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EXTENDED ABSTRACTS**

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Universidade do Porto, Faculdade de Engenharia, Departamento de Engenharia Química, Rua Dr. Robertos Frias s/n 4200 - 465 Porto, PORTUGAL

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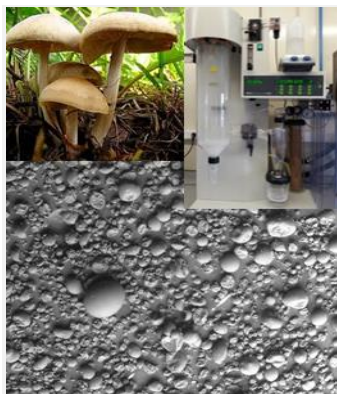
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A. Ribeiro^{1,2}, G. Ruphy³, L. Barros², J. C. Lopes³, M. M. Dias³, I. C. F. R. Ferreira², M. F. Barreiro¹. (1) Laboratory of Separation and Reaction Engineering (LSRE) – Associate Laboratory LSRE/LCM, Bragança Polytechnic Institute, Bragança, Portugal; (2) Mountain Research Centre (CIMO), School of Agriculture, Bragança, Portugal, (3) Laboratory of Separation and Reaction Engineering (LSRE) – Associate Laboratory LSRE/LCM, Faculty of Engineering, University of Porto, Porto, Portugal; *barreiro@ipb.pt.



Mushrooms are widely appreciated all over the world for their nutritional and pharmacological value being sources of important bioactive compounds. In this study the antioxidant potential of *Suillus luteus* and *Coprinopsis atramentaria* mushroom alcoholic extracts, and its synergistic effect, was evaluated before and after being spray-dried with maltodextrin, aiming at its exploitation as functional food ingredients. The antioxidant activity of free and spray-dried extracts was studied *in vitro* by DPPH radical scavenging activity, reducing power and inhibition of β -carotene bleaching (this only applied with free extracts assays). The encapsulation yield of the spray-drying process was around 50% and efficiency, determined in terms of cinnamic acid, comprised between 40-60%. The evaluation of the antioxidant activity of the combined extracts pointed out for synergistic effects.

Introduction

Mushrooms are widely appreciated all over the world for their nutritional and/or medicinal properties. Mushrooms are rich in water, minerals, proteins, fibres and carbohydrates, but possess a low fat content (low caloric value). Their reported bioactive properties can be associated with the phenolic, polysaccharidic and lipidic fractions. In what concerns phenolic compounds, they can exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, and vasodilatory effects, which have been partly related with their antioxidant activity [1].

Apart from its instability at high temperatures, presence of oxygen and light, mushroom extracts are characterized by a strong odour and flavour. One way to ensure its viability as functional food ingredients is to proceed with their microencapsulation, providing protection against oxidation and masking odour and flavour. Despite the numerous available microencapsulation possibilities, spray-drying is still one of the most used processes to encapsulate food ingredients. The main advantages are related to its easy industrialization and continuous production. Nevertheless, care must be taken to avoid prolonged contact with high temperatures, which can compromise bioactive properties [2,3].

Among several possibilities, maltodextrin (MD), a hydrolysed starch, offers various advantages as microencapsulation material. It has a low cost, neutral aroma and flavour, high water solubility,

low viscosity at high solids contents and provides an effective protection against oxidation [4,5].

In the present work, the antioxidant potential of *Suillus luteus* (L.: Fries) Gray and *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys & Moncalvo mushroom alcoholic extracts, and its synergistic effect, was evaluated before and after being spray-dried with maltodextrin, aiming at its exploitation as functional food ingredients. The potential synergistic effects were primarily evaluated based on the free extracts (DPPH radical scavenging activity, reducing power and inhibition of β -carotene bleaching) and thereafter with the encapsulated homologue. Powders obtained by spray-drying were characterized by SEM (morphological and size analysis), FTIR, encapsulation yield (EY), encapsulation efficiency (EE) and antioxidant activity (DPPH radical scavenging activity and reducing power).

Materials and methods

Preparation of alcoholic extracts: The lyophilized mushroom samples (1.5 g) were extracted with methanol (30 mL) at room temperature during 2h under stirring. The extract was filtered through a Whatman paper filter N° 4 and the remaining residue subjected to an additional extraction. The combined methanolic extracts were evaporated at 40 °C in a rotary evaporator until dryness, re-dissolved in methanol at a concentration of 20 mg/mL and stored at 4 °C for antioxidant properties evaluation. For spray-drying purposes, the extract was lyophilized and stored in a desiccator protected against light. Two

mushroom extracts have been produced: *Suillus luteus* (Sl) and *Coprinopsis atramentaria* (Ca).

Preparation of solutions for spray-drying: The mushroom extracts (1 g) were dissolved firstly in ethanol (10 mL) followed by water addition (90 mL) to achieve a homogenous solution. Thereafter 20 g of MD with a dextrose equivalent (DE) of 18 was added under stirring. The used conditions were adapted from the best ones achieved in the work of Wu and co-workers [2] resulting in a ratio extract/maltodextrin of 1/20 (w/w) and a solids content around 20% (w/w). Solutions of MD alone, the two individual mushrooms and a combination of both (Sl:Ca of 1:1) were prepared.

Spray-drying of solutions: A Büchi mini spray dryer B-290 was used operating at the following conditions (also based on [2]): inlet temperature 170 °C, outlet temperature 95 °C, aspiration at 90% and pump at 20% (6 mL/min). The equipment was connected to a condenser and nitrogen was used due to the present of organic solvents in the atomized solutions. The nozzle size was 0.7 mm and the atomized volume 100 mL.

Evaluation of antioxidant properties of free and spray-dried extracts: Antioxidant properties were evaluated through DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging activity, reducing power and inhibition of β -carotene bleaching following the methodologies well established at CIMO and found elsewhere [1]. The inhibition of β -carotene bleaching method was only applied with the free extracts. The results are expressed as the extract concentration (mg/mL) yielding 50% of antioxidant activity or 0.5 of absorbance in reducing power assay (EC_{50}).

Characterization of spray-dried extracts: In addition to antioxidant activity, powders obtained by spray-drying were characterized by SEM (morphological and size analysis) and FTIR. Encapsulation yield (EY) was calculated as the ratio between the obtained microcapsules weight and the atomized one. Encapsulation efficiency (EE) was determined by HPLC-DAD as the ratio between the experimentally determined encapsulated extract, in terms of cinnamic acid (the major compound identified in the extracts), and the theoretical one.

Results

DPPH radical scavenging activity, reducing power and inhibition of β -carotene bleaching EC_{50} values obtained with free extracts are presented in the Table 1. Comparing the individual mushroom extracts, we can notice that Sl has the highest antioxidant activity, as shown by the obtained lower EC_{50} values for all the performed assays. Additionally, the combination Sl:Ca of 1:1

exhibited a synergistic effect. This tendency was corroborated by the tests performed with other Sl:Ca ratios (1:2 and 2:1) (data not shown).

The microparticles produced by spray-drying showed a good encapsulation yield and encapsulation efficiency (Table 2). The obtained powders were lightly yellow in colour and the mushroom aroma was masked (qualitative evaluation). Although the FTIR analysis was inconclusive due to the predominant influence of MD in the obtained spectra (data not shown) the HPLC analysis showed that the extract was retained after spray-drying. Moreover based on preliminary tests, bioactivity was also retained.

In what concerns SEM analysis (Figure 1), the obtained microparticles showed a heterogeneous size distribution (particles with sizes comprised between 2 and 50 μ m were registered). A mixture of particles with round shape, both with smooth or rough surface, can be noticed. The appearance of teeth or invaginations on the particles surface can be attributed to a rapid evaporation of water during the drying process [2]. Nevertheless, differences were observed as a function of the encapsulated extracts. Particles with rough surface are predominant in the assay performed with Sl extract, being almost absent in the one done with the mixture Sl:Ca (1:1). Also, a higher particle size was registered for this last case.

Table 2. Encapsulation yield (EY) and efficiency (EE) obtained with individual extracts and a combination.

Extract	EY (% w/w)	EE(% w/w)
Sl	55.2	62.6
Ca	51.8	43.5
Sl:Ca (1:1)	47.1	59.8

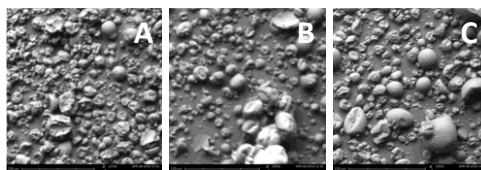


Figure 1. SEM analysis of microparticles containing Sl (A), Ca (B) and the mixture Sl:Ca (1:1) (C) under a magnification of 1300X.

Conclusions

In this work, the antioxidant activity of Sl and Ca alcoholic extracts was evaluated being observed that their combination results in synergistic effects. The spray-drying of the extracts using an extract/maltodextrin ratio of 1/20 and an inlet temperature of 170 °C resulted in a good yield (around 50%) and good encapsulation efficiency (40-60%). In conclusion, this study could help to promote the use of mushroom extracts as functional food ingredients.

Table 1. Antioxidant activity values expressed as EC₅₀ (mg/mL) for free individual extracts and a combination of both (SI:Ca (1:1)). Results are presented as average±SD calculated from 3 replicas.

Assay	SI	Ca	SI:Ca (1:1)		
			Theoretical (*)	Experimental	Effect
DPPH scavenging activity	2.86±0.02	4.62±0.10	3.70	3.49±0.13	SE
Reducing Power	0.97±0.02	1.11±0.02	1.00	1.05±0.02	AE
Inhibition of β-carotene bleaching	1.64±0.20	5.28±0.36	3.50	1.46±0.04	SE

(*) The theoretical values were calculated as the weighted average of the individual values ($EC_{50,theoretical}=0.5*EC_{50,SI}+0.5*EC_{50,Ca}$); **SE** (Synergistic Effect) means that the experimentally determined antioxidant activity is 5% higher (EC₅₀ value 5% lower) than the theoretical one, **AE** (Additive Effect) means that the difference is lower to 5%.

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