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Ultrasound-assisted extraction of carotenoids produced by *Rhodotorula araucariae* using manipueira as substrate

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Carotenoids are lipophilic bioactive compounds with a range of health benefits present in plants, animals and microorganisms. *Rhodotorula* yeasts synthesize carotenoids inside their cells via fermentation under certain conditions of stress in the environment and the extraction of these compounds from their cell compartment may represent a challenge for industrial use. Therefore, the aim of this work was to determine the optimized conditions for the extraction of carotenoids of *Rhodotorula araucariae* biomass through ultrasound-assisted extraction (UAE). In this study, the biotechnological production of carotenoids was carried out using manipueira as low-cost substrate. Manipueira was extracted after pressing the crushed cassava roots followed by concentration to 12 °Brix by boiling (≈100°C). The concentrated *manipueira* was sterilized in autoclave (121°C for 15 min) and stored at -20°C until use. The *R. araucariae* yeasts (CCT 2185) was reactivated (YEPD agar), incubated at 28°C for 120 h and inoculated into the concentrated manipueira in a bench-top bioreactor. The fermentation process was carried out for 5 days at 35°C, with shaking (150 rpm), pH 5.0. and presence of intense fluorescent light (2086 lux). The optimized conditions for extracting carotenoids from the biomass was determined by response surface methodology with a central composite rotational design 2^3 varying the time of ultrasound exposure (1.6 to 8.4 min), solid/liquid ratio (1:16 to 1:184, w/v) and number of extraction cycles (1 to 5 times), using ethanol. Manipueira presented high-water content (89.6 ± 0.2%) and high total sugars level (7.21 ± 0.05%). The best condition for the UAE of carotenoids from *R. araucariae* biomass were 7 extractions of 4 min each in a solid-liquid ratio of 1:150 (w/v) resulting in a total carotenoid contents of 616 ± 31 µg/g (dry biomass), which was 35% higher than the levels obtained by the conventional maceration technique with acetone (456 ± 11 µg/g). During the optimization, the solid-liquid ratio and the number of extractions showed the greatest influence on the total carotenoid contents. In addition, the liquid extract obtained in the best UAE condition (0.50 ± 0.05 µg carotenoids mL^{-1}) was able to scavenge about 12.67% of ABTS radicals. Therefore, UAE allowed the extraction of high yields of carotenoids from the biomass of *Rhodotorula araucariae* providing an extract with high antioxidant potential to be investigated for the use in both food and physiological systems.

Keywords: Challenges in the analysis of bioactive compounds, *Rhodotorula*, response surface methodology, biotechnology, fermentation.

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