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Valorization of mushroom species in the cosmetic industry using natural deep eutectic systems

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1. Introduction

Nowadays, many natural resources are limited, and because of that there is a growing concern in achieving a sustainable development. All around the world, industries are putting more focus on being sustainable at every step of a product's life cycle.[1,2] The cosmetics industry is also seeing a shift toward consumer preference for natural ingredients. A possible solution involves adopting sustainable technologies, methodologies, and natural-origin ingredients. [3,4]

Mushrooms are rich in several bioactive components but are an underused resource. Their cultivation has minimal environmental impact, requiring less water and energy, and emitting less CO_2 compared to traditional crops. [1,5] They contain a variety of bioactive compounds, such as vitamins, phenolic compounds, and polysaccharides, that offer health benefits including antimicrobial, anti-inflammatory, and antioxidant properties. These compounds make mushrooms promising sources of innovative natural cosmeceutical ingredients, offering anti-aging, moisturizing, and skin-brightening effects. [1,6,7]

Currently, the extraction of bioactive compounds from mushrooms primarily depends on conventional methods. These techniques often require large volumes of costly solvents and extended processing times, resulting in low yields. The use of organic solvents also raises concerns due to their toxicity, environmental impact, and the substantial waste they produce.[1,8,9]

Recently, a new class of green solvents has emerged, the Natural Deep Eutectic Systems (NADES). NADES are mixtures of two or more naturally occurring molecules, a hydrogen bond donor and a hydrogen bond acceptor, when mixed in a certain molar ratio have a lower melting point compared to its individual compounds, becoming a liquid at room temperature.[10] Natural deep eutectic solvents (NADES) are easy to prepare, biodegradable, biocompatible, and non-toxic. They also eliminate the need for purification steps and can enhance the stability of natural compounds. [4,11,12] Increasingly, NADES are being explored as alternative solvents for extracting bioactive compounds, demonstrating higher extraction efficiency in comparison to conventional organic solvents, as reported in the literature.[12] Despite being a relatively

new technology, NADES hold great promise for extracting bioactives from mushrooms and creating valuable cosmetic extracts. [7,13]

This research aims to develop bioactive-rich extracts from mushroom species easily available in Portugal using NADES, which could be incorporated directly into cosmetic topical formulations. These extracts can deliver substantial benefits for human skin while advancing sustainability in the cosmetic sector. Available mushroom species were analyzed for their health benefits, cosmetic potential, and chemical composition, quantifying their bioactive compounds. Different NADES were prepared and characterized, followed by solid-liquid extractions. Extract analysis highlighted their suitability for topical formulations. Compared to hydroalcoholic extracts, NADES-based extractions yielded better results, demonstrating their capacity to stabilize phenolic compounds and serve as an alternative to conventional solvents.

2. Materials and Methods

2.1 Materials

Brown *Agaricus Bisporus* mushroom were obtained from a local supermaket in Lisbon, Portugal. Sorbitol, Glycerol (purity 99.5%), L-Proline (purity 99%), Citric Acid (purity 99.5%), Sodium Carbonate solution, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA). Betaine (purity 98%) was purchased from Glentham Life Science (UK). Ethanol (purity 99.5%), Methanol (purity 99.8%), and Trichloroacetic Acid (purity 99.5%) were purchased from Scharlau (Spain). Folin-Ciocalteu reagent was purchased from MERK (Germany).

2.2 Methods

2.2.1 Preparation of mushroom samples.

The mushroom samples were subjected to pre-treatments such as washing and lyophilization. The dried mushrooms were roughly milled using a blender and kept at -20°C in hermetically vacuum-sealed plastic bags. An elementary analysis of the dried mushroom sample was performed and the protein content was determined using a nitrogen-to-protein conversion factor of 4.55.[14]

2.2.2 Preparation of NADES.

Different NADES were synthesized via a heating and stirring method, with different organic acids, sugars and aminoacids. The required amounts of the individual components, according to their molar ratio, were weighed using a precision balance and placed in a sealed glass vial with a magnetic stirring bar. The mixture was agitated at approximately 50°C in a water bath on a hot plate until a homogeneous liquid formed. An aqueous ethanol solution (ethanol/water 1:1) was also prepared and used as a conventional solvent for comparison purposes.

2.2.3 Preparation of Mushroom Extracts

The dried mushroom sample was added to the NADES in a solid−liquid ratio of 1:20 (g/mL). Samples were extracted for 1 h at 40°C in a water bath with stirring. The samples were then centrifuged for 15 min at 6000 rpm to ensure complete phase separation. Before analysis, the extracted samples were filtered to remove any remaining solids. The same procedure was followed for the conventional aqueous ethanol extract.

2.2.4 Determination of Total Phenolic Content

The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteau method, as previously described.[15] Briefly, the first step is the protein precipitation. An amount of 120 mL of 100% (w/v) trichloroacetic acid was added to 800 mL of the sample, and the mixture was thoroughly mixed before being stored for 5 min at 20ºC and sub-sequently during 15 min at 4ºC. After centrifugation (12000 rpm, 15 min) the precipitate was discarded. Test tubes were filled with 1580 µl of distilled water, 20 µl of the sample, and 100 µl of Folin-Ciocalteu reagent before being agitated on vortex, and then were 5 minutes at room temperature. Then to the mixture was added 300 µl of a sodium carbonate solution, followed by agitation. Tubes were placed bath at 40ºC during 30 minutes. After they were analyzed by UV/Vis spectroscopy using a spectrophotometer at 750 nm.

2.2.5 Determination of Antioxidant activity

The antioxidant potential of the phenolic extracts was determined using the DPPH method, as previously described.[15] This method assesses the ability of serial dilutions of the extracts to donate hydrogen atoms or scavenge free radicals. Distilled water was used as the control, and all samples were prepared in triplicates. To make the stock solution, was dissolved 24 mg of DPPH in 100 mL of methanol, and then was kept it at -20ºC for at least two hours. The absorbance was then determined at 517 nm by diluting the solution by adding 10 mL to 45 mL of methanol. The extracts were produced with serial dilutions. A mixture of 150µL of the diluted extracts and 4ml of DPPH solution was incubated in amber vials, at room temperature protected from light during 40 min, before measuring the absorvance at 517 nm. The following equation was used to evaluate the inhibition of the free radical by each sample. The inhibition of free radical production was calculated using eq 1, where A_{DPPH} is the absorbance of the blank and A_{Sample} is the absorbance of the sample with the extract, both at 517 nm.

$$\%inhibition = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \quad (1)$$

The antioxidant activity of the extracts was expressed as EC_{50} (g/mL), the concentration required to cause 50% DPPH inhibition calculated from the inhibition curves obtained.

2.2.6 Stability over Time Assays

The TPC was quantified over a span of several days using the Folin-Ciocalteau method described previously. The antioxidant potential of extracts over a span of several days was determined by using the DPPH method, as described previously. For the stability assay, extract samples were stored in glass flasks and tested under two different conditions: at room temperature in ambient light, and incubated in a heating oven at 40 °C. Quantification of the samples was performed at specific time intervals: 0, 2 and 4 weeks.

3. Results

The mushroom sample was characterized through elemental analysis to determine its carbon, hydrogen, nitrogen, and sulfur content. The results showed that the sample contained 39.23% carbon, 6.38% hydrogen, 0.18% sulfur, 4.96% nitrogen, and 22.57% proteins. Different NADES were prepared and the extraction of phenolic compounds was performed for the selected NADES and the TPC was determined. The results showed that NADES 1 and NADES 2 in study presented better results than the conventional hydroalcoholic extract. The yield was two times higher, and there were no significant differences between the two NADES. The TPC was quantified over a span of 4 weeks, as shown in Table 1.

Table 2. Stability assays: TPC for Mushroom Extracts

Sample	Storage Temp.	Time	TPC (%)
AqEtOH	40°C	**0 weeks**	100.0
		2 weeks	56.7
		4 weeks	42.0
	Room Temp.	**0 weeks**	100
		2 weeks	65.2
		4 weeks	52.2
A	40°C	**0 weeks**	100
		2 weeks	94.1
		4 weeks	78.9
	Room Temp.	**0 weeks**	100
		2 weeks	83.9
		4 weeks	81.2
B	40°C	**0 weeks**	100
		1 weeks	114.4
		2 weeks	136.6
	Room Temp.	**0 weeks**	100
		1 weeks	124.4
		2 weeks	128.4

4. Discussion

The elemental analysis of the dried *Agaricus Bisporus* is in concordance with the previous studies. [16][17][18] The sample shows a high carbon content, primarily due to carbo-hydrates such as chitin and glucans, as well as other organic compounds. According to the literature, carbon levels typically range between 38.3% and 48.9%.[14][16] Hydrogen is present as a component of both water and organic compounds, while nitrogen is mainly found in amino acids and proteins. Protein content in mushrooms is significantly influenced by the nitrogen-to-protein (NP) conversion factor used. In mushrooms, a notable portion of nitrogen exists in non-protein forms, such as chitin and free aminoacids. As a result, the commonly applied NP factor of 6.25 tends to overestimate the actual protein content. Therefore, a more accurate conversion factor of 4.55 was used in this analysis.[14] Mushrooms are considered a valuable source of protein, and the protein content obtained in this study is consistent with previously reported values, which range from 11% to 29.14%.[16]

The total phenolic content (TPC) in *Agaricus bisporus* can vary widely depending on the type of organic solvent used (such as ethanol, methanol, or acetone) and the specific extraction conditions, including temperature, solid-to-liquid ratio, and extraction time. When compared to previous studies that employed ethanolic solutions, the obtained TPC values are consistent with the results obtained in this study. [19][20] NADES 1 and NADES 2 had higher yield of phenolic compounds when compared to the conventional hydroalcoholic extract. The yield was two times higher, and there were no significant differences between both NADES.

Mushroom species like *Agaricus bisporus* have been shown to contain phenolic compounds with potent antioxidant properties, making them promising alternatives for the development of novel natural antioxidants. The results of this study further demonstrate that using natural deep eutectic solvents (NADES) as alternative extraction media can enhance the TPC. This

improvement may be attributed to the chemical affinity between phenolic compounds and the components of the selected NADES. These solvents have proven to be more effective than conventional solvents in increasing the solubility of natural products, boosting their bioactivity, and enhancing both the stability and shelf-life of extracts.[16] [21]

One of the main objectives of this study was to assess the long-term stability of extracts prepared with NADES under both normal and extreme conditions. Conventional extracts exhibited poor stability over time, after two weeks, the EC_{50} could no longer be determined, indicating a significant reduction in TPC, likely due to the degradation of phenolic compounds, which are known to be heat-sensitive. The TPC decreased more than 50% over time. In contrast, the two NADES-based extracts maintained the TPC stability over a four-week period, with variations lower than 20%. Over time the EC_{50} remained stable for both NADES, with variations considered normal and non-significant. These results are in agreement with previous studies that reported the ability of NADES to stabilize bioactive compounds under various storage conditions.[4]

5. Conclusion

This study demonstrated that NADES-based extracts have valuable properties that support their application in cosmetic formulations. Compared to conventional hydroalcoholic extracts, the mushroom extracts obtained with NADES showed greater stability over time. The stability studies conducted under various storage conditions suggest that NADES can effectively stabilize the phenolic compounds present in mushrooms. These findings indicate an extended shelf life for the extracts, making them a powerful asset for the cosmetic industry. Moreover, NADES offer a sustainable and natural alternative, aligning with current trends toward eco-friendly and innovative formulation strategies.

6. References

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