Optimization of key growth parameters involved in biomass production of Pavlova gyrans and their effects on the fatty acid profile and the protein content

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Microalgae have been presented as an interesting source of bioactive compounds for human consumption in several industries, such as nutraceutical, pharmaceutical or cosmeceutical. Their rich composition in anti-inflammatory and antioxidant compounds (e.g., polyunsaturated fatty acids (PUFAs), proteins) have shown protective effects against several human diseases (e.g., cardiovascular, cancer). Despite their increasing popularity, current limitations related to microalgae production hinder their cost-effectiveness and their widespread application and consumption, falling short of the market demand. Among the main bottlenecks, it is possible to highlight the proper control of the growth conditions targeting the improvement of both biomass production and biochemical composition, according to the microalga species/commercial application.

This work aimed at maximizing the biomass production of the marine microalga *Pavlova gyrans*, commonly used in aquaculture for feed application due to its high content of n-3 fatty acids and phytosterols, through a two-step approach. Firstly, a Plackett-Burman design (PB) was used to identify the most significant abiotic factors ($p < 0.10$), among the seventeen tested. Then, a central composite rotatable design (CCRD) was applied to optimize the key parameters found in PB experiments, being the optimal formulation validated against the control conditions (Walne´s medium). The cultures resulting from the CCRD assays were further characterized regarding their content of protein and lipids, as well as the fatty acid profile.

The PB design identified light intensity, NaNO3 (both with positive effects), and CuSO4.5H2O (negative effect) as the critical parameters ($p < 0.10$) involved in the biomass productivity of *P. gyrans*. These variables, together with NaH2PO4.H2O – a biologically important nutrient with considerable significance ($p = 0.13$) - were optimized in the CCRD design, which determined 700 µmol.photons.m^{-2}.s^{-1} of light intensity, 1500 mg.L^{-1} NaNO3, 6 µg.L^{-1} CuSO4.5H2O, and 40 mg.L^{-1} NaH2PO4.H2O as the optimal conditions for enhanced maximum biomass production (*Xmax*). Compared to the Walne's medium, the optimal formulation allowed a 3.8-fold increase ($p < 0.05$) in *Xmax* (2.26 g ash free dry-weight.L^{-1}), along with improved biochemical composition (p =0.05), namely increased protein content (10.59-30.76 % DW), PUFAs content (37.13-47.11 %TFA), n-3 fatty acids (26.49-38.27 %TFA), DHA (5.73-10.33 %TFA), and EPA (17.09-20.69 %TFA – $p > 0.05$).