Evaluation of different methodologies to extract colorants from microbial cell wall

Patrícia R.H.¹, Maria F. S. T.², André G.S.¹, Valéria C. S. E.¹

¹ UNESP- Univ. Estadual Paulista (Rodovia Araraquara-Jaú, km, 1,0 - Campus Ville- 14801-992- Araraquara - SP); ²Culture Collection DPUA/UFAM. Federal University of Amazonas (Av. Gal. Rodrigo Octávio Jordão Ramos, 3000, 69077-000, Manaus, AM).

Abstract:
The colorants are used in industrial sectors such as additives to enhance or restore the appearance of products. Currently there is a tendency to use natural colorants, which can be produced by microorganisms as intra- and extracellular compounds. The aiming of this work was to evaluate combined extraction methods to recovery intracellular colorants presented in cell wall of Penicillium purpurogenum. To this purpose, the production process occurred at orbital skaker using P. purpurogenum in CYA liquid medium during 216 hours at 150 rpm/30ºC. The fermented broth was filtered and the biomass retained in the filter was used in the extraction studies. It was analyzed six methods of extraction in triplicate as follow: (a)Extraction using acetone; (b)Extraction using methanol-dichloromethane-ethyl acetate and ultrasonic lysis; (c)Extraction with ethanol 70% and ultrasonic lysis; (d)Extraction with ethanol 70%, ultrasonic lysis and temperature; (e)Extraction using 90% methanol; (f)Extraction using ethyl acetate, isopropanol and ultrasonic lysis. All extractions were filtered at the end of the process, and then the absorbance was measured at 400, 470 and 490 nm, which represents the wavelengths absorption maxima for yellow (YC), orange (OC) and red colorants (RC), respectively. The results were expressed in Units of Absorbance (UA). The extraction using acetone was not effective since it not caused a disrupt of cell wall. All the others extractions presented suitable results. The best results of colorants extraction decreased in following sequence: d>b>e>c>a. In the methodology (d) the extraction resulted in 0.50 UA₄₀₀nm for YC, 0.42 UA₄₇₀nm for OC and 0.42 UA₄₉₀nm for RC. In this condition the cell wall was disrupt by ultrasonic lysis and the colorants, as a polar molecule, had good attraction by the solvent. Furthermore, the shaker provided greater contact between the solvent and the cell membrane, resulting in larger amount of colorants extracted. In this way, the colorants extraction from cell wall is dependent of solvent polarity and the integration of this process with ultrasonic lysis and/or temperature can improve the results. Anyway, aiming to obtain high amounts of natural colorants to be used in industrial products is of great interest combine the extracelullar colorants with intracellular ones and this work evaluated different methodologies to achieve this purpose.

Keywords: intracellular colorants, microbial cell wall, extraction