6th WORKSHOP

Green Chemistry and Nanotechnologies in Polymer Chemistry

July 15-17, 2015

Polytechnic Institute of Bragança | PCT-TMAD Brigantia EcoPark

Bragança · Portugal

WORKSHOP PROCEEDINGS

Eds. - M. F. Barreiro, O. Ferreira, A.I. Pereira

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LABORATÓRIO ASSOCIADO LSRE LABORATORY OF SEPARATION AND REACTION ENGINEERING

Laboratório de Catálise e Materiais lcm

Brigantia EcoPark

Title
6th Workshop - Green Chemistry and Nanotechnologies in Polymer Chemistry

Edited by
M.F. Barreiro, O. Ferreira, A.I. Pereira

Graphic Design
Soraia Maduro - CIPweb IPB

First edition 07/2015
ISBN 978-972-745-188-3

Edition
Instituto Politécnico de Bragança

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Green Chemistry and Nanotechnologies in Polymer Chemistry

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P52. MICROENCAPSULATION OF PLANT EXTRACTS RICH IN APIGENIN TO BE USED AS CHEMOPREVENTIVE AGENTS IN FUNCTIONAL FOODS

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Introduction

Angiogenesis is the process by which new blood vessels are formed from pre-existing vasculature, being a key process that leads to tumor development. Excessive angiogenesis occurs when diseased cells produce abnormally large amounts of angiogenesis factors (e.g. vascular endothelial growth factor (VEGF)) [1]. Some studies recognize phenolic compounds as chemopreventive agents; flavonoids seem to suppress the growth of tumor cells modifying the cell cycle and inducing apoptosis in several tumor cell lines [2]. Among them, apigenin derivatives have been recognized as having antiangiogenic effects on tumor cells being this related to a decrease in VEGF expression or to a VEGFR inhibition [3]. According to Ferreira et al. previous research, *Arenaria montana* L. is rich in apigenin derivatives [4]. Therefore, this plant source is ideal to prepare apigenin rich extracts to be used as chemopreventive agents in functional foods. However, bioactive compounds are generally recognized as presenting problems of instability, which can weaken their bioavailability and potential benefits. Therefore, the use of microencapsulation is studied here as a way to confer protection and increasing the efficacy of functional foods incorporating these extracts [5].

In this work, a hydroethanolic extract prepared from *A. montana* was evaluated for its *in vitro* antiangiogenic effects, being further microencapsulated to be used as chemopreventive agent in functional yogurts.

Experimental

The bioactive ingredient was obtained from *Arenaria montana* L. through an extraction with ethanol: water 80:20 (v/v). The obtained extract was evaluated in terms of inhibitory activity of the tyrosine kinase intracellular domain of the Vascular Endothelium Growth Factor Receptor-2 (VEGFR-2) through an enzymatic assay. The microspheres were prepared by using an atomization/coagulation technique where a solution of sodium alginate containing the extract (10 mL, extract/sodium alginate ratio of 50/400 (mg/mg)) was atomized through a nozzle (0.35 mm diameter) and coagulated in a calcium chloride solution (250 mL, 4% (v/v)). The forming microspheres were characterized by optical microscopy (OM) during the microencapsulation process to monitor morphology evolution. The encapsulation efficiency (EE) was evaluated by HPLC-DAD based on apigenin (the major extract's aglycone). Additionally, free and microencapsulated extracts were incorporated into yogurt samples that were thereafter evaluated in terms of *in vitro* antiangiogenic activity and nutritional composition at two different storage times (0 and 3 days).

Results and discussion

The *A. montana* extract showed capacity to inhibit the phosphorylation of VEGFR-2 (IC_{50}=63.13 µg/ml), according to the performed enzymatic fluorescence resonance energy transfer (FRET)-based assay. This extract was microencapsulated and the obtained microspheres were observed by OM immediately after the atomization and after 4 hours in contact with the coagulation solution (Fig 1). This analysis confirmed that the process was conducted successfully. The observed microspheres had a spherical morphology and no agglomeration was detected (they were presented as individualized structures). The determined EE pointed out a value reaching 100% since no apigenin (the major extract's aglycone) was detected, both in the coagulation and washing solutions.

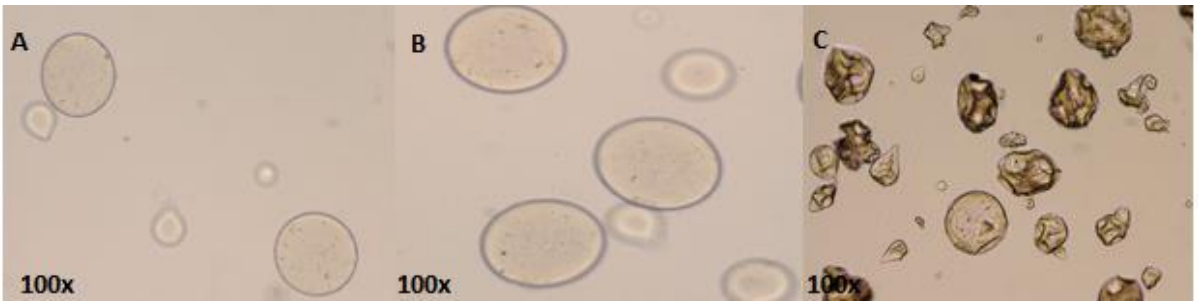

Fig. 1. Optical microscopy of the microcapsules along the microencapsulation process: A- Microspheres after being sprayed (initial time of the coagulation process); B- Microspheres after coagulation; C- Microspheres after lyophilization (storage form).

The amount of the incorporated extract in the yogurt samples (free or microencapsulated) was twice the IC_{50} value which is in accordance with the apigenin daily recommended dose and its relative concentration in the extract (48%, w/w). For the yogurt added with the free form, and comparatively with the microencapsulated form, an initially higher antiangiogenic activity was observed. Nevertheless, a decrease was observed for t3 (3 days), which can be associated with the extract degradation. On the contrary, in the case of using the microencapsulated form, an increase of antiangiogenic activity was observed from t0 (initial time) to t3. This could be correlated with an effective protection provided by the used microencapsulation process, together with a sustained release of the extract with time.

Conclusions

The atomization/coagulation technique allowed the production of viable microspheres enriched with the plant extract. This final ingredient was effectively incorporated into yogurts, protecting the extract and envisaging the development of novel functional foods with chemopreventive effects. The evaluation of the nutritional composition is under progress.

Acknowledgements

FCT for financial support to CIMO (Project PEst-OE/AGR/UI0690/2014). FCT/MEC and FEDER under Program PT2020 for financial support to LSRE (Project UID/EQU/50020/2013). QREN, ON2 and FEDER (NORTE-07-0124-FEDER-000014) and PRODER (Project nº 46577- PlantLact). The authors also thank Ana Maria Carvalho for providing *Arenaria montana* L. samples.

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