EXTRACTION OF CAROTENOIDS FROM YEAST BIOMASS USING DEEP EUTECTIC SOLVENTS

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ABSTRACT

Carotenoids are colored molecules that can present antioxidant, anti-inflammatory, anti-obesity and anti-cancer properties, making them highly sought after by various industry sectors, such as food, pharmaceutical and cosmetical. In this study, the carotenoid extract comes from the red yeast *Rhodotorula glutinis*. This yeast accumulates these molecules inside its cells, requiring a cell rupture process for its removal. Conventional extraction methods use volatile organic solvents, which can present toxic, mutagenic and carcinogenic properties. Additionally, these solvents can destabilize the carotenoids, consisting in an obstacle for the industries. To achieve efficient and safe carotenoid extraction, it is considered the use of deep eutectic solvents (DES), promoting a sustainable green extraction process. So, this work analyzed the extraction of intracellular carotenoids from *R. glutinis* biomass using DES.

Keywords: Microbial carotenoids. Industrial applications. Deep eutectic solvents. Green extraction.

1 INTRODUCTION

Carotenoids are natural pigments, ranging in color from orange, yellow and red. These molecules can promote important biological properties, especially antioxidant, anti-inflammatory, anticancer and anti-obesity. Biotechnological carotenoids are obtained as secondary metabolites synthetized by microalgae, bacteria, filamentous fungi and yeast, and have been explored by industries for applications in food, feed, pharmaceutical and cosmetical products[1], reducing the negative impact of synthetic colorants in humans and environment, consisting in a more eco-friendly alternative[2].

When produced by the yeast *Rhodotorula glutinis,* these pigments are intracellular, which means that, for its recovery, it is necessary to perform cell rupturing methods[3]. One common method for extracting carotenoids from microbial biomass is solid-liquid extraction using volatile organic solvents (VOCs). These substances present toxicity and can prejudice the commercialization of products with carotenoids in its composition, due to the carotenoids molecular destabilization besides the loss of color and biological properties[4]. Therefore, deep eutectic solvents (DES) which consists in a mixture of a hydrogen bonding acceptor substance, such as L-menthol,[5,6] and a hydrogen bond donor, such as organic acids, come as a more sustainable alternative for carotenoids extraction. These compounds are classified as green solvents, non-polluting and safer when considering the application of the extracted molecules in industrial products[7].

The objective of this work was to evaluate the stability of carotenoids produced by *R. glutinis* in the presence of DES, followed by its application on the extraction process, aiming to establish an eco-friendly methodology compared to the ones already applied and available industrially.

2 MATERIAL & METHODS

A priori, a total of six DES was synthetized using L-menthol as hydrogen bonding acceptor and several organic acids as hydrogen bond donor. The organic acid was chosen and evaluated by the thermodynamic predictive model COSMO-SAC, as shown in Table 1.The synthesis was performed as described in Figure 1, adapted from Li *et al.* (2022) [8].

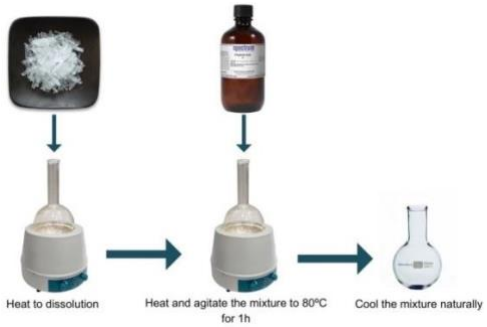

Figure 1 Synthesis of DES.

The *R. glutinis* CCT 2186 yeast was acquired from the Tropical Culture Collection André Tosello (Campinas, SP, Brazil), which was isolated from the leaves of a kaki fruit (Diospyros). The production of biomass used in this project was based on the methodology described by Mussagy *et al*. (2021),[9] and illustrated by Figure 2.

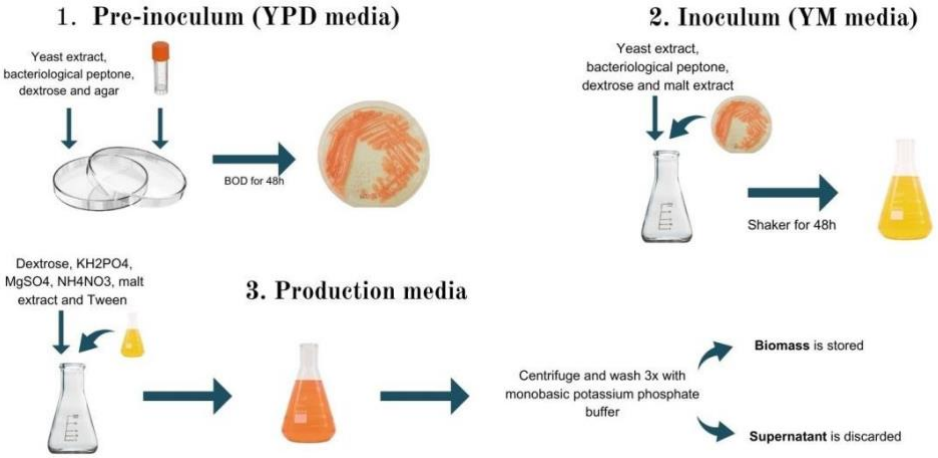

Figure 2 Production of *R. glutinis* biomass containing the carotenoids by submerged culture.

The obtained biomass was divided for two different procedures. In the first one, the produced carotenoids were extracted from the biomass by a methodology described and adapted by Mussagy et al. (2019),[10] which consists in a series of extractions by macerating the freeze-dried biomass with acetone. Subsequently, the acetone was evaporated using a rotaevaporator equipment, and the final dry carotenoid extract was stored for future stability tests in contact with DES. The second procedure used the dry biomass in extraction tests. The produced carotenoids were extracted from the biomass by a methodology described and adapted by Silva *et al.* (2023),[11] which consists in a series of extractions in a stirrer hot plate mixer at 65 °C during 5 minutes. Extraction was performed by aqueous solutions (50 % v v^{-1}) of all synthetized DES, in contact with freeze-dried biomass.

3 RESULTS & DISCUSSION

The synthetized DES were L-menthol:propionic acid, L-menthol:octanoic acid, L-menthol:acetic acid, L-menthol:decanoic acid, L-menthol:hexanoic acid and L-menthol:dodecanoic acid. These compounds were characterized by nuclear magnetic resonance (NMR) spectroscopy, and the results were compared to the ones found in literature [12,13] as shown in Figure 3. From the NMR can be seen that the DES was successfully synthesized.

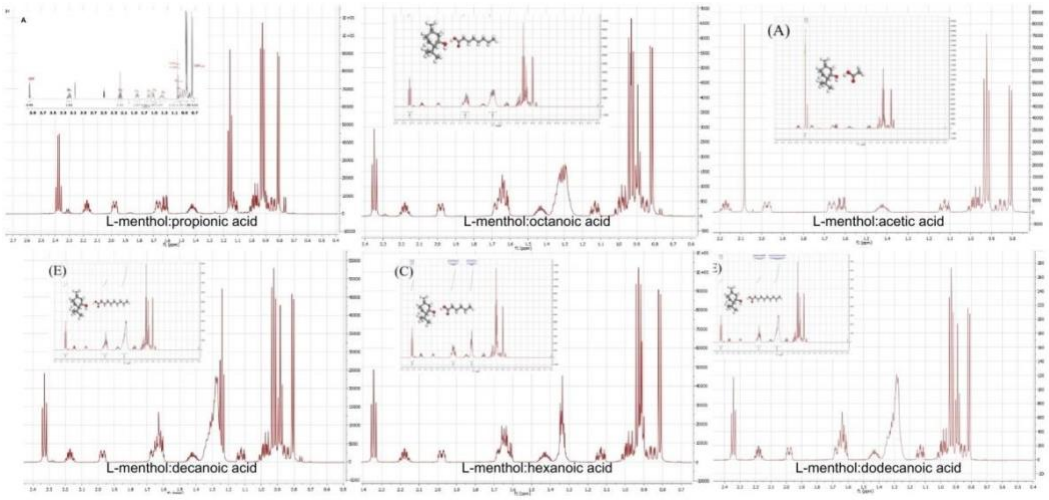

Figure 3 Results of the NMR spectra for the synthetized DES.

Among the carotenoids present in the *R. glutinis* biomass, special attention has been given to torularhodin due to its high antioxidant activity. So, DES were chosen based on torularhodin infinite dilution activity coefficient (γ) as presented at Table 1. The natural logarithm of γ is inversely proportional to the molecule solubility in solvents. The more negative the ln (γ) the higher the compatibility between solute and solvent. In this way, DES with negative value of ln γ was chosen for extraction studies. The results of the extraction using aqueous solutions of DES are exhibited in Figure 5.

Table 1 Natural logaritm of activity coefficient (γ) from torularhodin at 298 K calculated using the JCOSMO software.

DES	Ln γ
L-menthol:propionic acid	- 0.9001
L-menthol:octanoic acid	- 0.5762
L-menthol:acetic acid	- 0.4709
L-menthol:decanoic acid	- 0.4670
L-menthol:hexanoic acid	- 0.4102
L-menthol:dodecanoic acid	- 0.2966

As can be seen from Figure 4, acetone promoted the highest extraction of torularhodin. This solvent is used in the literature to extract carotenoids from yeast biomass and this solvent present an ln γ of -7.1192. However, this solvent present several problems considering a sustainability approach such as contribution to air pollution and the formation of ground-level ozone. A component of smog, acetone exposure can cause health issues, including respiratory problems, skin irritation, and neurological effects besides acetone is highly flammable. Considering the carotenoids extract can be used in food and pharmaceutical products is important to replace this solvent. In this sense, the DES L-menthol:hexanoic acid presents a potential to extract the carotenoids in a solid-liquid procedure considering that in this experiments it was used only 5 minutes of biomass contact with the solvent.

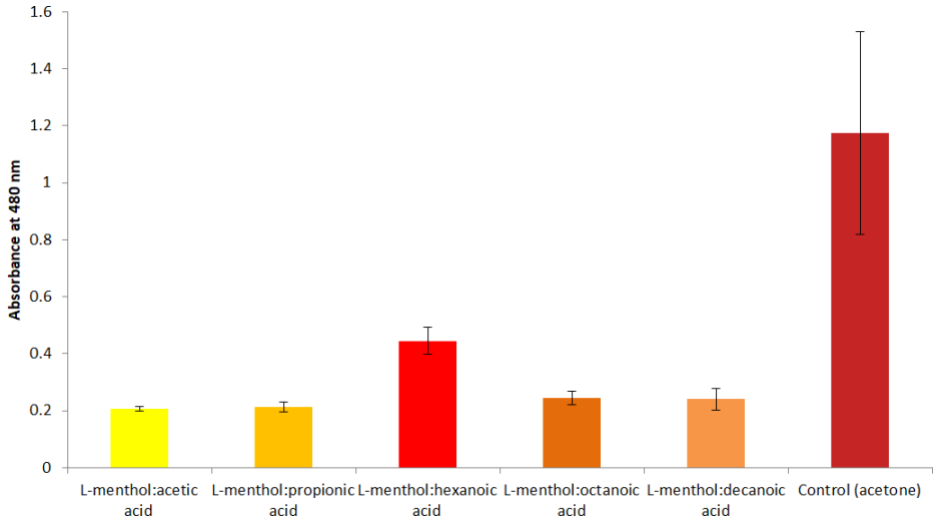

Figure 4 Results of torularhodin extraction from *R. glutinis* biomass using aqueous solutions of DES (50 % v v^{-1}) and acetone (standard). The error bars represents standard deviations from 3 cycles of extractions.

4 CONCLUSION

The aqueous solution of hexanoic acid was satisfactorily in the process of carotenoid extraction, and, to improve its capacity, variants such as temperature, number of cycles, and solid-liquid ratio will be changed, in order to discover the best conditions for solid-liquid extraction of carotenoids; In the final steps of this work, stability of carotenoid extract will be analyzed in the presence of L-menthol:hexanoic acid.

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