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Encapsulation of thyme (*Thymus serpyllum* L.*)* aqueous extract in Ca-alginate microbeads

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INTRODUCTION AND OBJECTIVES

The current focus of life science community is naturally derived antioxidants, especially plant polyphenols, which exhibit high antioxidant properties and therefore they are highly recommended for consumption (Langley-Evans 2000). A wide array of positive health effects has been ascribed to plant polyphenols, such as antimicrobial properties and ability to protect against cancer and cardiovascular diseases.

One way to preserve the health-beneficial properties of plant extracts is to encapsulate them within a matrix or a membrane in the particulate form to achieve this one or other desirable effects. Except to improve stability during transport, storage or processing, the purpose of encapsulation is to achieve superior handling of the active compounds by conversion of liquid actives into solid forms. In addition, those solid formulations could be used as food additives or food supplements.

MATERIALS AND METHODS

Preparation of thyme aqueous extract Dried thyme was purchased at a special market – House of tea (Zagreb, Croatia). Tea sample (10g) was infused in 200 mL of distilled water heated up to 100ºC and occasionally stirred. After extraction (30 min) the infusion was filtered through a tea strainer.

Preparation of hydrogel microbeads encapsulating thyme extract The obtained thyme extract was used to dissolve sodium alginate powder (0.015 g mL^{-1}). Spherical droplets were formed by electrostatic extrusion of the polymer-extract solution (voltage of 6.5 kV, neddle diameter 22 gauge). The collecting solution was tea extract containing calcium chloride in concentration 0.015 g mL^{-1} where 1g of microbeads was allow to gel in 2 mL of the solution.

A part of microbeads was dried in a drying-oven at 50 °C for 24h, while the other part was freeze dried.

Scanning electron microscopy The surface morphology of beads was imaged using a scanning electron microscope (SEM, model Jeol JSM 6460LV).

Determination of total phenol content (TPC) Total phenol content (TPC) was determined spectrophotometrically using Folin–Ciocalteu's reagent according to a modified method of Lachman (1998).

Determination of free radical-scavenging ability The Trolox equivalent antioxidant capacity (TEAC) was estimated by the ABTS radical cation decolourization assay (Re 1999). The results, obtained from triplicate analyses, were expressed as Trolox equivalents, and derived from a calibration curve determined for this standard (100-1000 μM).

Release studies The release studies of polyphenols were performed at room temperature in a 500-mL glass containing microbeads in distilled water under magnetic stirring. At define time intervals, 1 mL aliquots were taken. The content of polyphenols and free radical-scavenging activity of aliquots were analyzed as previously described.

RESULTS AND DISCUSSION

Table 1 shows the effect of preparation method on TPC encapsulated or absorbed by 1g of Ca-alginate microbeads. Except hydrogel, dried forms were also investigated since dried hydrogel beads are stronger than non-dried gel beads and they are important as carriers for food and bioprocess systems. Also, inulin was investigated as a filler to improve final properties of alginate microbeads. Fillers are known to have ability to induce changes in terms of influencing and increasing brittleness (i.e. creating a crunchier product) (Rassis 2002). Especially alginate-inulin hydrogel has potential for food applications, since inulin can be used as a functional food and as a dietary fiber. Depending on the water content and preparation procedure, TP content in microbeads ranged from 1.7 to 4.1 mg GAE g^{-1}. The TPC in hydrogel beads correspond to the theoretical values (i.e. TP in the original thyme aqueous extract). The results also indicate that dried alginate beads are dosage sources richer in phenolics, i.e. the entrapment loading improved 2-fold when they were dried to a 5% of their original mass.
According to the obtained results free radical-scavenging ability expresed per weight unit of microbeads rised up to 13 times after freeze-drying process (Table 1). This result is in agreement with those found by Chan (2009) who claimed that Ca-alginate beads made of alginate with high content of G-units express higher antioxidant capacity when water content is reduced.

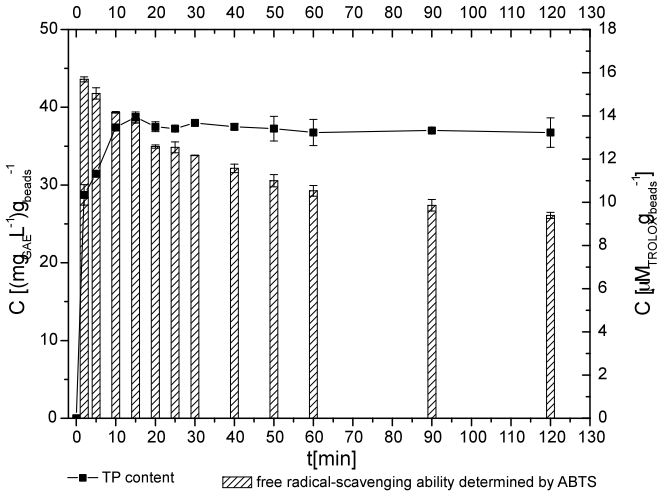
Table 1: Total phenol content (TPC) and antioxidant capacity of thyme extract and Ca-alginate microbeads.

	TPC [mg_{GAE} g_{beads}^{-1}]	ABTS [µmol Trolox g_{beads}^{-1}]
Original extract	2.01 ±0.02[a]	8.60 ± 0.05[b]
Hydrogel microbeads	2.04 ± 0.06	8.02 ± 0.31
Dried microbeads	2.52 ± 0.09	56.2 ± 9.12
Lyophilized microbeads	4.15 ± 0.13	108 ± 11.2
Hydrogel microbeads with inulin	1.75 ± 0.06	11.30 ± 0.65

[a]the unit is [mg_{GAE} mL^{-1}]
[b]the unit is [μM_{Trolox}]

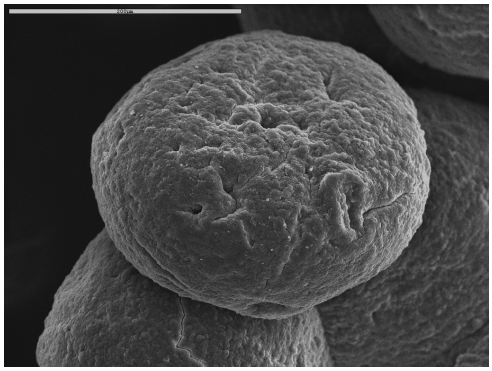
The release profile for hydrogel microbeads containing inulin as a filler and encapsulating tea extract is shown on Figures 1. The most of polyphenolic compounds was released relatively rapidly from native hydrogel beads and reaches a plateau after about 10 min (not shown). In case of microbeads containing inulin as a filler, the release was extended to 15 min (Figure 1). Simultaneously with the increase in TPC of the surrounding medium, the antioxidant capacity continuously increases, which confirms that polyphenolic compounds are responsible for the antioxidant effect of obtained microbeads. Actually, the release of polyphenols is consistent with difussion controlled release through the alginate gel matrix.

Figure 1: Release of polyphenols from Ca-alginate-inulin microbeads.



Morphological properties of microbeads are important since the surface of the bead is the first part to come into contact with its fluid, solid or gaseous environment and together with its internal structure, will influence, if not determine, its suitability to a predetermined task. Alginate gel microbeads encapsulating tea extract appear spherical with a relativley smooth surface. Lyophilization may create artefacts by collapsing the walls of pores so that beads appear less spherical and the process caused the beads to shrink. The surface liophylized alginate beads (not shown) have spongy texture that was expected from freeze-drying process. Numerous cavities are formed as a result of the freeze-drying process. During freezing, there are localized areas of expansion (ice crystals) and localized areas of contraction (most other constituents), and thus the volume change is not uniform throughout the system. The problem of the gel collapse we partially reduced by addition of inulin as a filler to hydrogel prior to liophylization (Figure 2).

Figure 2: SEM micrographs of liophylised Ca-alginate-inulin microbeads encapsulating tea extract.



CONCLUSIONS

Microencapsulation of thyme aqueous extract within Ca-alginate hydrogel matrix by electrostatic extrusion has been assessed. Freeze-dried microbeads appeared to be suitable dosage forms since they exhibit significantly higher antioxidant capacity compared to hydrogel or heat-dried forms. The inclusion of a filler substance such as inulin or sugar contributes not only to improved cellular bead structure but also to enhanced biological activity.

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