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Functionalization of yogurts with *Agaricus bisporus* extracts encapsulated in spray-dried maltodextrin crosslinked with citric acid

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ABSTRACT

Mushroom extracts contain bioactive compounds potentially useful to functionalize foodstuffs. Herein, alcoholic extracts of *Agaricus bisporus* were studied for their bioactivity and viability as functional ingredients in a food product with high water content (yogurt). Extracts were microencapsulated (to improve their stability and hydrophilicity) by spray-drying, using maltodextrin crosslinked with citric acid as encapsulating material. The effect of thermal treatment (after atomization) on crosslinking and bioactivity of microspheres was tested. The incorporation of free and thermally untreated forms resulted in yogurts with higher initial antioxidant activity (EC_{50} values: 214 and 272 mg.mL^{-1}) that decreased after 7 days (EC_{50} values: 248 and 314 mg.mL^{-1}). Contrarily, thermally treated microencapsulated extracts showed higher antioxidant activity after the same period (EC_{50} values, 0 days: 106 mg.mL^{-1}; 7 days: 48.7 mg.mL^{-1}), in result of an effective protection provided by microencapsulation with crosslinked maltodextrin and citric acid. Functionalized yogurts showed an overall maintenance of nutritional properties.

1. Introduction

The extensive use of synthetic additives in foods, some of them recognized as carcinogenic substances, has alerted consumers towards the need of adopting healthier habits. Moreover, their appetence for functional foods, i.e. foods promoting positive effects on health in addition to their basic nutritional purposes, is progressively increasing (Granato, Nunes, & Barba, 2017). In fact, functional foods are integrating the daily diet of consumers and are gaining prominence worldwide. The potential global market for functional foods and beverages has been estimated to value $192 billion by 2020 (Kaur & Singh, 2017).

Mushrooms represent interesting sources of bioactive compounds with potential application in functional foods. They can provide immunomodulating, antitumour, anti-hypercholesterolemic, antibacterial, antifungal, anti-inflammatory, antiviral, anti-diabetic, and cardioprotective effects to consumers (Reis, Martins, Vasconcelos, Morales, & Ferreira, 2017). *Agaricus bisporus* is the species with the highest consumption worldwide, showing a great potential to be used to enrich food matrices, mainly due to its high amount of ergosterol, a mycosterol with several bioactivities such as antioxidant, anti-inflammatory, antitumoral and antimicrobial, in addition to hypocholesterolemic effects (Gao et al., 2007; Gil-Ramírez, Ruiz-Rodríguez, Marín, Reglero, & Soler-Rivas, 2014; Shao, Hernandez, Kramer, Rinker, & Tsao, 2010; Yasukawa et al., 1994).

Besides selecting the bioactive ingredient (mushroom extract in the study reported herein) it is also important to choose a suitable food product suitable to be functionalized. Yogurt is among the most studied products, most likely because it is a cheap product, generally appreciated by most consumers, besides having intrinsic beneficial properties (Pereira et al., 2016; Pereira et al., 2016). Nevertheless, due to their high water content yogurts are mainly appropriate to be functionalized with hydrophilic extracts (Caleja et al., 2016; Ghorbanzade, Jafari, Akhavan, & Hadavi, 2017; Karaaslan, Ozden, Vardin, & Turkoglu, 2011; Karam, Gaiani, Hosri, Burgain, & Scher, 2013; Santillán-Urquiza, Méndez-Rojas, & Vélez-Ruiz, 2017; Singh & Muthukumarappan, 2008). Still, the formulation of functional foods, namely dairy beverages, with

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A. bisporus extracts was reported to increase their bioactivity, as indicated by *in vitro* studies that validated the antioxidant activity and cytotoxicity against tumor cells (Heleno et al., 2017). However the poor solubility of its major compound, ergosterol, both in lipophilic and hydrophilic media, may limit the application of those extracts (Barreira, Oliveira, & Ferreira, 2014; Corrêa, Peralta, Bracht, & Ferreira, 2017). This solubility constraint might be overcome by microencapsulating the extract with a material showing improved water compatibility such as maltodextrin, which could also allow a better stability during storage.

Among different encapsulating materials used to protect bioactive ingredients and increase their bioaccessibility, maltodextrin is a competitive solution. It was already tested with success with different bioactives such as mussel hydrolysates and several phenolic compounds (Breternitz, de V. Fidelis, Silva, Eberlin, & Hubinger, 2017; Moser et al., 2017) and it was also studied in combination with other cross-linking molecules (*e.g.*, cinnamyl alcohol, transglutaminase and octenyl succinic anhydride) showing the advantages of following such strategies (Khandal, Aggarwal, Suri, & Coqueret, 2015; Nawong, Oonsivilai, Boonkerd, & Truelstrup Hansen, 2016; Wang et al., 2014).

The application of maltodextrin itself, without any hardening or reticulation treatment, results in the ready release of the active component upon contact with the hydrophilic matrix, especially in the case of low viscosity products. Among different options, some recent works have shown the possibility to conduct maltodextrin reticulation with citric acid under certain conditions of temperature and time, resulting in materials with improved thermal and mechanical resistance (Awadhiya, Kumar, & Verma, 2016; Castro-Cabado, Parra-ruiz, Casado, & Román, 2016; Menzel et al., 2013). This strategy is worth investigating since it combines natural products with a low-cost process with potential implementation at industrial scale, resulting in an attractive and viable alternative to produce mycosterol-derived ingredients to functionalize food products, such as yogurts (Dias, Ferreira, & Barreiro, 2015; Ribeiro et al., 2015). To the best of our knowledge this approach was never tested with spray-drying,

Therefore, the aim of this work was the development of a microencapsulation process to obtain microspheres containing *A. bisporus* extracts by spray-drying using maltodextrin cross-linked with citric acid, a novel material in the present context, as the carrier material. In order to guarantee an effective crosslinking reaction, a post thermal treatment was also studied. The obtained microcapsules were used as functionalizing agents of yogurt.

2. Materials and methods

2.1. Standards and reagents

Methanol and acetonitrile of HPLC grade, and chloroform and ethanol of analytical grade were acquired from Fisher Scientific (Lisbon, Portugal). Maltodextrin was provided by Cargill (Wayzata, MN, USA) with a dextrose equivalent of 18. Citric acid was acquired from Panreac (Barcelona, Spain). Trolox (6-hydroxy-2,5,7,8-tetra- methylchroman-2-carboxylic acid), the sterol standards (ergosterol (98%) and cholecalciferol (98%)), acetic acid, phosphate buffered saline (PBS), sulforhodamine B (SRB), trypan blue, trichloroacetic acid (TCA) and tris-(hydroxymethyl)aminomethane (TRIS) were purchased from Sigma (St Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM) and RPMI-1640 medium, fetal bovine serum (FBS), Hank's balanced salt solution (HBSS), l-glutamine, nonessential amino acid solution (2 mM), penicillin/streptomycin solution (100 U mL^{-1} and 100 mg mL^{-1}, respectively), and trypsin- EDTA (ethylenediaminetetraacetic acid) were from Hyclone (Logan, UT, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA) before use. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common suppliers.

2.2. Obtainment and characterization of A. bisporus extract

2.2.1. Source material

The samples of *Agaricus bisporus* L. were provided by a local mushroom producer "Mogaricus mushrooms – Sociedade Unipessoal Ltda". They correspond to exemplars with non-conformities (e.g. small visual defects, low size etc.) to be commercialized constituting biowaste for the company. Samples were weighed, frozen, freeze-dried (Freezone 4.5 freeze dryer model 7750031, Labconco, Kansas City, MO, USA) and then reduced to powder (20 mesh).

2.2.2. Extraction procedure

Ultrasound-assisted extraction (UAE) was the chosen technique to obtain the extracts. It was carried out using an ultrasonic device (QSonica sonicators, model CL-334, Newtown, CT, USA), which comprise an ultrasound probe working in the range of 100–500 W at 20 kHz and a digital timer. Extractions were carried out according to the procedure described by Heleno et al. (2016). Briefly, the lyophilized powdered mushroom samples (3 g) were extracted with 100 mL of ethanol using previously optimized conditions (15 min, 375 w). After extraction, the extract solution was filtered through a Whatman paper filter No. 4 and the solvent evaporated under reduced pressure to obtain the *Agaricus bisporus* raw extract (ABRE). The obtained composition, performed according to the one reported by Heleno et al. (2016), comprised a content of 19.4 mg.g^{-1} in ergosterol.

2.2.3. Characterization

A. bisporus extract was characterized in what concerns antioxidant and anti-inflammatory activities and cytotoxicity. Antioxidant activity was evaluated using the DPPH radical scavenging activity assay. For this, the *A. bisporus* extracts were re-dissolved in ethanol at 20 mg.mL^{-1}. These stock solutions were successively diluted to determine EC_{50} values (sample concentration providing a value of 50% in the DPPH assay). DPPH radical scavenging activity was performed according to the methodology described by Heleno et al. (2012), and evaluated using an ELX800 microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). The percentage of DPPH discoloration was calculated using Eq (1), where A_S is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution.

$$\text{DPPH scavenging activity}(\%) = 100 \times \frac{A_{DPPH} - A_S}{A_{DPPH}} \quad (1)$$

Anti-inflammatory activity was evaluated following the procedure reported by Taofiq et al. (2015). Briefly, the LPS-induced NO production by Murine macrophage (RAW 264.7) cell lines was determined as nitrite concentration in the culture medium. The *A. bisporus* extract was dissolved at 8 mg.mL^{-1} in water and the nitric oxide (NO) production was determined using the Griess Reagent System kit and dexamethasone as a positive control.

For the toxicity evaluation, a primary culture cell was prepared from a porcine liver (PLP2), according to a procedure previously established by Abreu et al. (2011). The *A. bisporus* extract was dissolved in water at 8 mg.mL^{-1} and successive dilutions were performed from the stock solution. PLP2 cells were treated for 48 h with the different sample solutions using sulforhodamine B assay and ellipticine was used as positive control.

2.3. Microencapsulation of A. bisporus extract

2.3.1. Microencapsulation procedure

Microencapsulation was performed by the spray-drying technique using maltodextrin crosslinked with citric acid as the encapsulating material. The used procedure and operating conditions were adapted from those described by Ribeiro et al. (2015) with some modifications. This comprised the preparation of a solution with a ABRE/maltodextrin