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WORKSHOP PROCEEDINGS
Eds. - M. F. Barreiro, O. Ferreira, A.I. Pereira
P48. MICROENCAPSULATION OF *Ceratonia siliqua* L. EXTRACT FOR FOOD PURPOSES: EFFECT OF EXTRACT/ALGINATE RATIO

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**Introduction**

Human health, nutritional status and well being can be enhanced through consumption of foods containing specifically desired nutrients and bioactive agents [1]. Popularly known as St John’s Bread, *Ceratonia siliqua* L. (carob) has a long history of use in food (over 4000 years). It has a good nutritional value and its polyphenolic extract shows high antioxidant capacity and even higher antiradical activity than well-aged red wines. Its reducing power can also be four-fold higher than many well known potent antioxidant agents such as gallic acid, caffeic acid and catechin [2]. Nevertheless, preparing high quality nutritious food is critically dependent on availability of effective delivery systems. Such systems should preserve the specific nutritional, biological, chemical and functional properties of the sensitive constituent, and should effectively release the compounds, in a desired mode, after ingestion. Nowadays, the most promising technology that can allow overcoming the stated difficulties is microencapsulation [1]. In this context, a hydroethanolic (80:20, v/v) extract obtained from carob pulp by ultrasound extraction was microencapsulated for further use in the development of functional yogurts.

**Experimental**

The bioactive extracts were obtained from powdered carob pulp through an ultrasound extraction process (testing different times - 5, 10 and 15 min and amplitudes - 50, 75 and 100%). The extracts were evaluated in terms of antioxidant activity (free radicals scavenging activity, reducing power, β-carotene bleaching inhibition and lipid peroxidation inhibition in brain homogenates - TBARS assay) and the most promising was encapsulated for food purposes. Three microencapsulation trials were conducted by an atomization/coagulation technique using different extract/sodium alginate ratios (50/400, 75/400 and 100/400, mg/mg, 10 mL) in order to choose the most suitable one. The solutions were then atomized through a nozzle (0.35 mm) and coagulated in a calcium chloride solution (250 mL, 4% (v/v)). The obtained microspheres were characterized by optical microscopy (OM) during the microencapsulation process to monitor morphology evolution and after being lyophilised. The encapsulation efficiency (EE) was evaluated by HPLC-DAD.

**Results and discussion**

The hydroethanolic extract obtained by ultrasonication using 75% amplitude during 10 minutes gave the highest antioxidant activity (data shown in Table 1).

**Table 1.** EC₅₀ values (mg/mL) obtained according to different methodologies (effective concentrations providing 50% of antioxidant activity or 0.5 of absorbance in reducing power assay).

<table>
<thead>
<tr>
<th>Activity</th>
<th>EC₅₀ ± SE</th>
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<tbody>
<tr>
<td>DPPH scavenging activity</td>
<td>2.96 ± 0.05</td>
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<tr>
<td>Reducing power</td>
<td>0.78 ± 0.01</td>
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<tr>
<td>β-Carotene bleaching inhibition</td>
<td>1.59 ± 0.14</td>
</tr>
<tr>
<td>TBARS inhibition</td>
<td>0.33 ± 0.02</td>
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The evaluation of the obtained microspheres by OM, during different stages of the encapsulation process and after being lyophilised for storage purposes, revealed changes in their shape. In fact, at the end of the atomisation step (Fig. 1 A, D and G) and coagulation processes (Fig. 1 B, E and H),
microspheres with various dimensions and a clear spherical form were observed. After being dried (Fig. 1 C, F and I), the microspheres corresponding to the different ratios applied, showed a ruffled form being apparently glued to each other possibly due to the absence of water. Another perceptible point is the presence of a growing number of small microcapsules as the ratio extract/alginate increases. The HPLC-DAD analysis of the coagulation and the washing solutions, which revealed to present none or only traces of extract, let to estimate an EE around 100% for all the tested extract alginate/ratios.

![Fig. 1. OM analyses with magnifications of 100X: microspheres after atomization (A, D and G, respectively for 50/400, 75/400 and 100/400 extract/alginate ratios); microspheres after four hours in contact with a solution of calcium chloride under stirring at 200 rpm (B, E and H, respectively for 50/400, 75/400 and 100/400 extract/alginate ratios); freeze-dried microspheres (C, F and I, respectively for 50/400, 75/400 and 100/400 extract/alginate ratios).](image)

Conclusions

Viable microspheres were produced through the atomization/coagulation technique using different extract/alginate ratios, being obtained for all the produced samples, an EE of 100%. This final product will be incorporated into natural food matrices, specifically yogurts (work under progress). The main objective will be to infer the impact of using microcapsules with different extract concentrations, which will be reflected on the used microcapsules’ amount, on the extract delivery and bioactivity maintenance. With this strategy the delivery of bioactive phenolic compounds can be tailored enhancing the bioavailability of extracts and related health promoting properties of the developed food product.

References