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Study of the carotenoid production kinetics by oxidative stress in *Sporobolomyces ruberrimus* and pigments extraction techniques

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Carotenoids are natural pigments produced by a wide variety of bacteria, yeast, filamentous fungi, plants, and algae. These pigments are natural antioxidants and represent a group of valuable molecules for applications in the pharmaceutical, chemical, food and feeding industries. Carotenoids are synthesized by *Sporobolomyces ruberrimus* in the intracellular environment and cell wall rigidity limits the extractability of such compounds, making the application of methods for recovery of these pigments necessary. The objective of this study was to study the carotenoid production kinetics in *Sporobolomyces ruberrimus*, seeking to enhance torularhodin biosynthesis and investigate pigment extraction methods (mechanical, chemical and high pressure). Three concentrations of dissolved oxygen (20%, 50%, and 80%) were used in submerged batch cultivation in bioreactor. The cell morphology, after the application of these methods, was verified by scanning electron microscopy. Besides it, after 72 h of fermentation, the biomass was concentrated and lyophilized for the application of supercritical extraction, a recent technique applied for the extraction of pigments. HPLC analyses were used for carotenoids identification and quantification. Results showed that the best condition for torularhodin biosynthesis (11.95 mg.L-1) was 50% of dissolved oxygen, with X_{max} 13.13 $g.L^{-1}$, μ_{max} of 0.12 h-1, a maximum production of carotenoids total of 30.61 g.L-1 and productivity of metabolites of 0.42 $mg.L^{-1.h-1}$. The use of supercritical extraction using lyophilized cells pretreated by soaking with liquid nitrogen and the use of ethanol as co-solvent proved to be the best treatment in terms of yield (3.11%) and extracted carotenoids (28.99 $\mu g.g^{-1}$ of dry cell).

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Direct analysis of some fatty acids in food oils using ULPC technology

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A simple, fast, highly efficient and direct method using ultra-performance liquid chromatography coupled to mass spectrometry has been established for the simultaneous separation, identification and quantitation of a few saturated and unsaturated fatty acids in olive oils from various countries. No sample pretreatment techniques were employed such as extraction or derivatization for the analysis of target acids from oil samples, as the oil samples were just diluted, filtered and then directly injected to the instrument. The chromatographic separations of all target fatty acids were achieved on a Hypersil Gold C18 column of particle size 1.9 µm, 50×2.1mm I.D, while the gradient elution using a binary mobile phase mixture of acetonitrile and water at a flow rate of 1.5 mL/min was adopted for achieving optimum separations. The identification and quantitation of target compounds was accomplished using selected ion reaction monitoring mode. The recoveries of the fatty acids were obtained higher than 89% with good validation parameters; linearity (r2>0.992), detection limit between 0.09 and 0.24 µg/ml, run to run and day to day precisions with percent relative standard deviation lower than 2.4% at both low (1 µg/ml) and medium (10 µg/ml) concentration levels. The total content of fatty acids in each individual oils was found in the range of 472.63 to 7751.20 µg/ml of olive oil, while oleic acid was found to be the major fatty acid among all analyzed oils with the amount 3785.94 µg/ml (maximum) in Syrian olive oil. The obtained validation parameters confirm that the proposed analytical method is rapid, sensitive, reproducible and simple and it could be applied for the successful evaluation of fatty acids in various oils and other matrices. All the fatty acids were efficiently eluted in a time of less than 8 min with well resolved peaks by employing the proposed method.

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