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Chitosan microbeads for encapsulation of thyme (*Thymus serpyllum* L.) polyphenols

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ABSTRACT

In this work chitosan microbeads were prepared by emulsion technique and loaded with thyme polyphenols by diffusion from an external aqueous solution of *Thymus serpyllum* L. The effects of concentrations of chitosan (1.5–3% (w/v)) and GA (glutaraldehyde) (0.1–0.4% (v/v)), as a crosslinking agent on the main properties of microbeads were assessed. The obtained microgel beads from ~220 to ~790 μm in diameter were exposed to controlled drying process at air (at 37 °C) after which they contracted to irregular shapes (~70–230 μm). The loading of dried microbeads with polyphenols was achieved by swelling in the acidic medium. The swelling rate of microbeads decreased with the increase in GA concentration. Upon this rehydration, thyme polyphenols were effectively encapsulated (active load of 66–114 mg GAE g_{beads}^{-1}) and the microbeads recovered a spherical shape. Both, the increase in the amount of the crosslinking agent and the presence of polyphenols, contributed to a more pronounced surface roughness of microbeads. The release of encapsulated polyphenols in simulated gastrointestinal fluids was prolonged to 3 h.

1. Introduction

In recent years polyphenols, as naturally derived antioxidants, have gained growing interest in the functional food domain. Species of genus Thymus (*Lamiaceae*), rich in polyphenols, were commonly used as spices and it was reported that they manifest specific biological effects: antimicrobial (Hazzit, Baaliouamer, Verissimo, Faleiro, & Miguel, 2009), antifungal (Giordani, Hadef, & Kaloustian, 2008), antibacterial (Essawi & Srour, 2000), as well as antioxidant (Mkaddem et al., 2011; Safaei-Ghomi, Ebrahimabadi, Djafari-Bidgoli, & Batooli, 2009). Particularly, the aqueous *Thymus serpyllum* L. extract is proved to have pronounced antioxidant and free radical scavenging activity in vitro and antihypertensive effect in spontaneously hypertensive rats (Mihailovic-Stanojevic et al., 2013).

The conventional way of plant polyphenols consumption involves an aqueous extraction process of herbs and preparation of tea (herbal infusion). During this process the biological effects of active compounds can be impacted due to presence of oxygen, moisture or inappropriate storage conditions (Fang & Bhandari, 2010). Further, unpleasant taste of polyphenolic compounds often limits their intake to small amounts (Fang & Bhandari, 2010). The difficulties mentioned here could be solved by utilization of encapsulated instead of free polyphenols. Furthermore, by encapsulation the active compounds from liquids are converted to solid forms, which are more easily to handle with. Finally, encapsulation enables regulation of the release of such compounds in the gastrointestinal tract. Encapsulated formulations of aqueous extracts (such as *T. serpyllum* L.) have a potential to be used as additives in new functional food products; upon intake of such products it is possible to achieve a synergistic action of different polyphenolic compounds. There are a number of reports on encapsulation of essential oils derived from plants, but very few studies are focused

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on encapsulation of particularly *T. serpyllum* L. aqueous extract (Stojanovic et al., 2012). The use of natural plant extracts instead of purified compounds is a cost-effective approach.

Chitosan, the cationic (1-4)-2-amino-2-deoxy-β-D-glucan, is industrially produced in food grade from marine and fungal chitin (Muzzarelli, 1996, 2010; Muzzarelli et al., 2012). It is being used in the food industry owing to filmogenicity and absence of toxicity. Chitosan has been used for the encapsulation of probiotics and prebiotics (Chavarri et al., 2010), aromatic compounds (Higuera-Ciapara, Felix-Valenzuela, Goycoolea, & Arguelles-Monal, 2003), enzymes (Žuža, Obradović, & Knežević-Jugović, 2011) and antioxidants (Belščak-Cvitanović et al., 2011; Goncalves da Rosa et al., 2013). Although systems chitosan/polyphenols could be very promising as a functional food additive (combining polymer protective, mucoadhesive and antimicrobial properties with antioxidant activity of polyphenols), to our knowledge up to now there has been no study on entrapment of thyme polyphenols into chitosan microbeads prepared by particularly emulsion crosslinking technique. In addition, in the existing studies incorporation of polyphenolic compounds in chitosan micro/nano-particles has been performed by spray drying or ionic gelation in the presence of polyphenolic compounds (Harris, Lecumberri, Mateos-Aparicio, & Meng, 2011; Liang et al., 2010), while other encapsulation technologies have not been yet explored enough. Thus, in this study an attempt was made to load polyphenolic compounds into the chitosan microbeads obtained by emulsion crosslinking technique (ready-made support) by the so-called post-loading entrapment. Since GA has toxic effects above a certain level (Ghanem & Ghaly, 2004), it was important to know how to design the most efficient formulations with the minimum amount of crosslinking molecules in order to enhance the safety of these systems. Oven-dried forms were under scrutiny as they are reported to be more useful carriers for food applications than swollen samples; they are mechanically stronger and more stable during storage (Nussinovitch & Gershon, 1996).

2. Materials and methods

2.1. Chemicals

Chemicals used in the study: chitosan – Sigma, Japan; itaconic acid, GA, Folin-Ciocalteu reagent, ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and Trolox((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) – Sigma Aldrich Steinheim, Germany; paraffin oil – Centrohem, Serbia; Tween 80 – Riedel-de Haën, Germany.

The molecular weight of the chitosan was determined using a viscometric method and the degree of deacetylation was 92.5% (mean value) (Milosavljević, Kljajević, Popović, Filipović, & Kalagasidis Krušić, 2010). The experimentally determined value for the limiting viscosity number (Kuhn–Mark–Houwink–Sakurada equation; Abdelaal, Abdel-Razik, Abdel-Bary, & El-Sherbiny, 2007) and calculated value for molecular weight were 1.03 mL/mg, and 1537 kDa, respectively.

2.2. Preparation of chitosan microbeads

Chitosan microbeads were synthesized applying the emulsion technique, with addition of GA as a crosslinking agent. The water phase was prepared by adding chitosan solution (1.5–3% (w/v)) in 5 mL of itaconic acid solution (7.5% (w/v)). Water and oil phases (50 mL of paraffin oil) were mixed in 1:10 ratio with the addition of 2.0% (v/v) Tween 80 (1 mL) as an emulsifier. After homogenization, GA solution (0.1–0.4% (v/v)) was added, drop-by-drop, and stirring (750 rpm) was resumed for the next 24 h. Depending on the content of chitosan and GA, the obtained microbeads had different composition, as follows: S(1.5/0.2) – means 1.5% (w/v) of chitosan and 0.2% (v/v) of GA; S(1.5/0.4) – means 1.5% (w/v) of chitosan and 0.4% (v/v) of GA; S(2.0/0.1) – means 2.0% (w/v) of chitosan and 0.1% (v/v) of GA, S(2.0/0.2) – means 2.0% (w/v) of chitosan and 0.2% (v/v) of GA; S(2.0/0.4) – means 2.0% (w/v) of chitosan and 0.4% (v/v) of GA; S(3.0/0.1) – means 3.0% (w/v) of chitosan and 0.1% (v/v) of GA; S(3.0/0.2) – means 3.0% (w/v) of chitosan and 0.2% (v/v) of GA and S(3.0/0.4) – means 3.0% (w/v) of chitosan and 0.4% (v/v) of GA. In order to remove all residues of the surface active agent, the microbeads were washed out three times, using water, ethanol and petroleum ether, respectively. The microbeads were dried in the oven at 37 °C for 48 h and kept until further use in a desiccator at 25 °C.

2.3. Preparation of thyme aqueous extract

Thyme (The Institute of Medicinal Plants Research "Dr Josif Pančić", Belgrade, Serbia) extract was prepared by conventional water extraction process, by pouring 200 mL of boiled distilled water over 10 g of ground plant at room temperature. The extraction was carried out for 30 min with stirring, and the extract was filtered through medical gauze.

2.4. Encapsulation of thyme polyphenols in chitosan microbeads

Dry chitosan microbeads (around 0.1 g) were immersed in the flask containing 10 mL of aqueous thyme extract and 2.0% (w/v) of itaconic acid. Itaconic acid was added in order to adjust pH (at 3.4). Chitosan microbeads were held in thyme extract for 24 h with mild orbital shaking (Rotamax 120, Heidolph, Germany). After that, the microbeads with encapsulated polyphenols were dried and kept in desiccator until further use, as described in Section 2.2.

2.5. Characterization of microbeads

2.5.1. Swelling degree studies

In order to simulate the gastrointestinal (GI) tract temperature and pH conditions, as well as the average time spent in GI tract, the swelling studies were carried out at 37 °C, by immersing the microbeads in a buffer solution of pH values of 2.20 ± 0.01 (HCl/KCl) for a period of 2 h and subsequently, in a buffer solution of pH 6.80 ± 0.01 ($NaH_2PO_4 \times 2H_2O/Na_2HPO_4 \times 12H_2O$) for the next 22 h.

After immersion, microbeads weight was measured in predetermined time intervals. The degree of swelling was calculated according to the following equation:

$$q = \frac{w_t}{w_0} \quad (1)$$

where w_t corresponds to the weight of swollen microbeads at time t and w_0 to the weight of dried microbeads. The swelling process was monitored gravimetrically.

2.5.2. Bead size and size distribution

The size and size distribution of the obtained chitosan microbeads (native and with encapsulated polyphenols) was determined using Mastersizer 2000 (Malvern Instruments, Worcestershire, UK), equipped with the Hydro 2000S dispersion unit. The mean diameter over volume (also called DeBroukere mean) was used as representative diameter. The breadth (span) of the size distribution was calculated as follows:

$$\text{Breadth} = \frac{D_{90} - D_{10}}{D_{50}} \quad (2)$$

where D_{90}, D_{10} and D_{50} correspond respectively to the beads diameters bellow which 90, 10 and 50% of beads volume were found.

Data related to macroscopic structure of beads were obtained using optical microscope Olympus CX41RF, equipped with picture analyzing software "CellA" (Olympus, Tokyo, Japan). Microbeads were examined immediately after formation (relaxed state of polymer chains) – native microbeads, in dry and in rehydrated form (dry beads immersed in buffer of pH 2.20 ± 0.01, until the equilibrium degree of swelling was achieved).

Microscopic picture analysis was used to calculate the sphericity of the hydrogel, oven-dried and rehydrated microbeads according to the following expression described in literature (Chan, 2011b):

$$\text{Sphericity factor}\,(SF) = \frac{D_{max} - D_{per}}{D_{max} + D_{per}} \quad (3)$$

where D_{max} presents the largest dimension passing through the microbead centroid, while D_{per} presents the dimension perpendicular to D_{max} passing through the microbead centroid.

The reduction in microbead size after oven drying process was calculated by the following equation (Chan et al., 2011a):

$$k_{(SF)} = \frac{D_h - D_o}{D_h} \quad (4)$$

where $k_{(SF)}$ stands for shrinkage factor related to the oven drying process; D_h is the mean diameter of the hydrogel microbeads, while D_o is the mean diameter of the microbeads after oven drying.

2.5.3. Scanning electron microscopy

The surface microbeads morphology was evaluated by means of scanning electron microscopy (Tescan Mira3 XMU, Cranberry Township, USA). Samples were analyzed in three different forms: native, dry and rehydrated microbeads. Prior to SEM analysis samples were coated with gold/platinum alloy (15/85) under vacuum conditions, using Polaron SC502 vacuum sputter coater.

A simple method for obtaining cross-sections was developed utilizing alginate solution/hydrogel as a microbead carrier. In brief, dry microbeads were mixed with 1.73% w/v sodium alginate solution and the mixture was poured in a Petri dish, left to dry at room temperature for 48 h in order to obtain dry film with incorporated dry microbeads. Films were cut across the incorporated microbeads and used for FE-SEM analysis. After a deposition of a thin gold/palladium layer on the film cuts, a TESCAN MIRA 3 XMU field emission scanning electron microscope (FE-SEM), operated at 20 keV, was used to analyze the morphology of the microbeads cross-section.

2.5.4. Bead porosity

Nitrogen adsorption–desorption isotherms were determined using a Micromeritics ASAP 2020 instrument. Samples were degassed at 105 °C for 10 h under reduced pressure. The specific surface area of samples was calculated according to the Brunauer, Emmett, Teller (BET) method from the linear part of the nitrogen adsorption isotherms. The total pore volume was given at $p/p_0 = 0.998$. The volume of the mesopores was calculated according to the Barrett, Joyner and Halenda method from the desorption branch of isotherm.

2.6. Determination of total phenol content and encapsulation efficiency

Total phenol content (TPC) was determined spectrophotometrically using Folin-Ciocalteu reagent, as described by Stojanovic et al. (2012).

Encapsulation efficiency was calculated from the actual loading of the microbeads with polyphenols and the theoretical loading. Encapsulation efficiency, *EE* (%) was calculated as shown in Eq. (5):

$$EE(\%) = TPC_e/TPC_i \quad (5)$$

where TPC_e is the total phenol content encapsulated in beads, while TPC_i is the total phenol content in the initial extract solution used for the encapsulation process. TPC_e was calculated as a difference between total amount of polyphenols (in the initial thyme extract used for encapsulation) and the free amount of polyphenols left in the extract solution after encapsulation process.

2.7. Release studies

The release of polyphenols from dried chitosan microbeads with encapsulated polyphenols was studied for 24 h at 37 °C in buffer solutions simulating GI conditions. Around 0.1 g of dried chitosan microbeads with encapsulated polyphenols were placed in the flask containing 10 mL of pH 2.20 ± 0.01 solution for 2 h under mild magnetic stirring in order to simulate gut movement. Successive aliquots of a sample were taken from the solution at pre-determined time intervals. After 2 h microbeads were immediately transferred to appropriate quantity of pH 6.80 ± 0.01 solution and sampling was continued for 22 h. The total phenol content of aliquots was determined as previously described (Section 2.6.).

2.8. Statistical analysis

Experiments were carried out in duplicate or triplicate and expressed as means with SD. The Tukey test was used to determine the significant difference between sample at $p < 0.05$ level. All analyses were done using Microsoft Office Excel 2007 software (Microsoft Corporation, WA, USA).

3. Results and discussion

3.1. Swelling studies

In this study loading of microbeads with polyphenols proceeded with simultaneous swelling process. Fig. 1 shows dynamic swelling behavior of all samples in simulated gastric fluid of pH 2.20 ± 0.01 for 2 h and then in simulated intestinal fluid of pH 6.80 ± 0.01 up to 24 h. In acidic environment, the swelling rate increased since the chitosan amino groups were protonated (NH_3^+); according to literature data (Kim, Shin, Lee, & Kim, 2003) these cationic charges of the polymer molecules act as cationic repulsive forces between polymer chains, enabling higher degree of swelling. Within the first 2 h, swelling rate was lower as the concentration of GA in microbeads was higher, since there are less free amino groups available for protonation. Namely, the aldehyde groups of the GA appeared to form covalent imine bonds with the amino groups of chitosan, due to the resonance established with adjacent double ethylenic bonds via a Schiff reaction (Goncalves, Laranjeira, Favere, & Pedrosa, 2005). Thus, with increasing degree of crosslinking with GA, most likely fewer amino groups became available on the chitosan backbone for protonation leading to a lower swellability of chitosan microbeads. After being transferred to the medium with pH 6.80 ± 0.01, microbeads of all samples shrunk, since hydrogen bonds tended to associate by changing $-NH_3^+$ into $-NH_2$ groups, as a result of low concentration of H^+ (Kim et al., 2003). Importantly, after 24 h, the swelling of microbeads remained constant. This implied that swollen microbeads remained stable and thus, can be considered as useful for delivery of low molecular weight molecules such as polyphenols.

3.2. Size of microbeads

The mean diameter of native chitosan microbeads varied in the rather wide range, from ~220 to ~790 μm, depending on the content of GA and chitosan (Table 1). Obviously, the formation of covalent imine bonds between GA and chitosan

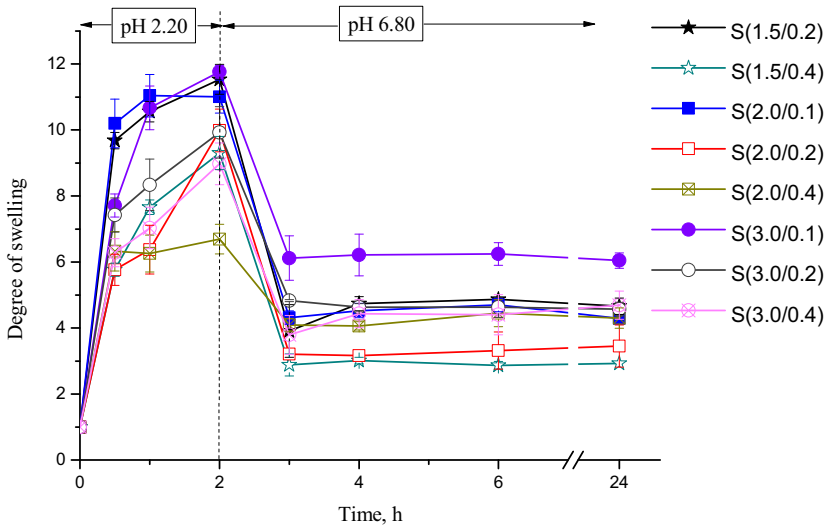

Fig. 1. Swelling kinetics of chitosan microbeads.

Table 1
Mean diameter (D), sphericity (SF) and shrinkage factors (k_{SF}) for hydrogel, oven-dried and microbeads after rehydration in a buffer of pH value 2.20 ± 0.01.

Microbeads sample	Hydrogel microbeads		Oven-dried microbeads		Rehydrated microbeads with encapsulated polyphenols	
	D[a] (μm)	SF[b]	D (μm)	k_{SF}[c]	D (μm)	SF[b]
S(1.5/0.2)	792.2 ± 44.4	0.056	233.3 ± 21.2	0.706	890.9 ± 90.3	0.080
S(1.5/0.4)	616.6 ± 29.2	0.067	209.8 ± 24.3	0.771	618.9 ± 14.0	0.112
S(2.0/0.1)	721.7 ± 34.7	0.028	168.9 ± 15.1	0.766	435.5 ± 49.3	0.050
S(2.0/0.2)	602.4 ± 65.4	0.043	140.7 ± 18.8	0.766	359.1 ± 37.9	0.069
S(2.0/0.4)	602.6 ± 62.6	0.046	135.4 ± 15.8	0.775	343.9 ± 30.3	0.074
S(3.0/0.1)	427.5 ± 24.4	0.011	99.3 ± 11.4	0.768	445.4 ± 25.9	0.057
S(3.0/0.2)	299.1 ± 25.9	0.014	72.1 ± 9.9	0.759	367.8 ± 39.5	0.079
S(3.0/0.4)	220.3 ± 25.7	0.016	68.2 ± 5.9	0.763	358.6 ± 34.4	0.081

[a] $n = 3$.
[b] The average absolute deviation less than 11% ($n = 30$).
[c] The average absolute deviation less than 4% ($n = 30$).

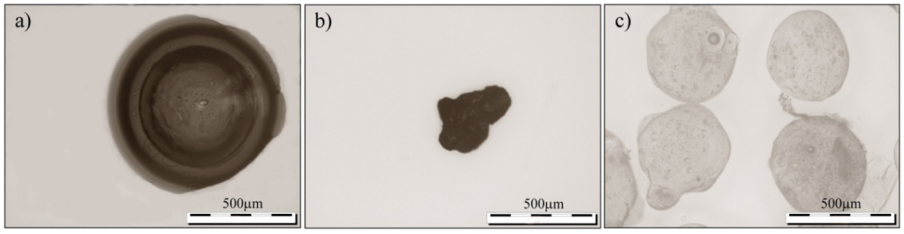

Fig. 2. Microphotographs of S(2.0/0.4) chitosan microbeads (a) native microbead; (b) an agglomerate of dry microbeads; (c) rehydrated microbeads with encapsulated thyme extract ('bar' 500 μm).

affected the microbeads size. Therefore, the non-consistent trend in the microbeads diameters was observed for both, native and microbeads after rehydration. The microbeads prepared with higher concentration of GA and/or higher concentration of chitosan, were found to be smaller in size compared to microbeads with lower concentrations of chitosan and GA ($p > 0.05$). This clearly indicates that microbeads were more compact if the degree of crosslinking was higher. Hydrogel microbeads appeared as perfectly round, shown in Fig. 2a and proved by SF values in Table 1 (particles with $SF \leq 0.05$ are considered as spherical, Chan et al., 2011a) with an exception of the S(1.5/0.2) and S(1.5/0.4) samples. The content of chitosan had an effect on shape as evidenced by a change in the sphericity factor calculated using Eq. (3). A decrease in sphericity factor with the increase in chitosan content (at the same concentration of GA) is confirmation of strong molecular packing. This effect surpassed the influence of increased viscosity of the chitosan dispersed phase (during microbeads preparation); the last usually reflects on efficiency of the emulsification process in a way of enlargement of the forming beads. On the other hand, the increase in the amount of GA added led to a decrease in sphericity, at the same concentration of chitosan. It was assumed that GA, if present in higher concentrations, occupied amino groups of chitosan more rapidly leading to a faster formation of the polymer network; thus, the time remaining for formation of regular spheres became less sufficient. After drying the microbeads shrunk to irregular shapes (Fig. 2b) which had 22–34% of the initial size (shrinkage factor between 0.706 and 0.775) and SF values far from the values for spherical symmetry (data not shown).

After they were re-immersed in a buffer solution (pH 2.20 ± 0.01) microbeads recovered a round shape distorted from

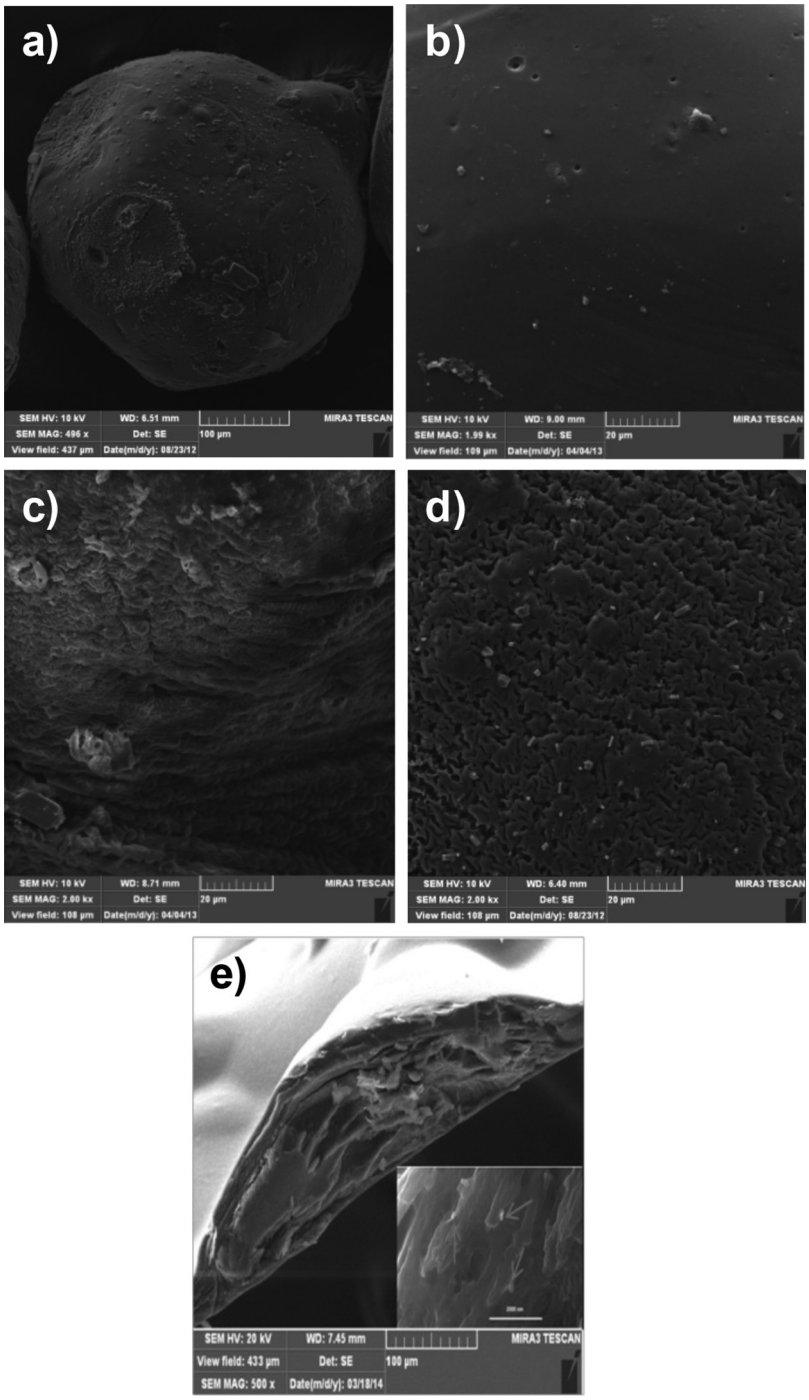

Fig. 3. SEM micrographs of oven-dried chitosan microbeads: (a) S(2.0/0.4) microbeads with encapsulated polyphenols (496×); (b) S(2.0/0.1) empty microbead – surface (2000×); (c) S(2.0/0.4) empty microbead – surface (2000×); (d) S(3.0/0.1) microbead with encapsulated polyphenols – surface (2000×); (e) S(2.0/0.4) microbead with encapsulated polyphenols – cross-section (496×); inset: (30,000×).

the perfect sphere (Fig. 2c) ($SF \geq 0.05$, Table 1) having 57–163% of the initial diameter. In addition, according to breadth of microbeads size distribution, polydispersity of samples differs insignificantly both among empty microbeads (0.813 ± 0.091) and microbeads with encapsulated polyphenols (1.678 ± 0.432) ($p>0.05$). The considerable difference was noticed only between the empty chitosan microbeads and microbeads with encapsulated polyphenols, which indicated the reduction in microbeads uniformity upon encapsulation.

It can be assumed that during the drying stage, a strong reduction in the average distance among crosslinking sites occurred, which promoted a substantial increment in the actual crosslinking density of the beads, probably through additional interpolymeric hydrogen bonding (Barreiro-Iglesias, Coronilla, & Concheiro, 2005).

3.3. SEM analysis and porosity of microbeads

SEM analysis was performed in order to obtain information about morphology of chitosan microbeads. Fig. 3 shows the micrographs of oven-dried microbeads, as well as the appropriate surfaces. Oven-dried microbeads appeared as spherical with dense and smooth morphology (Fig. 3a and 3b). The observations under

Table 2
Encapsulation efficiency (*EE*) and total phenol content (TPC) of oven-dried microbeads with encapsulated thyme polyphenols.

Sample	*EE*[a] (%)	TPC[a] (mg GAE g_{beads}^{-1})
S(1.5/0.2)	61.8 ± 1.2	105.1 ± 12.0
S(1.5/0.4)	62.3 ± 1.2	105.7 ± 18.0
S(2.0/0.1)	59.3 ± 0.8	97.3 ± 1.8
S(2.0/0.2)	61.8 ± 1.1	104.6 ± 4.1
S(2.0/0.4)	67.2 ± 1.0	114.5 ± 1.9
S(3.0/0.1)	44.8 ± 0.9	66.4 ± 2.3
S(3.0/0.2)	54.6 ± 0.9	80.5 ± 5.0
S(3.0/0.4)	56.5 ± 1.1	91.5 ± 7.9

[a] $n = 3$.

high magnification showed rough surface of microbeads when high amount of GA (0.4% (v/v)) was used for crosslinking (Fig. 3c). It has been reported that GA crosslinking often leads to the formation of rough microbeads, which can be overcome by using toluene saturated GA (Gohel, Sheth, Patel, Jani, & Patel, 1994); however, scientists are still seeking the appropriate crosslinking agent which will give well designed spheres and, at the same time, be safe for oral consumption.

The encapsulation had an effect on the surface morphology of the microbeads, as it is clear from the SEM micrographs for microbeads without encapsulated polyphenols (Fig. 3c) and microbeads when polyphenols from thyme aqueous extract were encapsulated (Fig. 3d). The encapsulation of polyphenols imparted a high degree of surface roughness associated with surface deposition of polyphenolic crystals. An oven-dried microbead with encapsulated polyphenols has been broken to reveal the architecture of the pores in the bulk of the matrix (Fig. 3e).

The SEM image of a cross-sectional fracture shows the presence of polyphenols crystals (as indicated by arrows). Porosity measurements also confirmed that upon encapsulation polyphenols fulfill cavities of the matrix, therefore causing reduction in porosity. Thus, empty microbeads are far more porous (the average pore diameter 35.0 nm, specific surface 2.18 m^2/g) in comparison to microbeads with polyphenols (e.g. for S(2.0/0.4) the average pore diameter 12.3 nm, specific surface 0.59 m^2/g).

3.4. Efficiency of encapsulation

The polyphenols content and encapsulation efficiency appeared to be affected by both concentration of the polymer and the amount of GA added. As can be seen from the results in Table 2, the highest polyphenols encapsulation efficiency was achieved with concentrations of chitosan and GA of 2.0% (w/v) and 0.4% (v/v), respectively. It seems that inferior results were obtained for 3% (w/v) chitosan while, in general, the increase in GA concentration led to a better entrapment of polyphenols. The result for encapsulation efficiency is consistent with total phenol content in microbeads (Table 2), since the same quantity of the extract solution was spent in all preparations. As the absorption process was conducted in the excess of polyphenols, by reducing the extract expenditure (and keeping the same amount of microbeads) it is possible to increase encapsulation efficiency. The question is down to what level is possible to reduce extract-microbeads ratio while still having encapsulation process feasible. Therefore, the more relevant parameter to assess successfulness of encapsulation is the actual load, i.e. total phenol content in microbeads. Total phenol content in microbeads varied in the range 66–114 mg GAE g_{beads}^{-1} depending on the sample (Table 2) while the TPC in the original thyme extract amounted 2.4 mg GAE mL^{-1}. In general, encapsulation efficiency is a complex function of several factors influencing absorption of polyphenols, such as hydrophilicity of the polymer matrix, porosity of the network and interactions between

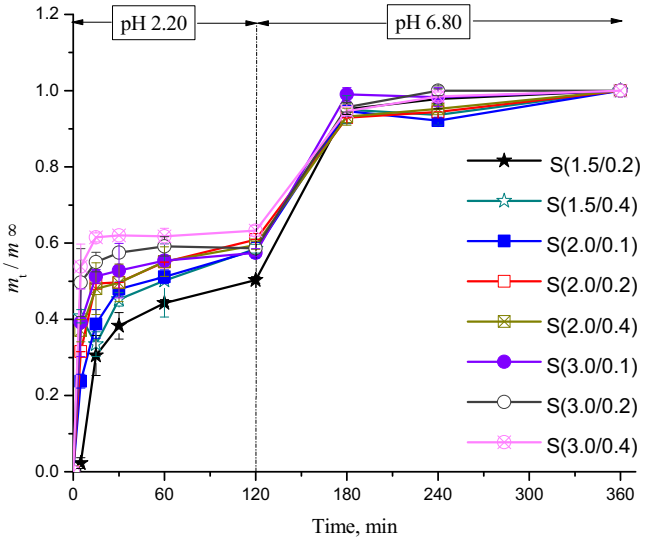

Fig. 4. Release of thyme polyphenols from chitosan microbeads in simulated GI conditions.

matrix components and the extract compounds. GA contributes to hydrophobic nature of the matrix, as reported in literature, since dialdehydes alter the species that are responsible for water binding (Beppu, Vieira, Aimoli, & Santana, 2007) and probably, for binding of other molecules as amino groups are blocked with aliphatic chains (as confirmed by FT-IR analysis; data not shown). On the contrary, higher GA content increases the number of binding cites (via methylene groups of GA shown by FT-IR spectra; data not shown) that might allow additional loading of polyphenols into the polymer matrix. Secondly, the swelling behavior of pre-formed dried chitosan microbeads plays an important role in loading mechanism. Herein, microbeads were less swellable if they contained more GA, as discussed previously (Section 3.2). Also, there is an influence of microbeads size on loading capacity; more precisely, a decrease in the beads size should lead to reduction in the amount of polyphenols that could be encapsulated.

3.5. Release studies

The release of water soluble polyphenolic compounds from crosslinked chitosan microbeads involves simultaneous absorption of water and desorption of the compounds via a swelling controlled mechanism (Dini, Alexandridou, & Kiparissides, 2003). The diffusion properties and the release kinetics of encapsulated extract compounds are functions of the shape, size and size distribution of microbeads, the crosslinked density of the polymer matrix, the content of extract compounds encapsulated into the microbeads and the kinetics of the swelling process. The fractional amount of polyphenols released from the chitosan microbeads m_t/m_∞ vs. time for different formulations is plotted in Fig. 4.

The release of polyphenols exhibited an initial burst effect which is more pronounced in some samples (e.g. S(3.0/0.4)) than in others (e.g. S(1.5/0.2)). The initial fast release was mainly caused by desorption of the non-incorporated polyphenols from the outer region of the microbeads. Swelling of the polymeric matrix occurred simultaneously with diffusion of polyphenols. The initial fast release was followed by tailoring-off behavior, characteristic for a release from the interior of the swollen hydrogel matrix through a more convoluted pathway (Forni, Vandelli, & Cameron, 1992).

The degree of swelling is governed mostly by the crosslinking density of the polymer network, as already described (Fig. 1) and as reported in literature (Dini et al., 2003). The expected effect was an

increase in polyphenols release rate with a decrease in the amount of crosslinking agent (due to the decrease in available free space for polyphenols diffusion). However, the obtained outcome was, actually, the decrease in release rate in the ranking order from S(3.0/0.4) (the smallest microbeads) to S(1.5/0.2) (the largest microbeads) (Table 1). Namely, the larger microbeads provide longer path for diffusion whereas those of smaller diameters exhibit higher specific surface, which additionally contributes to enhanced release of the entrapped compounds. About 70% of the total polyphenols content was released during the time period of 6 h, whereas about half of this amount was released in the medium of pH 2.20 ± 0.01. The incomplete release suggested that chemical interactions occurred between polyphenols and the matrix (as also confirmed by FT-IR analysis; data not shown) since polyphenols were completely soluble in an aqueous medium (Popa, Aelenei, Popa, & Andrei, 2000).

4. Conclusion

The suitability of chitosan microbeads prepared by emulsion technique as a carrier for thyme aqueous extract has been assessed. A high loading degree of thyme polyphenols was achieved when encapsulation was performed by swelling of dry microbeads in accommodated acidic environment. However, in order to increase the encapsulation efficiency it is necessary to further optimize the method proposed here. The features of the obtained chitosan microbeads were mostly affected by crosslinking degree. The prolonged release of polyphenols in simulated GI conditions is a confirmation that encapsulation system examined in this paper has strong potential to be used as a functional food additive. In order to enhance safety of the proposed system, a substitution of GA by an alternative crosslinking agent could be considered.

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