Food Chemistry 188 (2015) 612–618

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Spray-drying microencapsulation of synergistic antioxidant mushroom extracts and their use as functional food ingredients

Andreia Ribeiro [a,b], Gabriela Ruphuy [b,c], José Carlos Lopes [c], Madalena Maria Dias [c], Lillian Barros [a], Filomena Barreiro [b,*], Isabel C.F.R. Ferreira [a,*]

[a] Mountain Research Center (CIMO), ESA, Polytechnic Institute of Bragança, Campus Santa Apolónia Ap. 1172, 5301-855 Bragança, Portugal
[b] Laboratory of Separation and Reaction Engineering (LSRE), Associate Laboratory LSRE/LCM, Polytechnic Institute of Bragança, Campus Santa Apolónia Ap. 1134, 5301-857 Bragança, Portugal
[c] Laboratory of Separation and Reaction Engineering (LSRE) – Associate Laboratory LSRE/LCM, Faculty of Engineering, University of Porto, Porto, Portugal

ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 27 April 2015
Accepted 13 May 2015
Available online 14 May 2015

Keywords:
Mushroom extracts
Antioxidant activity
Synergistic effects
Microencapsulation
Cottage cheese

ABSTRACT

In this work, hydroalcoholic extracts of two mushrooms species, *Suillus luteus* (L.: Fries) (Sl) and *Coprinopsis atramentaria* (Bull.) (Ca), were studied for their synergistic antioxidant effect and their viability as functional food ingredients tested by incorporation into a food matrix (cottage cheese). In a first step, the individual extracts and a combination of both, showing synergistic effects (Sl:Ca, 1:1), were microencapsulated by spray-drying using maltodextrin as the encapsulating material. The incorporation of free extracts resulted in products with a higher initial antioxidant activity (t0) but declining after 7 days (t7), which was associated with their degradation. However, the cottage cheese enriched with the microencapsulated extracts, that have revealed a lower activity at the initial time, showed an increase at t7. This improvement can be explained by an effective protection provided by the microspheres together with a sustained release. Analyses performed on the studied cottage cheese samples showed the maintenance of the nutritional properties and no colour modifications were noticed.

1. Introduction

Mushrooms are widely appreciated all over the world for their nutritional properties (Kalač, 2009). They have a low fat content but are rich in water, minerals, proteins, fibres and carbohydrates (Heleno et al., 2012; Kalač, 2009; Reis, Barros, Martins, & Ferreira, 2012; Reis et al., 2011). Besides their nutritional value, it has been demonstrated that mushrooms have health promoting benefits (Palacios et al., 2011). They are effective as anti-inflammatory (Ma, Chen, Dong, & Lu, 2013), antitumor (Heleno et al., 2014), antibacterial (Alves et al., 2012) and antioxidant agents (Reis et al., 2011), extending their potential use as functional foods and applications in the biomedical field.

During natural cellular metabolism, reactive oxygen (ROS), nitrogen (RNS) and sulphur (RSS) species are produced (Carocho & Ferreira, 2013), ROS being the most abundant (Ferreira, Barros, & Abreu, 2009). When high concentrations of these species are present, an oxidative stress is generated. If in excess, ROS may oxidise and damage cellular lipids, proteins and DNA, leading to their modification and inhibition of normal functions (Ferreira et al., 2009). Given this scenario, the organism develops defence mechanisms such as endogenous defences, including enzymatic reactions such as the production of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase; or non-enzymatic reactions, resulting in species such as glutathione (GSH), α-tocopherol (vitamin E), ascorbic acid (vitamin C) and lipoic acid (Carocho & Ferreira, 2013; Ferreira et al., 2009). Both mechanisms are able to provide cells protection against excessive levels of free radicals (Carocho & Ferreira, 2013).

The exogenous antioxidant defence promoters can be ingested as part of the daily diet to help fight against high ROS contents. Therefore, mushrooms can play an important role since they contain diverse phenolic compounds, known to be excellent antioxidants due to their capacity for capturing free radicals by electron transferring, and to the excellent redox properties of the phenolic hydroxyls groups (Ferreira et al., 2009).

Apart from their instability at high temperatures, and in the presence of oxygen and light, some mushroom extracts are characterised by a strong odour and flavour. One way to ensure their viability as functional food ingredients is to proceed with their microencapsulation, providing protection against oxidation and masking their odour and flavour (Ersus & Yurdagel, 2007; Fang &

* Corresponding authors.
E-mail addresses: barreiro@ipb.pt (F. Barreiro), iferreira@ipb.pt (I.C.F.R. Ferreira).

http://dx.doi.org/10.1016/j.foodchem.2015.05.061
0308-8146/

Bhandari, 2010). Despite the numerous available microencapsulation possibilities, spray-drying is still one of the most widely used processes to encapsulate food ingredients (Fang & Bhandari, 2010). Among the main advantages of this technique are its easy industrialisation and the possibility of continuous production. Nevertheless, prolonged contact with high temperatures can compromise the bioactive properties of the mushroom extracts, and should be avoided.

Among several possibilities, maltodextrin (MD), a hydrolysed starch, offers advantages as a microencapsulation material (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). It is a low cost material with a neutral aroma and flavour, high water solubility and low viscosity at high solids content, being able to provide effective protection against oxidation (Ersus & Yurdagel, 2007; Saéns, Tapia, Chávez, & Robert, 2009).

Microencapsulation can be applied to protect bioactive natural extracts and some examples calling up this thematic can be found in literature (Dias, Ferreira, & Barreiro, 2015). Nevertheless, these studies are mainly related to the development of the microencapsulation process and do not proceed with the implementation of a final application as a functional food. (Ersus & Yurdagel, 2007; Kha, Nguyen, & Roach, 2010; Saéns et al., 2009; Silva, Stringheta, Teófilo, & Oliveira, 2013; Wu, Zou, Mao, & Liu, 2014). In fact, and according to the current research, the examples dealing with the full process development are scarcer. In this context, Çam, Içyer, and Erdoğna (2014) tested the incorporation of microencapsulated *Punica granatum* L., an extract from pomegranate, in ice creams, and Martins et al. (2014) studied the incorporation of *Rubus ulmifolius* Schoot (Rosaceae), a species of wild blackberry, microencapsulated in alginate microparticles in yogurt. The obtained results, rather preliminary, are encouraging interest in the development of foods enriched with natural extracts that are often referred as health promoters (Ramalingum & Mahomoodally, 2014).

In this work, extracts of two mushroom species, *Suillus luteus* (L.: Fries) and *Cooprinopsis atramentaria* (Bull.) were investigated for their synergistic antioxidant effects and a promising combination of both (in similar proportions) was chosen to be microencapsulated by spray-drying using maltodextrin as the encapsulating material. The obtained powders were characterised by Scanning Electron Microscopy (SEM) (to inspect morphology and particle size) and for their antioxidant activity (free radicals scavenging activity and reducing power). Encapsulation yield and efficiency were also estimated. As a final step in this work, the produced microspheres were incorporated into cottage cheese samples and their antioxidant activity, nutritional value and colour were determined and compared with the counterparts, using extracts in the free form and a control (sample with no added extracts).

2. Materials and methods

2.1. Standards and reagents

For antioxidant tests, 2,2-dipheny-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). For chromatographic analysis, HPLC-grade acetonitrile was purchased from Fisher Scientific (Lisbon, Portugal). The standards, such as fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U), β-carotene (98%) and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), were purchased from Sigma (St. Louis, MO, USA), along with formic acid. Maltodextrin (MD) was provided by Cargill (Wayzata, MN, USA) with a dextrose equivalent of 18. All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Preparation of mushroom extracts and evaluation of synergistic effects

2.2.1. Extract preparation

Mushroom samples, *S. luteus* (Sl) and *C. atramentaria* (Ca), were harvested in the Bragança region located in the North-east of Portugal, according to a previous report (Heleno et al., 2012; Reis et al., 2011). The chemical characterisation of both species can be found in the cited references.

Samples of individual species and combinations in three different proportions (Sl:Ca 1:2, 1:1 and 2:1, w/w) were extracted. To prepare the extracts, the lyophilized mushroom samples (1.5 g) were extracted with methanol/water (80:20, v/v, 30 ml) at room temperature for 1 h under stirring. The extract was filtered through a Whatman paper filter N° 4 and the remaining residue subjected to an additional extraction. The combined extracts were evaporated under reduced pressure in a rotatory evaporator (Büchi R-210, Flawil, Switzerland) until complete removal of methanol, lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) and stored in a desiccator protected from light until use.

2.2.2. Evaluation of antioxidant activity

The antioxidant activity of the obtained extracts was evaluated using the DPPH radical scavenging activity, reducing power, and inhibition of β-carotene bleaching assays. For the assays, the extracts were re-dissolved in methanol/water (80:20, v/v) at 20 mg/mL, and stored at 4 °C. These stock solutions were successively diluted to determine EC_{50} values (sample concentration providing a value of 50% in the DPPH and β-carotene bleaching assays, or an absorbance value of 0.5 in the reducing power assay).

DPPH radical scavenging activity (Heleno et al., 2012) was evaluated using an ELX800 microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA), and calculated as a percentage of DPPH discoloration using the formula: $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, 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. Reducing power (Heleno et al., 2012) was evaluated by the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching (Heleno et al., 2012) was evaluated by the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which is measured by the formula: (β-carotene absorbance after 2 h of assay/initial absorbance) × 100. Trolox was used as positive control.

2.2.3. Classification of additive, synergistic or antagonistic effects

Theoretical values for the antioxidant activity of the assayed mixture extracts were calculated as the weighted mean of the experimentally determined EC_{50} values of the individual extracts and considering additive contributions, e.g., for a mixture comprising 33% (w/w) of *S. luteus* and 67% (w/w) of *C. atramentaria*, $EC_{50} = EC_{50}$ *S. luteus* × 0.33 + EC_{50} *C. atramentaria* × 0.67.

The classification as additive (AE), synergistic (SE) or antagonistic (negative synergistic (NS)) effects was performed as follows: AE:EC_{50} theoretical and experimental values revealed differences lower than 5%; SE:EC_{50} experimental values were at least 5% lower than theoretical values; AN:EC_{50} experimental values were at least 5% higher than theoretical values. The limit of 5% was chosen taking into account the coefficients of variation obtained in the replications of each antioxidant activity assay. It should be noted that lower EC_{50} values means higher antioxidant activity.

2.3. Microencapsulation of mushroom extracts and characterisation

2.3.1. Microencapsulation

Microencapsulation was performed by spray-drying using the lyophilized extracts and maltodextrin (MD) as the encapsulating