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ANTIOXIDANT AND ANTIMICROBIAL CAPACITY OF ENCAPSULATED THYME ESSENTIAL OIL IN ALGINATE AND SOY PROTEIN-BASED CARRIERS

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The aim of this study was to develop a stable hydrogel carrier system for thyme essential oil (TEO) that could protect its sensitive polyphenol compounds. The impact of wall material (soy protein and alginate) on encapsulation efficiency and thymol release in simulated gastrointestinal conditions, was investigated. The release of thymol was ~ 80 % and 20 % in simulated gastric and pancreatic solutions, respectively. Thyme essential oil plays an important role as an antimicrobial and antioxidant agent. Results indicated that encapsulated TEO inside the hydrogel matrix exhibited antioxidant activity demonstrated by CUPRAC and ABTS analysis, even after thermal treatment of the beads, indicating the metal chelate effect as dominant. In vitro antimicrobial activity of encapsulated TEO has been studied against several pathogenic microorganisms such as Escherichia coli, Staphylococcus aureus, Bacillus cereus and Candida albicans. Beads coded as Ca-A1.5/SP1.5 showed anti-Candida albicans activity, while modified bead formulations Ca-A1.5/SP1.5 and Ca-A1.5/SP0.25** showed bactericidal activity against Escherichia coli and Staphylococcus aureus.*

Keywords: encapsulation, hydrogel carriers, thymol release, antioxidant activity, antimicrobial activity.

INTRODUCTION

Essential oils (EOs) are odoriferous, highly volatile substances mainly identified with terpenoids, aromatic and aliphatic compounds, having a strong interest in food, pharmaceutical, and agricultural industries. EOs have been proposed as an alternative to antibiotic growth promoters, regarding their antioxidant, anti-inflammatory, and antimicrobial properties. Numerous studies have shown that EOs possess great antimicrobial activities against bacteria and fungi (1). In specific, EOs are particularly effective against *Candida albicans* (2). Gram-negative bacteria are less susceptible than Gram-positive bacteria (3), as they contain a lipopolysaccharide component (LPS) in the outer membrane of their cell wall, which protects them from hydrophobic compounds with antimicrobial properties, present in essential oils (4, 5). Therefore, it could be challenging to deliver oil concentration to the targeted place in the human body which will be effective in controlling different bacteria species.

Both, antioxidant and antimicrobial effects of many EOs, such as thyme (*Thymus vulgaris*) essential oil, are associated with the presence of the major polyphenol compounds: thymol and carvacrol (6). Nevertheless, EO components tend to be thermolabile and volatile, and may easily oxidize which leads to a decrease in their biological potential (7).

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Recent strategies in the pharmaceutical and food industry have been focused on the design of bio-based carriers for entrapment and protection of essential oils (8, 9). Moreover, these essential oil-loaded polymer particles can be designed to carry bioactive substances to specific locations within the gastrointestinal tract (GIT) and release them at a controlled rate. During the last years, protein-based systems for encapsulation of bioactive agents have been studied due to their specific functional and nutritional properties (10, 11). Protein-based hydrogels have potential as controlled delivery systems due to their ability to produce a response to an environmental stimulus (e.g. changes in their physicochemical properties with a change in pH or temperature conditions) (12). For instance, protein unfolding and exposure of hydrophobic groups that lie on their surface make them potential surface-active molecules. Protein surface activity is related to the formation of a layer (i.e. adsorption) on a surface of oil droplets, thus providing oil stability and protection from droplets aggregation. Furthermore, proteins are known as oxidation inhibitors through free radical scavenging and chelation (13). However, high acidity in the stomach, as well enzymes presented in GIT which can initiate protein digestion; this poses a need for a combining with additional biopolymer to achieve protein hydrolysis at a slower rate and consequently slower oil release.

Blending natural biopolymers, protein and sodium alginate was found to be promising due to alginate's relatively slow degradation profile, which is related to shrinking in an acidic environment, thus providing protection from fast protein hydrolysis in simulated conditions of the stomach. According to the literature, this kind of blend has already been used for the preparation of microcapsules (14, 15). More precisely, the alginate/soy protein system for essential oil encapsulation has been partially characterized in our previous work (16).

This study represents the extension of our previous work (16) with the aim to investigate the potential of the encapsulation of thyme essential oil (TEO) in Ca-alginate/soy protein beads to achieve targeted release and protect its bioactive properties for application in food and pharmacy. Encapsulation efficiency (EE) and release profile of thymol, the main constituent of TEO, were calculated for different beads formulations by HPLC analysis. The ABTS and CUPRAC methods were used to confirm the antioxidant activity of prepared beads, as well test in suspension was carried out to confirm the antimicrobial activity of encapsulated oil against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans*.

EXPERIMENTAL

MATERIALS

Sodium Alginate (sodium;3,4,5,6-tetrahydroxyoxane-2-carboxylate) was purchased from PanReac AppliChem, Germany. Commercial soy protein isolate powder (SPI) was purchased from Brenntag, Ireland. Thyme oil was supplied from local pharmaceutical shop Prima Cosmetics doo. Thymol (5-methyl-2-propan-2-ylphenol), analytical standard, was supplied from Dr. Ehrenstorfer GmbH. Pancreatin 4X USP grade (from porcine pancreas) was purchased from MP Biomedicals, LLC, France. Bile salts were obtained from Biolife, Italia. Pepsin (from porcine gastric mucosa), Neocuproine (2,9-Dimethyl-1,10-phenanthroline), Trolox (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and ABTS (2,2'-

azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)diammonium salt), were obtained from Sigma-Aldrich, Germany. Calcium chloride dihydrate was purchased from Analytika, Ltd., Chech Republic. Methanol and acetonitrile (HPLC grade) were purchased from J.T. Baker, Netherlands. All reagents were used in accordance with the manufacturer's recommendations.

EMULSION AND BEADS PREPARATION

The Na-alginate/soy protein/thyme oil emulsions were prepared as follows. Na-alginate was dissolved and SPI was dispersed, both in water, after which protein solubility was enhanced by adjusting the SPI dispersion to pH 8.0. Finally, the protein denaturation step was carried out by heating the obtained SPI solution for 40 min at 80 °C. Both solutions (at room temperature) were mixed together, and thyme oil was added to the final Na-alginate/soy protein solution (polymer : thyme oil=5:1). The emulsion was homogenized with an UltraTurrax (IKA T25 digital, Germany) at 12,000 rpm for 3 min.

After preparation, the emulsion was extruded using a syringe pump (Razel, Scientific Instruments, Stamford, USA), for maintaining a constant flow rate, with an applied voltage of 5 kV (high voltage unit - Model 30R, Bertan Associates, Inc., New York). Thus formed droplets were trickled into a gelling bath solution containing $CaCl_2 \cdot 2H_2O$ (10 g/L), with gently stirring for the next 15 min to harden.

According to the Ca-alginate and SPI contents, obtained beads were coded as Ca-A1/SP1 (1 wt.% of Na-alginate and 1 wt.% of SPI), Ca-A1/SP1.5 (1 wt.% of Na-alginate and 1.5 wt.% of SPI), Ca-A1.5/SP1 (1.5 wt.% of Na-alginate and 1 wt.% of SPI), Ca-A1.5/SP1.5 (1.5 wt.% of Na-alginate and 1.5 wt.% of SPI), Ca-A2/SP1 (2 wt.% of Na-alginate and 1 wt.% of SPI) and Ca-A2/SP1.5 (2 wt.% of Na-alginate and 1.5 wt.% of SPI). Beads optimized for antimicrobial analysis were coded as Ca-A1.5/SP1.5* (1.5 wt.% of Na-alginate, 1.5 wt.% of SPI and 250 μL of TEO/4g of beads) and Ca-A1.5/SP0.25** (2 wt.% of Na-alginate, 0.25 wt.% of SPI and 250 μL of TEO/4g of beads).

CHARACTERIZATION OF THE BEADS

Size measurements of beads, wet and dry analogs, were evaluated under a digital light microscope (Motic BA210 Series), employing the image analyzer software (Motic Images Plus 2.0 ML). The shape of the beads was quantified by using the dimensionless shape indicator - Sphericity factor (SF), where the value zero of SF indicates a perfect sphere (17).

HPLC ANALYSIS

HPLC experiment was performed using a Nexera X2 with RID 20A detector (Shimadzu), with an ACE C18 (4.6 × 250 mm, 5 μm) column. UV-visible spectral properties were collected in the 200-900 nm range, extracting 274 nm for chromatograms. The mobile phase was an isocratic combination of acetonitrile (ACN):H_2O (50:50) with a flow rate of 1 ml/min. Stock solution of thymol (2 mg/ml) was prepared in ACN:H_2O (80:20) solvent, and different concentrations were made from stock solutions to plot the calibration curve of thymol.

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ENCAPSULATION EFFICIENCY (EE)

The encapsulation efficiency of hydrogel beads was estimated after beads hardening in the $CaCl_2$ solution. Aliquots were taken from the gelling bath, and concentration thymol was obtained by the HPLC method. Encapsulation efficiency was then expressed as Eq. [1]:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{actual amount of oil loaded in beads}}{\text{theoretical amount of oil loaded in beads}} \times 100 \qquad [1]$$

RELEASE OF THYMOL IN SIMULATED GASTROINTESTINAL CONDITIONS

The release of thymol from wet Ca-alginate/SPI beads was observed at 37 °C in solutions that simulated gastro-intestinal conditions. Solutions were freshly prepared before the analysis, according to the procedure described in our previous work (16). Firstly, the hydrogel beads (3 g) were kept in a flask containing gastric solution, under mild magnetic stirring, and aliquots of a sample were taken. After 1 h, the beads were filtered and transferred in the flask containing pancreatic solution, and sampling was continued until complete degradation of the beads. The release of thymol was analyzed using HPLC and results were expressed as the percentage of released thymol in SGF and SIF.

DETERMINATION OF FREE RADICAL - SCAVENGING ABILITY

The Trolox equivalent antioxidant capacity (TEAC) was estimated by the ABTS radical cation decolourisation assay. Free 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$), generated by chemical or enzymatic oxidation of ABTS, was produced according to the method published by Re *et al* (18). In order to test the TEAC of the beads, 1 g of freshly prepared beads were dissolved in 50 mL of 1% (w/v) Na-citrate solution and methanol (90:10). After complete degradation of the beads, a 30 µL aliquot of sample was added to 2.0 mL of the diluted $ABTS^{\bullet+}$ solution, and the absorbance readings were taken after exactly 6 min at 734 nm. The reagent blank was prepared by adding 30 µL of methanol to 2.0 mL of the diluted $ABTS^{\bullet+}$ solution. The results, obtained from triplicate analyses, were expressed as Trolox equivalents (µmol Trolox g_{beads}^{-1}) and derived from a calibration curve (62.5-1000 µmol L^{-1}) determined for this standard.

CUPRIC REDUCING ANTIOXIDANT POWER

The cupric reducing/antioxidant power (CUPRAC) assay was carried out according to a standard procedure by Apak *et al* (19). In brief, solutions of $CuCl_2$ (50 µL, 10 mM), neocuproine (50 µL, 7.5 mM) and CH_3COONH_4 buffer (60 µL, 1 M, pH 7.0) were prepared before the analysis. Around 3 g of hydrogel beads were degraded in Na-citrate solution and centrifuged at 700 rpm for 5 min. After complete degradation of the beads, a 400 µL aliquot of sample was mixed with reactive solutions and incubated for 1 h in a water bath at 30 °C. Absorbances were measured at 450 nm. A standard curve was prepared using different concentrations (31.25-250 µmol L^{-1}) of Trolox and results are expressed as µmol Trolox g_{beads}^{-1}.

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Original scientific paper

EFFECT OF THERMAL TREATMENT ON THE ANTIOXIDANT ACTIVITY

In order to evaluate the change in antioxidant activity of the beads (Ca-A1.5/SP1.5) after thermal treatment, beads were undergone three different temperatures (50 °C, 75 °C and 100 °C) during 4 h. After heating at predetermined temperatures, 1 g of the beads were completely degraded in Na-citrate solution. The antioxidant activity of the supernatant was determined using the previously described methods (CUPRAC and ABTS assays). Absorbance was measured and compared with an absorbance of the non-heated beads.

DETERMINATION OF ANTIMICROBIAL ACTIVITY

Antimicrobial activity of wet encapsulated thyme oil beads was estimated against two Gram-positive strains *Staphylococcus aureus* ATCC 25923 *and Bacillus cereus* ATCC 11778, one Gram-negative strain *Escherichia coli* ATCC 25922 and yeast strain *Candida albicans* ATCC 10259, all obtained from American Type Culture Collection. Sterile freshly prepared wet alginate/oil/protein beads were aseptically added in each flask, in the amount of 4 g and mixed with 10 ml of sterile physiological saline solution (0.85% w/v NaCl) supplemented with Tween 80 (polysorbate 80) at a final concentration of 0.5% (v/v) to enhance thyme oil solubility. Content of flasks is gently mixed and inoculated with one of the tested microorganisms (overnight cultures, not older than 18h), and the initial number of cells in suspension was in the range 10^5–10^6 CFU/ml. Afterward, the flasks were incubated in a shaking water bath at 37 °C for 24 h At the same time, alginate/protein beads without thyme oil were used as the control. After 24h of incubation, 100 µl of the liquid sample was aseptically withdrawn from each flask and directly transferred to Petri dishes. From them, serial (10-fold) dilutions were made in a physiological saline solution prior to 100 µl of appropriate dilution was added in Petri dish. Content in the Petri dishes was homogenized with melted nutrient agar (1.5 wt. %, 55 °C) and incubated at 37 °C for 24 h. After incubation, all Petri dishes were visually analyzed and those with formed colonies were counted. The experiments were performed in duplicates and the number of viable cells is expressed as colony-forming units (CFU).

STATISTICAL ANALYSIS

All experiments were expressed as means with standard deviations (SD). The mean values were analysed using one-way ANOVA. All analyses were done using software Origin Pro 8.5 (OriginLab Corporation, Northampton, USA).

RESULTS AND DISCUSSION

SIZE AND SHAPE OF THE BEADS

In this work, the effect of alginate and protein concentration on the size and shape of the beads after oil encapsulation was determined and presented in Table 1. Sphericity factor (SF) was used in order to estimate the shape of the beads. Wet hydrogel beads can be considered as spherical (SF≤0.05) (17), with exception of Ca-A2/SP1 and Ca-A2/SP1.5, which showed deformation in form of the elongated drops, as well noticed by Levic *et al* (20).

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Table 1. Dimensions (Maximum diameter and Minimum diameter), shape indicator (SF) and encapsulation efficiency of Ca-alginate/SPI complex beads with encapsulated thyme oil.

System code	Wet beads, µm		Dry beads, µm		$SF=(d_{max}-d_{min})/(d_{max}+d_{min})$		EE, %
	d_{min}	d_{max}	d_{min}	d_{max}	SF_w	SF_d	
Ca-A1/SP1	1561±12[a]	1688±34[a]	919±26[d]	1058±44[d]	0.040	0.070	70.04±0.1
Ca-A1/SP1.5	1601±17[b]	1769±42[b]	914±35[d]	958±15[b]	0.049	0.024	70.85±0.2
Ca-A1.5/SP1	1652±22[c]	1780±24[b]	729±36[a]	813±69[a]	0.037	0.054	72.90±0.4
Ca-A1.5/SP1.5	1679±45[c]	1838±31[c]	867±25[c]	960±29[b]	0.045	0.051	73.31±0.2
Ca-A2/SP1	1673±30[c]	1847±44[c]	837±28[b]	1002±28[c]	0.049	0.090	74.92±0.6
Ca-A2/SP1.5	1729±23[d]	1976±77[d]	868±37[c]	1144±64[e]	0.067	0.137	75.54±0.1

*Values with the same letter in each column showed no statistically significant difference.

The formulations were found to give an irregular shape of beads due to higher viscosity of blends with high polymer concentration. SPI concentration has shown no systematic influence on the shape of the beads. After drying, almost all beads showed irregular shape, probably due to intense shrinkage.

By using the electrostatic extrusion technique, the size of the hydrogel beads depends on many factors. Accordingly, beads were produced under the same conditions, thus the variation in their size dramatically depends on the concentration of polymers used for their production. The size of hydrogel beads ranged between ~1.56 mm and ~1.98 mm, while dried particles reduced in size compared to their hydrogel analogs (0.73 – 1.14 mm).

Both, alginate and protein content influenced the size of the beads. An increase in the bead size with an increase in polymer concentration has shown to be the most obvious by comparing the formulations Ca-A1/SP1 and Ca-A2/SP1.5. On the other side, a higher polymer concentration induced a higher amount of oil during bead preparation, making the matrix to be more pumped as more oil is inside.

ENCAPSULATION EFFICIENCY

The concentration of thymol encapsulated in the Ca-alginate/SPI beads was determined by HPLC analysis, and results were presented in Table 1. Depending on the content of alginate, SPI and consequently oil content, EE of thymol varied from 70.04 to 75.54 %. The concentration of both, alginate and SPI showed systematic dependence on encapsulation efficiency of the beads. Beads coded as Ca-A2/SP1.5 showed the highest EE, considering the alginate and protein concentration used for their production - in contrast to Ca-A1/SP1.

IN VITRO THYMOL RELEASE

As concerns the release kinetics of thymol from Ca-alginate/SPI delivery systems, Figure 1 shows the percentage of thymol released as a function of time. HPLC method was implemented in order to evaluate thymol concentration, and the impact of polymer concentration on thymol release was investigated (Ca-A1/SP1 vs. Ca-A2/SP1 and Ca-A2/SP1 vs. Ca-A2/SP1.5).

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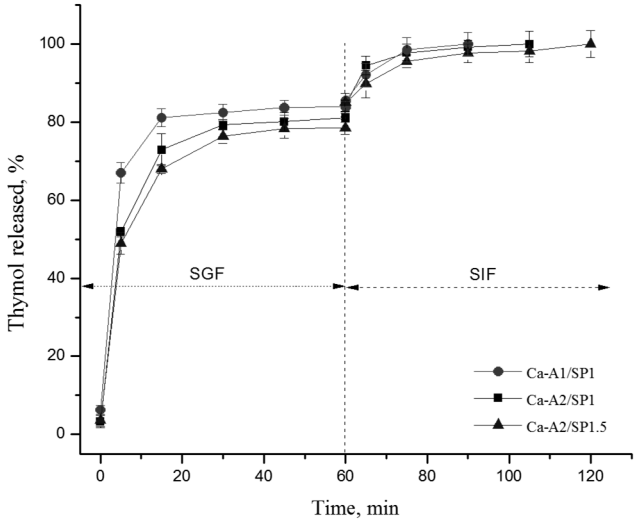

Figure 1. Release of thymol from Ca-alginate/protein beads in simulated SGF and SIF

ANOVA revealed a significant effect of polymer concentrations on thymol release (Figure 1) in simulated gastric conditions. The burst effect (in the first 10 min) was observed for all beads, which was characterized by the fast release of 'surface oil'. When the concentration of soy protein was 1.5 wt.%, in comparison to 1 wt.%, the release of thymol was lower. This could be due to changes in the density of the polymer matrix, resulted from the excess of protein in the continuous phase (non-adsorbed) (21). Moreover, a higher protein amount induces better emulsification (22), which leads to greater stability of emulsions and consequently of produced beads. However, the influence of alginate concentration was dominant. With an increase in alginate concentration (from 1 wt.% to 2 wt.%) polymer chains tend to be more densely packed, thus protecting the oil inside the matrix from fast leakage.

ANTIOXIDANT ACTIVITY

In this study, the antioxidant capacity of the beads with encapsulated thyme oil was determined using two analytical assays: (1) ABTS radical cation ($ABTS^{\bullet+}$) decolourisation assay and (2) Cupric (Cu^{2+}) reducing power activity.

According to the results of both tested assays (expressed per mass unit of beads), antioxidant activity was the lowest for the bead formulation with the lowest content of both alginate and protein (Ca-A1/SP1). In contrast, the highest Cu^{2+} reducing activity was achieved for the bead formulation with the highest network density (Ca-A2/SP1.5). According to cupric reducing power activity, an increase in protein content at the same concentration of alginate leads to higher antioxidant capacity; this is also true for ABTS radical cation scavenging activity, statistically significant for the formulations with 1 and 2 % alginate (Figure 2). It seems that antioxidant capacity correlates with the oil content (Table 1). However, it should have in mind that partially denatured proteins inside polymer matrix also express antioxidant activity (21).

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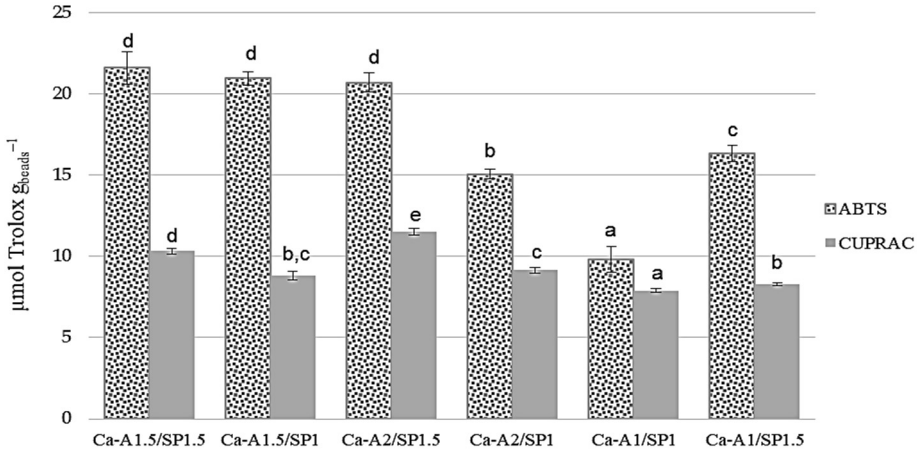

Figure 2. Comparison of the antioxidant activity of different beads formulations

The antioxidant activity of the beads has not been able to perform *in vivo,* probably due to enzyme interference in the applied method. Otherwise, enzymatic hydrolysis of proteins also increases the exposure of antioxidant amino acids, i.e. increases the antioxidant activity of the beads (23).

ANTIOXIDANT ACTIVITY OF THERMALLY TREATED BEADS

Initially, the air-dried beads were subjected to thermal treatment at 50 °C, 75 °C and 100 °C in order to evaluate the antioxidant capacity of beads after 4 h of heating. Both methods, CUPRAC and ABTS were applied in order to show the potential of a carrier (representative formulation Ca-A1.5/SP1.5) to preserve the antioxidant potential of encapsulated thyme essential oil.

Results are expressed as absorbance of compounds which has been interacted with free radicals, in comparison to the initial absorbance (non-treated beads), and presented in Figure 3.

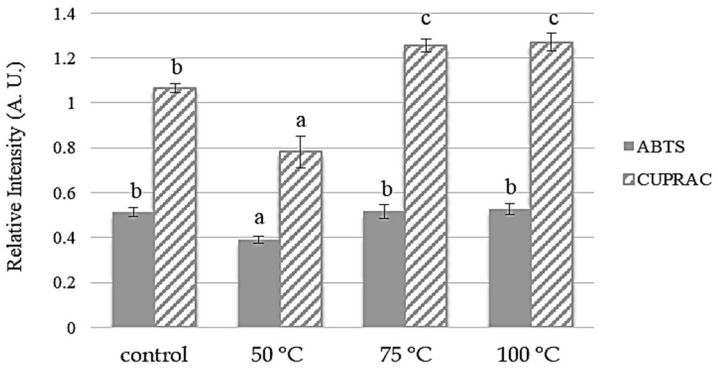

Figure 3. Antioxidant capacity of the heat-treated beads

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Original scientific paper

After 4 h of treatment at 50 °C, the antioxidant capacity of the beads (according to both methods) was lower than the initial, which indicated a decrease in the biological potential of encapsulated thyme oil under the act of temperature. The decrease in antioxidant activities can be associated mainly to volatilization of polyphenols rather than degradation, since degradation process occurs at higher temperatures (24).

On the other hand, ABTS radical scavenging ability remained unchanged, while cupric reducing power activity even increased upon heating at 75 °C and 100 °C. At these temperatures, proteins undergo denaturation process and disruption of protein tertiary structure, which increases the accessibility of the amino acids with antioxidant potential. It is known, when proteins are used in order to stabilize o/w emulsions, one fraction of them absorb on the oil surface, while the rest remains in the continuous phase. According to Yang and Xiong (25), it is that interfacial proteins express antioxidant properties by inhibiting lipid oxidation, while Faraji *et al* (21) assigned this potential to soy proteins in a continuous phase of the emulsion. Soy protein isolate may act as an antioxidant by free radical scavenging and metal chelation activity, due to the presence of certain amino acids and other compounds, such as isoflavone (21).

The results obtained in our work, which are related to the antioxidant capacity of both, heated and non-heated beads, indicated that the metal chelate effect was dominant.

ANTIMICROBIAL ACTIVITY

Essential oils with a high percentage of phenolic compounds possess the strongest antimicrobial activity. Many authors have demonstrated thyme essential oil as a potential agent for the inhibition of various pathogens (26), thanks to the presence of thymol and carvacrol, the main compounds responsible for its antimicrobial effect (6). Thyme oil compounds manifest their antimicrobial activities through several possible mechanisms, and the hydrophobic character of EOs compounds, allows interaction with the lipids of the bacterial cells membrane, which enhances its permeability to potassium ions and protons, and consequently leads to inhibition of ATP synthesis and finally to cell death (27).

In order to demonstrate the potential utility of encapsulated thyme oil beads, antimicrobial activity of wet beads was studied in suspensions of *S. aureus, E. coli, B. cereus,* and *C. albicans* cells in physiological solution (0.85% w/v NaCl) with 0.5% (v/v) Tween 80 as a surfactant to allow better dissolution of thyme essential oil. The results of antimicrobial activity are presented for the representative formulation Ca-A1.5/SP1.5 which provides the actual concentration of thyme oil of 4.5 µl/ml (Table 2).

According to the results, the microbicidal effect was observed only on *C. albicans* (colonies were not detected in the medium after 24h), while on other species a reduction of total viable count happened to a greater (a fall in *E.coli* and *B. cereus* concentrations for two orders of magnitude) or lesser (*S. aureus*) degree. Therefore, another formulation (Ca-A1.5/SP1.5*) was made with the same matrix composition but a higher oil loading thus providing the actual concentration of thyme oil of 20 µl/ml. This one induced a more significant reduction in *S. aureus* (from 10^6 to 10^1 CFU/ml), and *E. coli* (10^4 to 10^1 CFU/ml) populations than Ca-A1.5/SP1.5. Here it should be emphasized that as opposed to thyme oil, released proteins promoted cell proliferation due to proteolytic activity of bacteria, as evidenced by a somewhat increase in cell number in control samples (blank beads without thyme oil). To optimize formulation which will have a microbicidal effect,

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the content of protein was decreased (0.25 %) while keeping the same content of thyme oil and the new formulation (Ca-A1.5/SP0.25**) completely inactivated three of four tested pathogens. *B. cereus* strain appeared to be less sensitive than others although its initial inoculum size was the smallest.

Table 2. Antimicrobial activity of different bead formulations

	Initial number of cells (CFU/ml)			
	S. aureus	*E. coli*	*B. cereus*	*C. albicans*
Control	1.78×10^6	1.32×10^6	7.58×10^4	1.14×10^4
Ca-A1.5/SP1.5	1.78×10^6	1.32×10^6	7.58×10^4	1.14×10^4
Ca-A1.5/SP1.5*	3.30×10^5	1.90×10^6	7.60×10^3	n.d.
Ca-A1.5/SP0.25**	3.10×10^5	1.80×10^6	6.10×10^3	n.d.
	Number of cells after 24h incubation (CFU/ml)			
	S. aureus	*E. coli*	*B. cereus*	*C. albicans*
Control	8.20×10^6	8.60×10^6	3.60×10^7	1.00×10^4
Ca-A1.5/SP1.5	1.51×10^6	4.80×10^4	1.02×10^2	0
Ca-A1.5/SP1.5*	2.00×10^1	2.00×10^1	1.63×10^2	n.d.
Ca-A1.5/SP0.25**	0	0	9.40×10^2	n.d.

n.d.- not determined

CONCLUSION

The beads with higher polymer content exhibited higher encapsulation efficiency, which implies an increase in their antioxidative potential. A dose-related response required to significantly affect bacterial growth and survival was achieved using prepared biopolymer beads with encapsulated thyme essential oil. A combination of Na-alginate and soy protein isolate has enabled effective protection of thyme oil during heat treatment at 75 °C and 100 °C, as well as controlled release, up to 120 min, depending on the biopolymer concentration.

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