, 100 μ L of film extract solution were mixed with 1 mL of DPPH solution (0.25 mM) and 1.9 mL methanol. The decrease in DPPH radical absorption at 516 nm was measured in the presence of antioxidants.

The antioxidant activity (radical scavenging activity) was calculated using the formula:

$$\% \text{ inhibition} = [(A_0 - A_s) / A_0] \times 100$$

where: A_0 = blank absorbance; A_s = sample absorbance.

g. Statistical analysis: The tests were carried out in triplicate and Microsoft Office Excel 2007 software was used for statistical analysis, the standard deviation (STDV) being <10.

RESULTS AND DISCUSSION

1. FTIR-spectroscopy

Fig. 1 shows the comparative FTIR spectra of CH-PVP membrane (a) and CH-PVP/EA (b) membrane.

the absorbance at 270 nm, at different time intervals: 1, 2, 3, 7 days, and at different pH: 3.0, 7.5, 8.0, 9.0. Small fragments of membranes (40–50 mg) were placed in 50 mL PBS of different pH, at 37°C, being permanently stirred. At fixed time intervals the buffer solution with immersed membrane fragments was analyzed for ellagic acid determination, at 270 nm, as well as for the determination of antioxidant activity.

The ellagic acid released from membrane was calculated based on a calibration curve (EA concentration range between 5–25 μ g/mL).

e. Determination of entrapment efficiency (EE)

The amount of ellagic acid entrapped in chitosan-polyvinylpyrrolidone membranes was calculated considering the initially used EA amount and the EA amount found in the buffer solution, according to the formula:

Fig. 1a shows the FTIR pattern characteristic of the CH-PVP membrane. The broad band due to the stretching vibration of $-\text{NH}_2$ and $-\text{OH}$ groups was observed at 3400–3500 cm^{-1} . The bands assigned to stretching vibration of $\text{C}=\text{O}$ and deformation vibrations of N-H from carboxamide ($\text{O}=\text{C}-\text{NHR}$) groups appeared at 1660 cm^{-1} and 1493 cm^{-1} respectively in pure chitosan spectrum and were preserved after membrane modification. Bands at 2881 cm^{-1} , 1424 cm^{-1} , 1320 cm^{-1} and 1291 cm^{-1} were assigned to CH_2 vibrations of the carbohydrate ring. Bands at 2881 cm^{-1} , 1424 cm^{-1} , 1320 cm^{-1} were assigned to CH_2 vibrations of carbohydrate ring and bands at 1291 cm^{-1} were assigned to tertiary amine C-N stretching from PVP.

Absorption bands at 1152 cm^{-1} (anti-symmetric stretching of the C-O-C), 1082 cm^{-1} and 1031 cm^{-1} (skeletal vibrations involving the C-O stretching vibrations) are characteristic of chitosan saccharide structure.³¹ The band assigned to C-N vibration appears in fingerprint region at 896 cm^{-1} .

The characteristic bands of CH-PVP membrane (Fig. 1a) appeared in the spectra of CH-PVP/EA membrane (Fig. 1b).

The FTIR spectrum of membrane with entrapped EA is similar to that of chitosan membrane due to the low quantity of EA included in membrane. Some changes appear in the CH-PVP/EA spectrum, the absorption band at 1592 cm^{-1} became smaller indicating the EA entrapment

into CH. It is also observed that the absorption bands are more prominent in the case of the membrane with entrapped ellagic acid.

2. Determination of swelling degree

The swelling behavior of chitosan membrane is of great importance due to its main potential applications, namely the contact to aqueous media or physiological fluids and, consequently, it will absorb the water with consecutive alteration of its physical properties.³²

Fig. 2 shows the swelling degree of chitosan membrane and its dependence to pH as follows: the highest swelling degree was obtained in the case of the membrane immersed at lowest studied pH (pH=3.0), then at highest pH (pH = 9.0).

3. *In vitro* release analysis

The ellagic acid amount released from membrane in function of duration (1, 2, 3, 7 days) and at different pH: 3.0, 7.5, 8.0 and 9.0 was calculated. In our experimental conditions the highest amounts of EA was released from the membrane M at lowest pH – pH=3.00, then at the most basic pH (pH=9.0) as it is observed in Fig. 3.

In similar studies in literature, it was also found a slow EA release from CH membranes, but after 3 weeks of incubation³³ or a low EA released from carbon microtubules at acid pH, while it was significant at neutral pH (pH=6.0 and 7.0).²⁵

Generally, the medicines which are chemically or physically entrapped in polymers are released by a combination of processes of diffusion through pores, as well as by degradation of polymer matrices.^{34,35}

It is known the fact that the degradation of chitosan films is dependent to the chitosan deacetylation degree (DDA) and to its molecular weight; the chitosan films with deacetylation degree higher than 73% have a slower biodegradation rate.^{33,36-38}

4. Determination of entrapment efficiency (EE)

The entrapment efficiency was assessed based on EA released in the buffer solution; the results were generally above 50% for the obtained membranes. Lower EE values were recorded even in the case of membranes where the ellagic acid was more rapidly released and in higher amounts – respectively at pH=3.0 and 9.0, at 24h and 48h (Fig. 4).

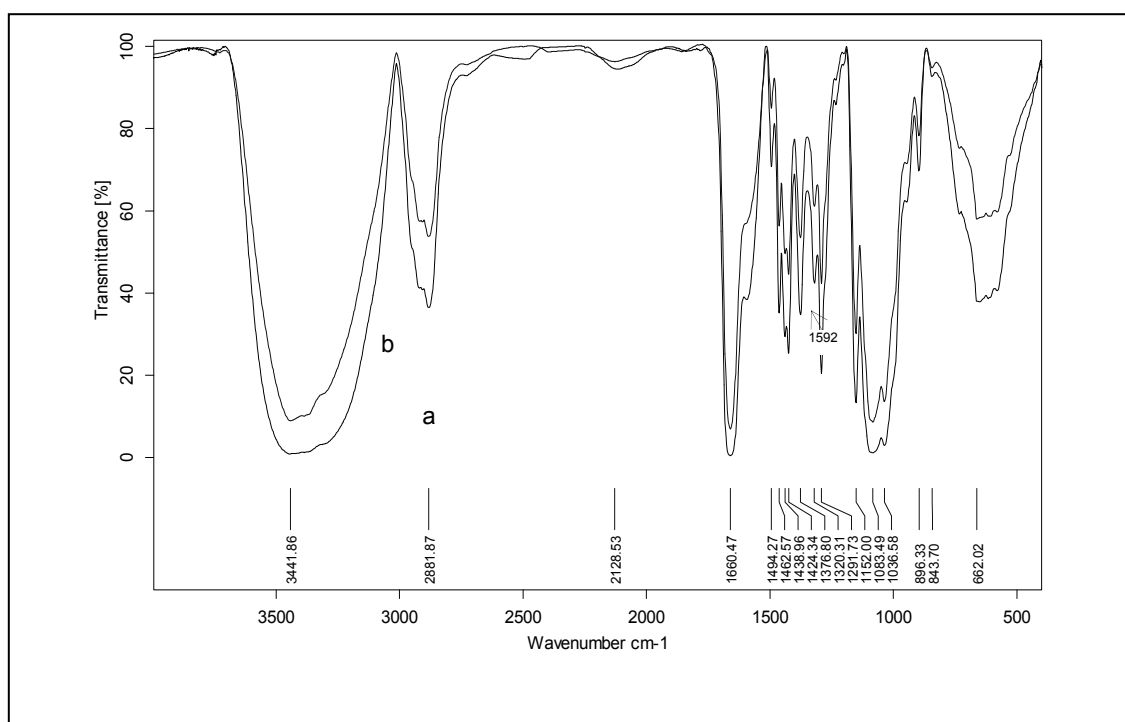


Fig. 1 – The FTIR pattern characteristic to the CH-PVP membrane (a) and CH-PVP/EA (b) membrane.

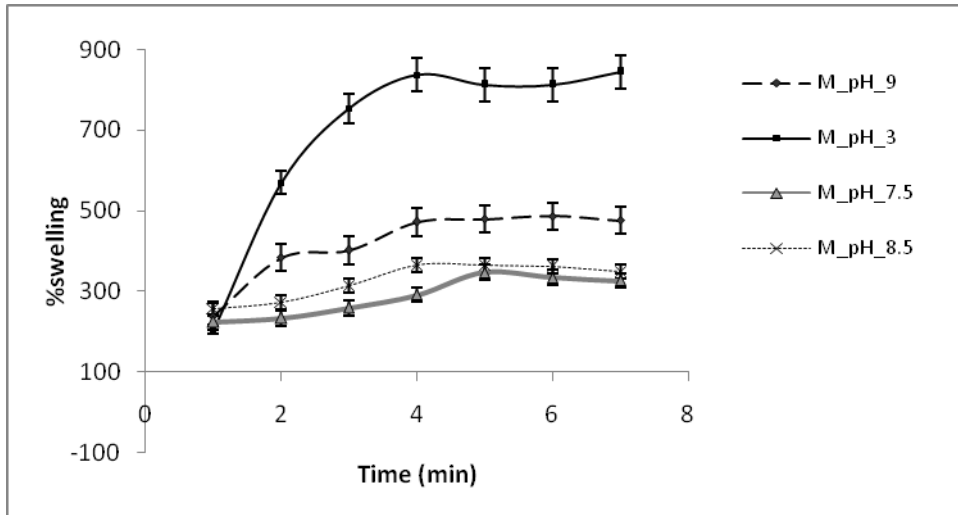


Fig. 2 – The swelling percent of chitosan membrane.

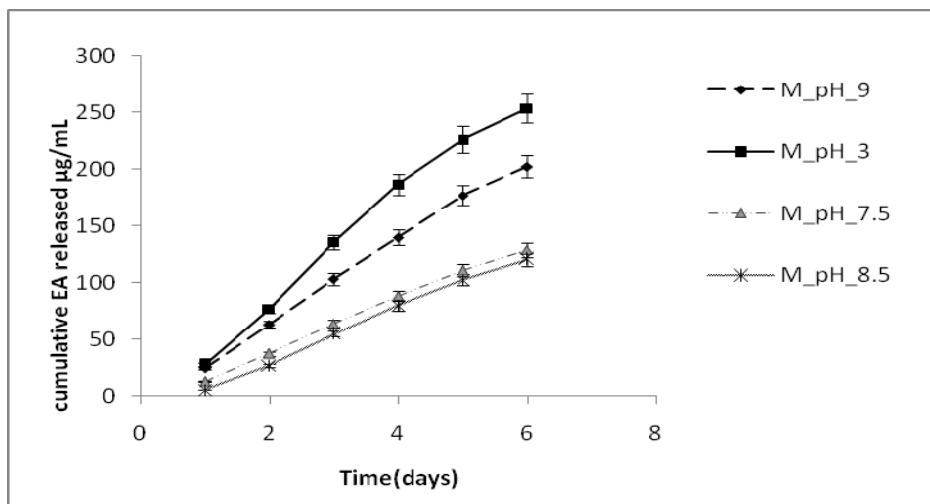


Fig. 3 – Cumulative release of the ellagic acid from the CH-PVP membrane.

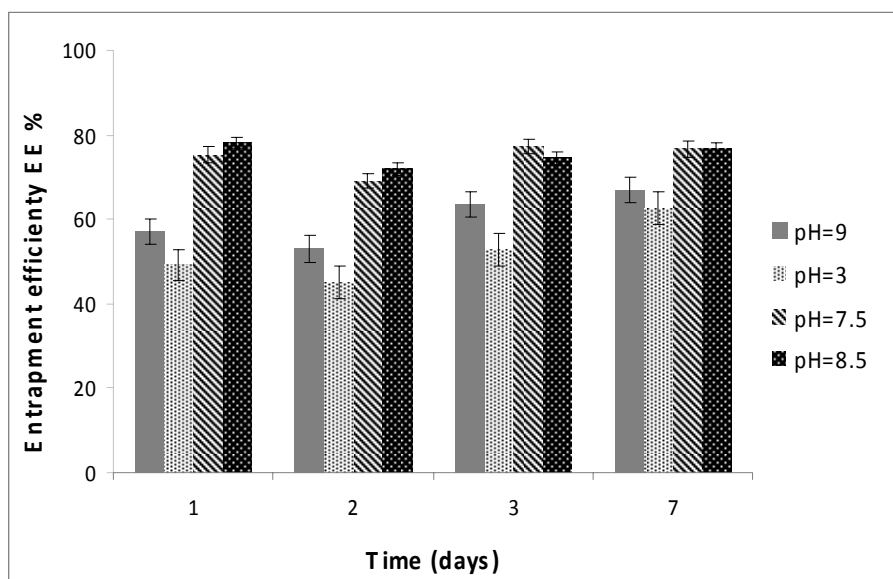


Fig. 4 – The entrapment efficiency (EE) percentage.

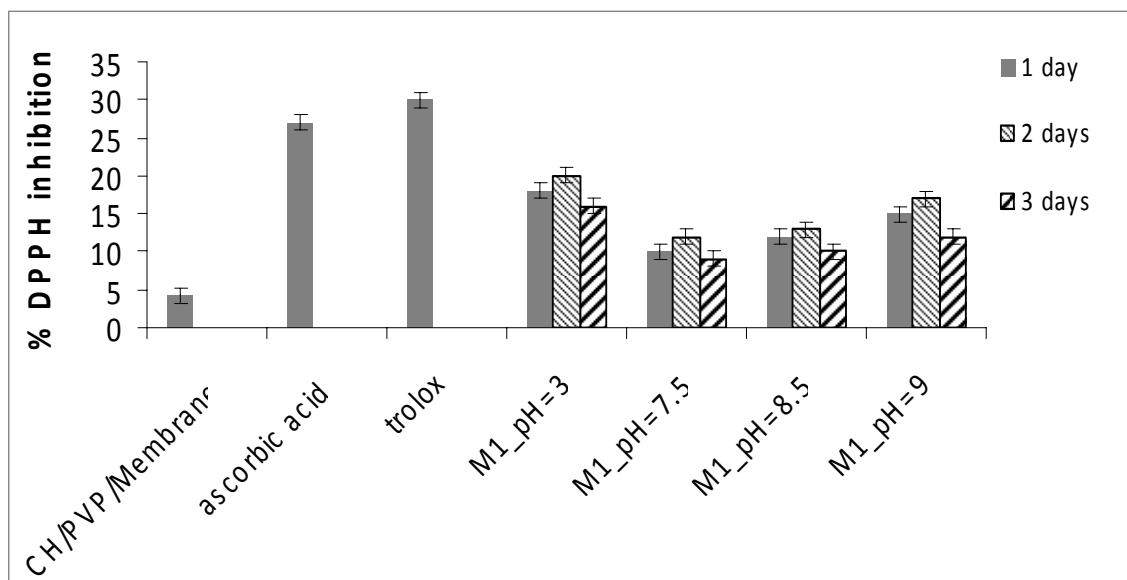


Fig. 5 – DPPH inhibition percentage.

5. Determination of antioxidant activity

The antioxidant activity of the obtained membranes was assessed by DPPH inhibition percent, using ascorbic acid and trolox as standards. The chitosan-polyvinylpyrrolidone membrane (CH-PVP) showed a low antioxidant activity (4.2% DPPH inhibition). The values obtained for the tested samples were < 20% DPPH inhibition, the highest results were obtained at 2 days, at pH=3.0 (20% DPPH inhibition) and pH=9.0 (17% DPPH inhibition). After the optimum time interval the antioxidant activity decreased, the values being lower than previous ones (Fig. 5).

CONCLUSIONS

In this study two types of membranes based on chitosan / polyvinyl pyrrolidone: simple – CH-PVP, and with ellagic acid entrapped – CH-PVP/EA were obtained. The controlled release of EA from this type of membranes in function of time and pH was analyzed. It was found that EA release in the case of this CH-PVP/EA membrane is pH dependent.

The CH-PVP/EA membrane was analyzed in terms of cumulative release of EA, the EA antioxidant activity and entrapment efficiency of EA.

The obtained membrane characterized by Fourier transform infrared spectroscopy (FTIR) had the absorption bands more prominent against

those of CH-PVP membrane without entrapped ellagic acid.

It was concluded that the highest EA amount was released from M membrane at lowest pH – pH=3.0, then at most basic pH – pH=9.0.

The antioxidant capacity of EA released from membrane, assessed by DPPH inhibition percent, was the best at pH=3.00 - 20% DPPH inhibition, at 48h. The results regarding the efficiency of EA entrapment into CH-PVP/EA membrane were generally above 50%.

The obtained membrane could be used to controlled release of some polyphenols with antioxidant activity in pharmaceutical or alimentary scopes.

Acknowledgments: This research was supported by the Roumanian National Center for Program Management – PN 09-360101/2009 projects.

REFERENCES

1. K. Hostanska, G. Jürgenliemk, G. Abel, A. Nahrstedt and R. Saller, *Cancer Detect Prev.*, **2007**, *31*, 129-139.
2. L. K. Shantha, L. D'ath and A. Lawrence, *Carbohydrate Polymers*, **2006**, *64*, 163-167.
3. A. T. Diplock, P. J. Aggett, M. Ashwell, F. Bornet, E. B. Fern and M. Roberfroid, *J Nutr.*, **1999**, *81*, 1-27.
4. J. M. Landete, *Food Res. Int.*, **2011**, *44*, 1150-1160.
5. T. Wu, F. Yen, L. Lin, T. Tsai, C. Lin and T. Cham, *Int. J. Pharm.*, **2008**, *346*, 160-168.
6. Y. Xu, C. Zhan, L. Fan, L. Wang and H. Zheng, *Int. J. Pharm.*, **2007**, *336*, 329-337.
7. L. Illum, *Pharm. Res.*, **1998**, *15*, 1326-1331.
8. H. S. Kas, *J. Microencapsul.*, **1997**, *14*, 689-711.

9. A. D. Sezer and J. Akbuga, *J. Microencapsul.*, **1999**, *16*, 195-203.
10. K. C. Gupta, M. N. Ravi Kumar, *Biomaterials*, **2000**, *21*, 1115-1119.
11. M. A. Augustin and Y. Hemar, *Chemical Society Reviews*, **2009**, *38*, 902-912.
12. K. G. H. Desai and H. J. Park, *Dry. Technol.*, **2005**, *23*, 1361-1394.
13. B. F. Gibbs, S. Kermasha, I. Alli and C. N. Mulligan, *Intern. J. Food Sci. Nutr.*, **1999**, *50*, 213-224.
14. I. Mourtzinou, F. Salta, K. Yannakopoulou, A. Chiou and V. T. Karathanos, *J. Agr. Food Chem.*, **2007**, *55*, 8088-8094.
15. P. Voos, M. Faas, M. Pasojevic and J. Sikkema, *Int. Dairy J.*, **2010**, *20*, 292-302.
16. Z. Fang and B. Bhandari, *Trends Food Sci. Tech.*, **2010**, *21*, 510-523.
17. L. Deladino, P. Anbinder, A. Navarro and M. Martino, *Carbohydr. Polym.*, **2008**, *71*, 126-134.
18. B. Hu, C. Pan, Z. Hou, H. Ye, B. Hu and X. Zeng, *J. Agr. Food Chem.*, **2008**, *56*, 7451-7458.
19. A. Belščak-Cvitanovi, R. Stojanović, V. Manojlović, D. Komes, I. J. Cindrić, V. Nedović, B. Bugarski, *Food Res. Int.*, **2011**, *44*, 1094-1101.
20. R. Paz, P. García, N. Reyes, J. Chávez and J. Santos, *Food Chem.*, **2012**, *134*, 1-8.
21. L. Ming-Wei, Y. Tzi-Peng, P. Hsiu-Hui and C. Jia-Wei, *Carbohydr. Polym.*, **2012**, *87*, 1749-1755.
22. W. Tzu-Hui, Y. Feng-Lin, L. Liang-Tzung, T. Tong-Rong, Lin Chun-Ching, C. Thau-Ming, *Int. J. Pharmaceut.*, **2008**, *346*, 160-168.
23. M. Larrosa, F.A. Tomas-Barberan and J.C. Espin, *J. Nutr. Biochem.*, **2006**, *17*, 611-625.
24. A. Malik, S. Afaq, M. Shahid, K. Akhtar and A. Assiri, *Asian Pac. J. Trop. Med.*, **2011**, *4*, 550-555.
25. N. S. Barnabya, K. R. Fathb, A. Tsiola and I. A. Banerjee, *Colloids Surfaces B: Biointer.*, **2012**, *95*, 154-161.
26. S. Madrigal-Carballoa, S. Limb, G. Rodriguez, A. O. Vilac, C. G. Kruegerb, S. Gunasekaranb and J. D. Reedb, *J. Function. Foods*, **2010**, *2*, 99-106.
27. V.K. Lakshmanan, K. S. Snima, Joel D. Bumgardner, Shantikumar V. Nair and R. Jayakumar, *Adv. Polym. Sci.*, **2011**, *243*, 55-91.
28. K. De Yao, J. Liu, G.X. Cheng, X.D. Lu, H.L. Tu, J. A Lopes da Silva, *J. Appl. Polym. Sci.*, **1996**, *60*, 279-283.
29. V. Bondet, W. Brand-Williams and C. Berset, *Lebensm. Wiss. U. Technol.*, **1997**, *30*, 609.
30. U. Siripatrawana and B. R. Harte, *Food Hydrocolloids*, **2010**, *24*, 770-775.
31. A. Pawlak and M. Mucha, *Thermochim. Acta*, **2003**, *396*, 153-166.
32. C. Santos, P. Seabra, B. Veleirinho, I. Delgadillo and J.A. Lopes da Silva, *Eur. Polymer J.* **2006**, *42*, 3277-3285.
33. K. Sungwoo, M. W. Gaber, J. A. Zawaski, F. Zhang, M. Richardson, X. A. Zhang and Y. Yang, *Biomaterials*, **2009**, *30*, 4743-4751.
34. N. G. Avgeropoulos and T.T. Batchelor, *The Oncologist*, **1999**, *4*, 209-224.
35. M.A. Moses, H. Brem and R. Langer, *Cancer Cell*, **2003**, *4*, 337-341.
36. F.L. Mi, S.S. Shyu and C.T. Chen, *Polymer*, **2002**, *43*, 757-765.
37. C. Tangsadthakun, S. Kanokpanont, N. Sanchavanakit, T. Banaprasert and S. Damrongsakkul, *J. of Metals, Materials and Minerals*, **2006**, *16*, 37-44.
38. T.A. Khan, K.K. Peh and H.S. Ch'ng, *J. Pharm. Pharm. Sci.*, **2002**, *5*, 205-212.

