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MICROENCAPSLATION OF MUSHROOM EXTRACTS FOR COSMECEUTICAL DESIGN

Taofiq, O.[1,2,3], Heleno, S.A.[1,2], Barros, L.[1,2], Calhelha, R.C.[1], Alves, M.J.[1], Fernandes, I.P.[2], González-Paramás, A.M.[3], Barreiro, M.F.[2], Ferreira, I.C.F.R.[1]

INTRODUCTION & OBJECTIVES

The global cosmetic industry is constantly in search for bio based ingredients with less toxic effects but providing multifunctional properties, maintaining the structure and function of the skin. This will, thereby, reduce the presence of synthetic ingredients in personal care products (Taofiq, 2017).

Cosmeceuticals are the newest trend in the design of personal care products, presenting medical drug-like benefits through the supply of appropriate ingredients for the maintenance of a healthy skin. They include bioactive compounds such as phytonutrients, microbial metabolites, minerals, vitamins and animal proteins (Taofiq, 2016a).

Mushrooms are widely consumed due to their rich nutritional composition with recognized health promoting benefits. They contain several bioactive compounds such as polysaccharides, phenolics, terpenoids, nucleotides, steroids, fatty acids, proteins and glycol-peptides that are responsible for their antioxidant, antitumor, anti-inflammatory, antimicrobial anti-tyrosinase and other biological activities. Because of all the above-mentioned properties, there is an increasing interest in incorporating mushroom extracts and/or their isolated compounds in cosmetic formulations, expecting positive benefits on the skin. These extracts/compounds can act either as antioxidants, preservatives or as hyperpigmentation correcting agents (Taofiq, 2016a).

In a general way, the use of natural-derived extracts, including mushroom extracts, as cosmeceutical ingredients can present restrictions related with the lack of stability and ease degradation of some of their metabolites. To overcome these challenges, microencapsulation techniques have been utilised taking advantage of the protection conferred by polymeric materials, particularly natural-derived and biodegradable ones. This ensures protection against oxidation and degradation, controlled or target oriented release, guaranteeing that the bioactives effectively cross the biological membranes.

The specific objective of the present work was to obtain mushroom extracts-based ingredients for cosmeceutical applications. Soxhlet ethanolic extracts were prepared from *Agaricus bisporus* (J.E.Lange) Imbach (**A**) and *Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm (**P**), thereafter chemically characterised in terms of phenolic acids and ergosterol contents, and screened for their anti-inflammatory, antimicrobial, antioxidant, and anti-tyrosinase activities. The extracts were then microencapsulated in calcium alginate particles by an atomization/coagulation technique. The produced microparticles were characterised (morphology, particle size, encapsulation efficiency (EE%)) for subsequent incorporation in a base cosmetic cream. The developed formulations were evaluated for their bioactive properties, pH and colour evolution over a 6 months' period to ascertain the stability of the bioactives and monitor their *in vitro* release behaviour. The composition of the bioactive compounds in the creams was also monitored by HPLC.

MATERIALS & METHODS

Mushroom extracts were prepared by the soxhlet methodology using ethanol as solvent. Their antioxidant activity was evaluated by DPPH radical-scavenging activity, and reducing inflammatory activity was evaluated by quantification of NO production in RAW 264.7 macrophages cells. The microdilution method was used to determine the minimum inhibitory concentrations (MICs) of the extracts. The tyrosinase inhibition assay was evaluated using L-DOPA as substrate (Taofiq, 2016b).

The chemical characterization of both extracts and final formulations included the analysis of ergosterol by HPLC-UV, and of the phenolic compounds by HPLC-DAD.

Microencapsulation of the mushroom extracts was done by the atomization/coagulation method with sodium alginate coagulated with calcium chloride. Briefly, sodium alginate solutions (4%, w/v) containing the extracts were prepared under stirring at ambient temperature using a ratio extract:alginate of 100:400 (w/w). The solutions were atomized in a Nisco Var J30 unit and microparticles coagulated upon contact with a solution of $CaCl_2$ (4%, w/v). This coagulation solution was recovered after vacuum filtration to obtain the microparticles that were thereafter washed with deionized water.

The obtained microparticles were analyzed by optical microscopy (OM) and characterized in what concerns particle size distribution (Mastersizer 3000 based on laser diffraction technique). The EE% of the encapsulation system was deduced from the quantification of the non-encapsulated bioactives though the analysis of the recovered coagulation and washing solutions.

Free and microencapsulated extracts were incorporated in a Fagron Versatile™ vanishing o/w base cream. The formulations were stored in glass vials, and the bioactivities and chemical characterization carried out accordingly as previously described. The colour was measured using a colorimeter based on the *L*a*b** system and the pH using HI 99161 pH-meter.

RESULTS & DISCUSSION

The phenolic acids found in **P** were cinnamic, *p*-hydroxybenzoic and *p*-coumaric acids (Total- 584 ± 3 µg/g) while only cinnamic acid (90.1 ± 0.7 µg/g) was detected in **A**. The ergosterol content was 44.8 ± 0.4 and 78.2 ± 0.5 (mg/g) for **A** and **P**, respectively. The bioactive properties displayed by **A** and **P** were as follows (EC_{50} values in mg/mL): anti-inflammatory (0.18 ± 0.01 and 0.29 ± 0.03, respectively), anti-tyrosinase (0.16 ± 0.01 and 0.86 ± 0.07), DPPH radical-scavenging activity (7.0 ± 0.3 and 7.7 ± 0.2), reducing power (2.34 ± 0.05 and 2.36 ± 0.08). Up to 10 mg/mL, the growth of *E. faecalis*, MSSA and MRSA was inhibited. These bioactive properties of **A** and **P** highlight the obtained extracts as interesting cosmeceutical ingredients with multifunctional properties.

The concentration of phenolic acids in the coagulation and washing solutions was determined to calculate the EE% giving rise to values of 97.5 and 99.9 for **A** and **P**, respectively. These results showed that the extracts were successfully encapsulated. The analysis of the microparticles by MO showed the presence of spherical and individualized structures with little or no agglomeration.

The particle sizes were measured as *d10*, *d50* and *d90*

number were 0.719, 0.937 and 2.05 µm, while for **P** microparticles were 0.715, 0.920 and 1.96 µm respectively. Concerning the *d10*, *d50* and *d90* percentiles in volume, the values were 27.1, 100 and 254 µm for **A** and 27.7, 94.7 and 240 µm for **P** microparticles respectively. In conclusion, nevertheless the used active principle (**A** or **P**), the obtained microparticles showed similar properties in terms of size distribution. Additionally, the particle size distributions in number are unimodal, while distributions in volume are bimodal as it can be observed in Figure 1(a) and (b), respectively.

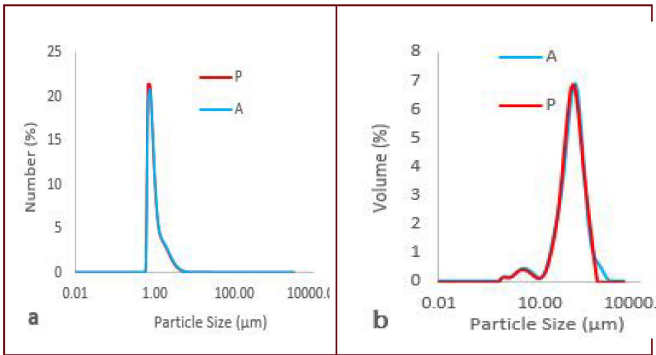

Figure 1. Microparticles size distributions expressed as mean (a) number distributions and (b) volume distributions.

The developed cosmeceutical formulations presented, along the storage time, a pH value comprised between 3.4 and 4.4, which is a suitable pH range for cosmetic preservation as most of the pathogenic bacteria thrive best at neutral pH. The *L** (lightness) parameter was the colour parameter showing the more pronounced variation among the three monitored variables. Significant colour changes could not be observed, which was attributed to the stability of the formulations, as degradation was not significant enough to produce end-products that may influence the colour of the formulations.

In the base creams with the **A** and **P** extracts, the total phenolic acids were 88 ± 2 µg/g and 509 ± 4 µg/g, respectively, immediately after incorporation. This means that the target compounds were successively incorporated, being detected up to 80% of the amount determined for the corresponding free extracts. Nevertheless, loss of bioactive compounds was found over the 6-month period of storage, but significant bioactivity was still achieved.

The cosmeceutical formulations prepared with the free extracts presented antioxidant, anti-tyrosinase and antimicrobial activities, while the formulations prepared with the microencapsulated extracts only displayed anti-tyrosinase and antimicrobial activities. These results show that even though the extracts have been well encapsulated, their *in vitro* release behaviour, particularly in what concerns the antioxidant activity, could not be rationally understood. This suggests the need to conduct further studies to better understand the showed behaviour.

CONCLUSION & PERSPECTIVES

The above findings reveal that ethanolic extracts of *A. bisporus* and *P. ostreatus*, display strong antioxidant, antibacterial, anti-inflammatory and anti-tyrosinase activities, mainly due to the contribution of their bioactive components, being suitable for multifunctional personal care ingredients. The atomization/coagulation technique was successfully applied to microencapsulated the extracts, giving rise to high encapsulation efficiencies (97.5 and 99.9, respectively for **A** and **P).** The incorporation of both free and protected (microencapsulated) extracts, into the base cosmetic creams, was able to impart bioactivity to the final products. Further studies need to be conducted to better understand the differences found between the two used forms (free and microencapsulated extracts). Additionally, this in course study will proceed with the study of other encapsulation materials. Also, *in vitro* skin permeation studies need to be conducted to determine the topical bioavailability of these bioactive ingredients.

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