Alginate-inulin microbeads encapsulating antioxidants from *Pterospartum tridentatum*

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INTRODUCTION AND OBJECTIVE

Pterospartum tridentatum L. is a European endemic species belonging to the subfamily Papilionoideae (Talavera, 1999) and known as carqueija or carqueja in Portugal. Various bioactive compounds, such as alkaloids and flavonoids, have been identified in aqueous extracts of *Pterospartum tridentatum*. However, most of bioactive compounds are very sensitive to many factors. The effectiveness of these natural antioxidants, namely polyphenols, depends on preserving the stability, which can be increased by microencapsulation. Microencapsulation is an effective method to protect bioactive components, preserve the stability during processing and storage and prevent undesirable interactions with food matrix (Nedović et al., 2011). The goal of the present study was to develop *P. tridentatum* extract formulations aimed at delivery of bioactive compounds in functional food products. The extract was encapsulated in alginate and alginate-inulin microbeads by electrostatic extrusion and the obtained microbeads were characterized from the aspect of TPC and antioxidant activity.

MATERIALS AND METHODS

Na-alginate (medium viscosity) was purchased from Sigma. Folin-Ciocalteu, Na-carbonate, Ca-chloride and Na-citrate were of analytical grade and supplied by Sigma-Aldrich (St. Louis, MO, USA). 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium chloride, potassium persulfate were obtained from Sigma -Aldrich (Germany). Inulin was generously gifted from a local milk factory.

Preparation of microbeads *P. tridentatum* aqueous extract (2 mg mL^{-1}) was added to Na-alginate water solution (1,5% w/v) to prepare extract-alginate solution. In addition, extract-alginate solution was mixed with 10 and 20 mass% of inulin to prepare extract-alginate-inulin solutions. Both types of solutions were submerged to electrostatic droplet generation to produce microbeads entrapping extract compounds. The solutions were extruded through a blunt stainless steel needle (23 G) at a constant flow rate of 25,2 mL h^{-1}, by a syringe pump (Razel Scientific Instruments, Stamford, CT, USA). The extrusion was performed under an applied electric field (7,0 kV). Collecting solution was mixture of *P.tridentatum* extract and Ca-chloride 1,5% (w/v). The hydrogel microbeads were left in the cross-linking solution for 30 min and then used for further analysis (Bugarski et al., 2004). Hydrogel microbeads were observed under optical microscope (Olympus CX41RF, Tokyo, Japan) and average diameter was measured with the image analysis program CellA (Olympus, Tokyo**,** Japan)**.** Hydrogel microbeads with encapsulated extract were suspended in 3 mL of distilled water. The samples were left on an orbital shaker operating at 100 rpm and when the extract was completely released the samples were analysed on TPC, DPPH and ABTS

Determination of total phenol content (TPC) TPC was determined using the Folin-Ciocalteau reagent, according to a modified method of Lachman et al (1998). Gallic acid was used as the standard and the results expressed as mg L^{-1} of gallic acid equivalents (GAE).

Encapsulation efficiency Encapsulation efficiency (EE%) was calculated as the amount of TPC encapsulated in microbeads (m_b) divided by the TPC of the solution used for the preparation of microbeads (m_s), as shown in equation 1: $EE\% = m_b/m_s \cdot 100$ (1)

Quantification of TPC in microbeads (m_b) was performed after dissolving microbeads in 2% (w/v) Na-citrate solution (in a weight ratio of 1:5).

Determination of free radical-scavenging ability The radical scavenging activity of the extract was determined using the DPPH radical assay according to Dudonne et al. (2009). Percentage of cation inhibition was calculated using equation 2:

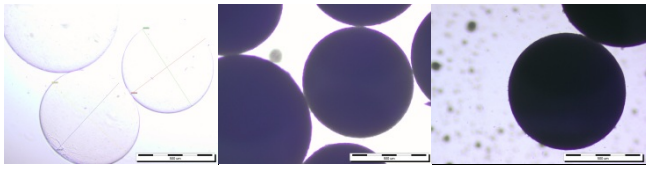
$$\% \text{ inhibition} = [(AB-AE)/AB] \cdot 100 \quad (2)$$

where (AB) is absorbance of the blank sample and (AE) is absorbance of the sample.

The free radical scavenging capacity of the extracts was also studied using the ABTS radical assay. ABTS• was produced according to Re et al. (1999). The percentage of inhibition of ABTS• was calculated using equation 2.

RESULTS AND DISCUSSION

Hidrogel microbeads encapsulating *P. tridentatum* extract appeared spherical with a quite smooth surface and average diameter of ~500 µm. Alginate microbeads with 10 mass% of inulin were slightly distorted from a perfect sphere and they are larger compared to alginate microbeads, ~700 µm. Alginate microbeads with 20 mass% of inulin had spherical appearance with the average diameter of ~800 µm.



(a) (b) (c)

Figure 1. Photos of alginate microbeads encapsulating *P. tridentatum* extract: (a) alginate microbeads; (b) alginate microbeads with 10 mass% of inulin; (c) alginate microbeads with 20 mass% of inulin.

The results of TPC and EE% for all microbeads are given in table 1. Depending on the addition on inulin, TPC in microbeads ranged from 0,24 to 0,33 mg GAE g_{beads}^{-1}. The highest amount of TPC was detected in alginate microbeads with 20 mass% of inulin. The results indicate that this type of microbeads has the highest encapsulation capacity. This can be explained by reduction of pore size of alginate in the presence of a filler such as inulin (Rassis et al., 2002).

Table 1. TPC and EE% of hydrogel microbeads encapsulating *P. tridentatum* extract.

Smple	TPC (mg GAE g_{beads}^{-1})	EE(%)
Fresh extract	0,35[a]	/
Alginate microbeads	0,24	49,0
Alginate microbeads with 10 mass% of inulin	0,30	63,8
Alginate microbeads with 20 mass% of inulin	0,33	73,8

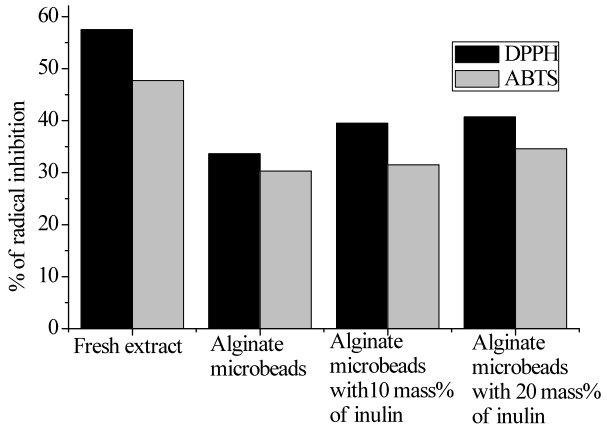


Figure 2. Radical inhibition of DPPH and ABTS radical cations for fresh extract and for alginate and alginate-inulinbmicrobeads

The antioxidant capacity of microbeads was determined by two analytical assays ABTS and DPPH (Fig.2). The results are expressed as the percentage of radical inhibition and compared to antioxidant activity of the fresh extract. The inhibition of the DPPH• radical with fresh extract (2 mg ml^{-1}) was around 57% and the inhibition of ABTS• was 47%. Upon encapsulation, antioxidative activity of extract compounds was preserved at a high level, as confirmed by both assays and alginate microbeads with 20 mass% of inulin showed the highest antioxidative potential.

CONCLUSIONS

Encapsulation of aqueous *P. tridentatum* extract within alginate and alginate-inulin microbeads has been assessed. The obtained microbeads displayed significant polyphenol content. The antioxidant activity of the extract was preserved after microencapsulation at a high level, especially in case of alginate microbeads with 20 mass% of inulin.

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