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Optimization of microwave-assisted extraction of ergosterol from *Agaricus bisporus* L. by-products using response surface methodology

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ABSTRACT

This work intends to valorize by-products of the industrial processing of mushrooms to obtain ergosterol as a value-added compound. *Agaricus bisporus* L. is the world's most consumed mushroom and one of the richest sources of ergosterol. Microwave-assisted extraction was used to replace conventional techniques that are time-consuming and need large amounts of solvent. Time (3–20 min), temperature (60–210 °C) and solid-liquid ratio (1–20 g/L) were found the relevant variables to analyze the extraction process. To maximize the ergosterol extraction yield, response surface methodology was used to optimize the process. The global optimal extraction conditions were determined and comprise: 19.4 ± 2.9 min, 132.8 ± 12.4 °C and 1.6 ± 0.5 g/L, yielding 556.1 ± 26.2 mg of ergosterol per 100 g of mushroom by-products. In the MAE optimal conditions, it was possible to obtain ergosterol in a similar value to the one obtained in other works when using the Soxhlet extraction method with a significant decrease in the time of extraction. The results show the potential of using the by-products of an agroindustry, mushrooms processing industry, as productive sources of ergosterol.

1. Introduction

Among two thousand species of edible mushrooms, only a handful is worldwide cultivated and processed at industrial level, in which *Agaricus bisporus* L. is included (Chou et al., 2013; Leiva et al., 2015; Royse, 2014). During the mushroom's manufacturing process a large amount of by-products is generated (Wu and Zivanovic, 2004). Some examples are the volva and bottom part of the stems due to their tough texture, or the organic matter present in the effluent generated in the washing and blanching stages. In addition, during mushrooms cultivation and harvest, the specimens with irregular dimensions and shape are discarded.

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Apart from the large amounts of the mentioned solid wastes, there is also a surplus production leading to a glut in the market, distress sale and low profit to the growers. Depending on the size of the mushroom industry, the amount of by-products ranges between 20 to 35% in weight of fresh mushrooms (Gil-Ramírez et al., 2013; Vasylenko et al., 2008). These by-products with a high nutritional value are being wasted, but also demand industries to deal with their environmental impact and associated managing costs (Leiva et al., 2015). Current industrial strategies for mushroom's by-products are based in low-economic income solutions (*e.g.*, animal feed and compost). Since mushrooms production/consumption rates are expected to increase in the next years, the environmental problem is expected to growth proportionally and new alternative and profitable solutions need to be explored (Royse, 2014). Several approaches have been postulated, including the production of biofuel, chitin and chitosan, β-glucan and sterols (Chou et al., 2013; Gil-Ramírez et al., 2013; Vasylenko et al., 2008; Wu and Zivanovic, 2004). However, innovative research is crucial to proper evaluate the most feasible and economically viable solutions for theses raising by-product materials from fungal sources.

Among the possibilities, ergosterol obtainment is quite attractive. It is the most abundant mycosterol, especially in *Agaricus bisporus* L. (90% of its sterols fraction) (Barreira et al., 2014; Gil-Ramírez et al., 2014), and has been related with different bioactive properties (Barreira and Ferreira, 2015; Villares et al., 2012). Moreover, ergosterol can be converted by irradiation, into vitamin D for sale as a dietary supplement and food additive (Asinghe and Perera, 2005; Teichmann et al., 2007).

A broad spectrum of solid liquid extraction techniques is widely used for the extraction of natural products. Conventional methods used for many decades include Soxhlet extraction, maceration, and percolation, among others. They are often time-consuming and require large quantities of solvents, including hazardous ones (Wang et al., 2013). Emerging technologies, such as supercritical fluid extraction, microwave-assisted extraction (MAE), pressurized solvent extraction (PSE) and ultrasound-assisted extraction (UAE) are fast and efficient extraction technologies that have been used in the last decades by the food processing industries and researchers with the purpose of extracting more efficiently major and minor compounds present in natural matrices, saving energy and reagents, avoiding losses and optimizing the extracting yields (Gil-Ramírez et al., 2013; Heleno et al., 2016; Villares et al., 2014).

According to literature data, the extraction yields of ergosterol can vary widely depending on the type of solvent, extraction time and technologies applied. Due to the low ergosterol concentration present in the cell membranes, it is necessary to study its performance during the extraction. Classical methods to optimize the process variables involve changing one variable at a time, keeping the others at fixed levels (Prieto et al., 2011). Single factor analysis are laborious and time-consuming methods and often does not guarantee the determination of the optimal conditions (Box et al., 2005; Wang et al., 2013). On the other hand, carrying out experiments using every possible combination of the test variables is impractical due to the associated large number of experiments required (Rodríguez-Nogales et al., 2007). One viable strategy consists on selecting, firstly, the variables playing a significant role in the extraction yield, and then applying the statistical multiresponse optimization using a response surface methodology (RSM).

The present study aimed at optimizing the MAE process to extract ergosterol from *A. bisporus* discarded by-products having in view applications in food, pharmaceutical and cosmetic industries. To the author's best knowledge, there are no other reports in the literature describing MAE optimization for ergosterol extraction. This technology has been applied mostly for phenolics and phytosterols extraction (Mustapa et al., 2015; Roselló-Soto et al., 2015). The results obtained in the present study will be compared with the ones obtained by our research group in the optimization of UAE in comparison with the conventional extraction technique, Soxhlet extraction (Heleno et al., 2016).

By means of RSM, the joint effect of the variables time (t), temperature (T) and solid/liquid ratio (S/L) on ergosterol extraction yield was described. With this study, where individual and interactive effects among variables were studied, the authors expect to give a contribution towards the understanding of the real potential of ergosterol extraction and related industrial applications.

2. Materials and methods

2.1. Samples

The *Agaricus bisporus* L. discarded by-products were obtained from a local mushrooms production company, Mogaricus Cogumelos - Sociedade Unipessoal Lda. All the samples were weighted, lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas City, MO, USA), and reduced to a fine dried powder (20 mesh) (Ultra Centrifugal Mill ZM 200, Porto, Portugal).

2.2. Standards and reagents

Methanol and acetonitrile of HPLC grade from Fisher Scientific (Lisbon, Portugal) were used. The standards of sterols (ergosterol, cholecalciferol) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA). All other chemicals and solvents were of analytical grade and purchased from common sources.

2.3. Settings and procedure for ergosterol microwave-assisted extraction

MAE process was performed using a Biotage Initiator Microwave (Biotage® Initiator+, Uppsala, Sweden) using closed vessels. The lyophilized powdered samples were extracted at different t, T and S/L ratio in the range defined by the RSM design. Before the extraction, an adequate volume of cholecalciferol (internal standard) was added to each sample. The main internal settings of the device during the MAE process were as follows:

1) Because pressure and T are correlated; moreover, the power applied is also linked to the t needed to reach the selected T or pressure. In consequence, the T variable was selected as the main controlled one and power variable was set to the maximum one (400 W) in order to ensure that the t to reach the selected T is the minimum one (less than 20 s for the highest T used). Therefore, to maintain T constant, the power would not be applied constantly along the duration of the experiment.