Supercritical CO_2 Extraction of secondary metabolites from *Agaricus blazei.* Experiments and modelling.

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The mycelium and young fruiting bodies of *Agaricus blazei* were submitted to supercritical CO_2 extraction, in a modified commercial flow apparatus, at temperatures from 40 to 80 ºC, pressures up to 600 bar and flow-rates from 2.0 to 9.0 g CO_2.min^{-1}.
The best extraction conditions of secondary metabolites, whereby the degree of solubilization (g extract/100 g of fungi) is the highest, was obtained with pure CO_2 at 400 bar, 70 ºC and a flow rate of 5.7g CO_2.min^{-1}.
In order to increase the extraction yield of secondary metabolites, which are mostly present in glycolipid fractions, a polar compound (ethanol) was used as co-solvent in the proportions of 5 and 10 % (mol/mol). The presence of ethanol increased the yield when compared with the extraction with pure CO_2. Moreover, a simple model was applied to the supercritical CO_2 extraction of secondary metabolites from *Agaricus blazei*.

INTRODUCTION

The fungi *Agaricus blazei* is a basidiomycete native from Brazil which has been used as nutraceutical due its medicinal properties. The antitumoral properties of the fungi were reference in several works [1,2,3]. Extracts obtain with hexane dichloromethane and methanol has been study recently [4]. The isolation and characterization of different components from Agaricus *blazei* were also obtain [5].
Supercritical fluid extraction is a separation technique where it is possible to obtain valuable lipids. Carbon dioxide is the most used supercritical solvent, because the obtained extracts are toxic solvent free and the degradation of thermal labile components is avoided due to the moderate temperature used in the process [6].
The objectives of this work are to carry out the supercritical CO_2 extraction of lipids, from *Agaricus blazei*, and to assess the influence of several parameters, namely pressure, temperature and flow rate.

MATERIALS AND METHODS

The supercritical fluid extraction experiments were performed in a flow apparatus (Figure 1). This equipment allows carrying out studies at a temperature up to 120ºC and a pressure up to 600 bar.
The liquid CO_2 flowing from the cylinder was compressed to the desire pressure (Applied Separations, Spe-ed™ SFE) into the extraction vessel, which is heated. Then CO_2 comes through a bed of glass spheres, propylene wool, sample, propylene wool and bed of glass spheres. The total volume of CO_2 was determined with a mass flow meter GFM and a totalizer (AALBORG). The CO_2 (99.995% purity) was supplied by Air Liquide (Portugal).
The sample, *Agaricus Blazei* mushroom supplement powder, was purchased (MRL – Mycology Research Laboratories, Ltd.) and appears as small spherical beans less than 0.3mm medium diameter, homogeneous and lyophilized.

Supercritical fluid extraction was carried out using 10g of biomass weight in an analytical balance (Sartorius CP224S). Conditions of extraction were: CO_2 flow rates of 2.0 g.min^{-1}, 5.7 g.min^{-1} and 9.0 g.min^{-1}, pressures up to 600 bar and temperatures up to 80 ºC. The conditions were supervised during all experiments. The extracts were collected in a U tube, at the atmospheric pressure and a temperature controlled with an ice bath. The amount of extract obtained was assessed gravimetrically.

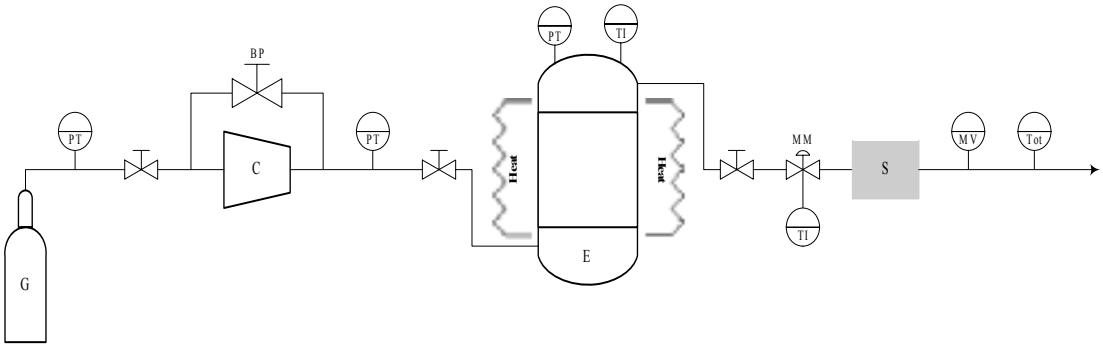

Figure 1:Diagram of the supercritical fluid extraction apparatus.G, CO_2; C, compressor; E, extractor; S, separator; BP, back-pressure regulator; MM, micrometer valve; MV, flow meter; Tot, totalizer; TI, temperature indicator, PT, pressure indicator.

RESULTS AND CONCLUSIONS

The biomass of *Agaricus blazei* was submitted to supercritical fluid CO_2 extraction at the following conditions: flow rates from 2.0, and 9.0 g.min^{-1} of CO_2, at a pressure of 400 bar and temperature of 70 ºC. Yield in lipids, collected at regular time intervals are shown in Figure 2 as a function of the CO_2 mass.

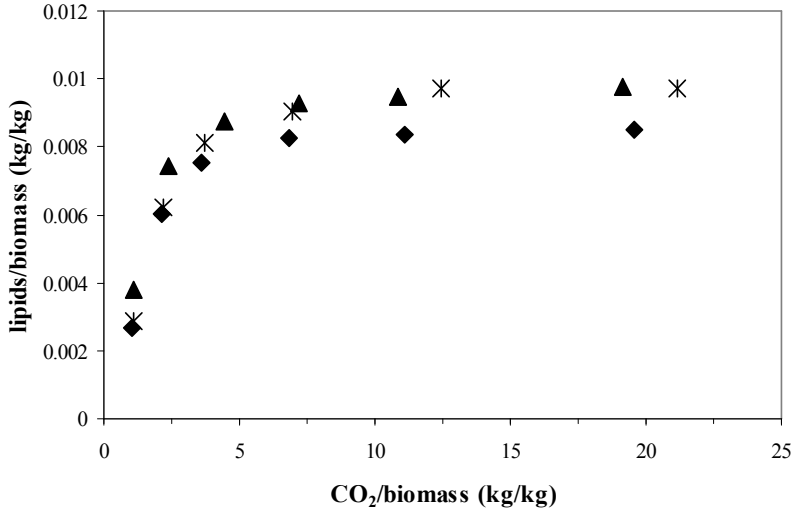

Figure 2: Yield of extracted lipids from *Agaricus blazei* as a function of carbon dioxide mass at 400 bar and 70 ºC, at several flow rates of CO_2: ◆ - 2.0 g.min^{-1}, ▲ – 5.7 g.min^{-1}, ✸ - 9.0 g.min^{-1}.

It can be seemed that the increase of flow rate increases the yield, but after 5.7 g.min^{-1} no significantly evaluation can be detected.

To assess the effect of pressure and temperature, at a flow rate of 5.7 g.min^{-1} experiments were carried out up to 600 bar and temperatures up to 80 ºC. The results are shown in Figure 3 and Figure 4.

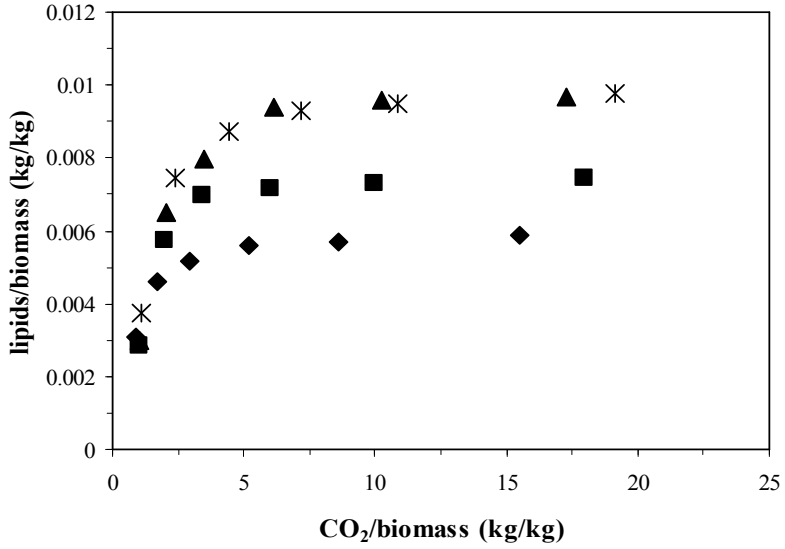

Figure 3: Yield of extracted lipids from *Agaricus blazei* as a function of carbon dioxide mass at 70 ºC and CO_2 flow rates of 5.7 g.min^{-1} for different pressures ◆ - 300 bar, ■ - 350 bar, ✸- 400 bar and ▲- 600 bar.

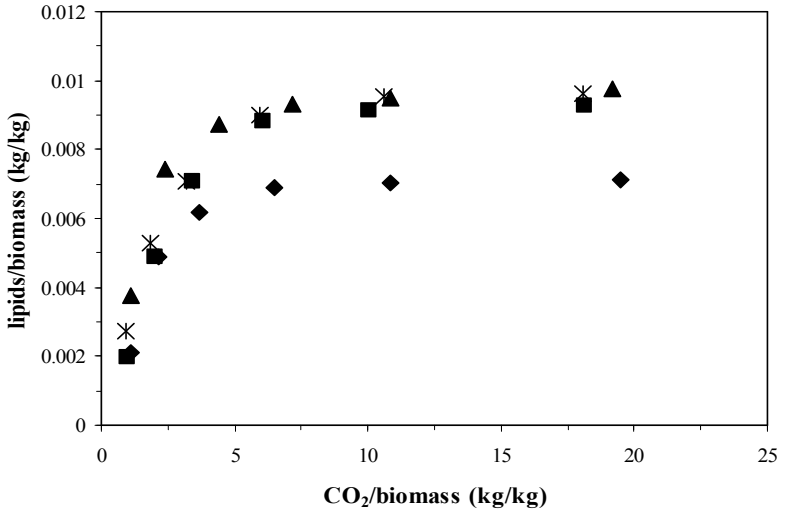

Figure 4: Yield of extracted lipids from *Agaricus blazei* as a function of carbon dioxide mass at 400 bar and CO_2 flow rates of of 5.7 g.min^{-1} for different temperatures ◆ - 40 ºC, ■ - 60ºC, ▲ –70 ºC and ✸- 80 ºC

At constant temperature and flow rate, the yield increased with the pressure (Figure 3) until 400 bar. The pressure improved the solvent power of the supercritical fluid, due to the

increase of its density, but it seems that the higher solubility of the lipids were obtain at 400 bar, since no improved in the yields of extracted lipids were obtained after that.
On the other hand, at constant pressure and flow rate, the yield increased with the temperature until 70 ºC (Figure 4). In fact, the temperature increases the vapor pressure of the lipids, leading also to its higher solubility. From the results that effect, seems to be more important until the temperature of 70 ºC.
With the aim of increasing the extraction yield in lipids from the dried biomass, a polar compound (ethanol) was used as co-solvent in the proportions of 5 and 10 % (mol/mol). The extractions were performed at 400 bar, 70ºC and flow rate of CO_2 of 5.7 $g.min^{-1}$. The maximum yields obtain were 0.011 and 0.013 kg of lipids by kg of biomass, respectively.
A empirical model based on a function of the Langmuir gas adsorption isotherm type was used: $Y = (Y_\infty\ t)/(B + t)$. Y_∞ is the yield after an infinite extraction time; t is the time extraction and B a constant.
Figure 5 shows experimental data and curves calculated with model. As shown, simple model previsions do fit with experimental data.

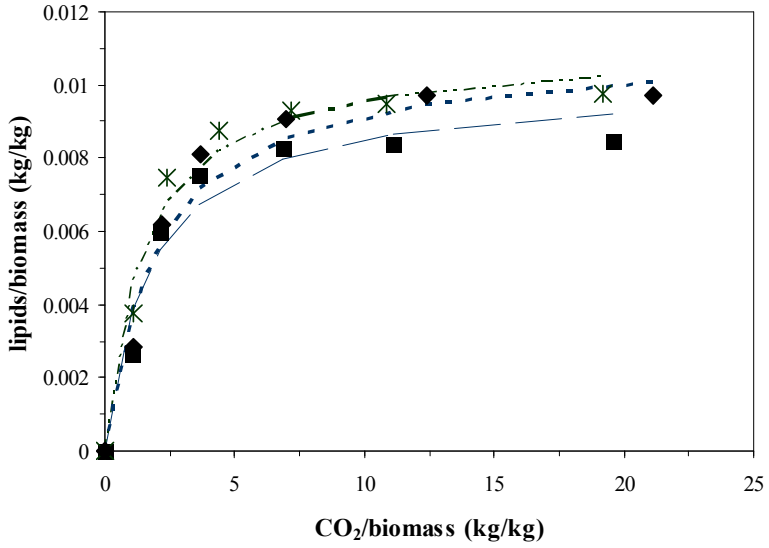

Figure 5: Yield of extracted lipids from *Agaricus blazei* as a function of carbon dioxide mass at 400 bar and 70 ºC, at several flow rates of CO_2: Experimental: ▲ - 2.0 $g.min^{-1}$, ✸– 5.7 $g.min^{-1}$, ◆- 9.0 $g.min^{-1}$; lines represent the model used.

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