

OPTIMISATION OF THE PROTEIN RECOVERY FROM *A. PLATENSIS* BY ULTRASOUND-ASSISTED ISOELECTRIC SOLUBILISATION-PRECIPITATION

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INTRODUCTION

The world's population is expected to increase from approximately 7.7 billion today to 9.7 billion in 2030, according to recent estimations reported by the United Nations. This expanded population is expected to consume twice as much protein than currently consumed today. The world's future protein supply needs new initiatives to address the environmental impact of food production and to produce enough quantities of high-quality protein. Proteins derived from microalgae have been suggested as potential protein sources for the future, not only because of the high protein content of some microalgal strains, but also for their high quality in terms of essential amino acids. Thus, the aim of the current study was to optimise the isoelectric solubilisation-precipitation extraction of proteins from the cyanobacterium *Arthrospira platensis* (Spirulina) using a response surface methodology.

MATERIALS & METHODS

Dried biomass of *A. platensis* was kindly provided by Biorizon Biotech (Almería, Spain).

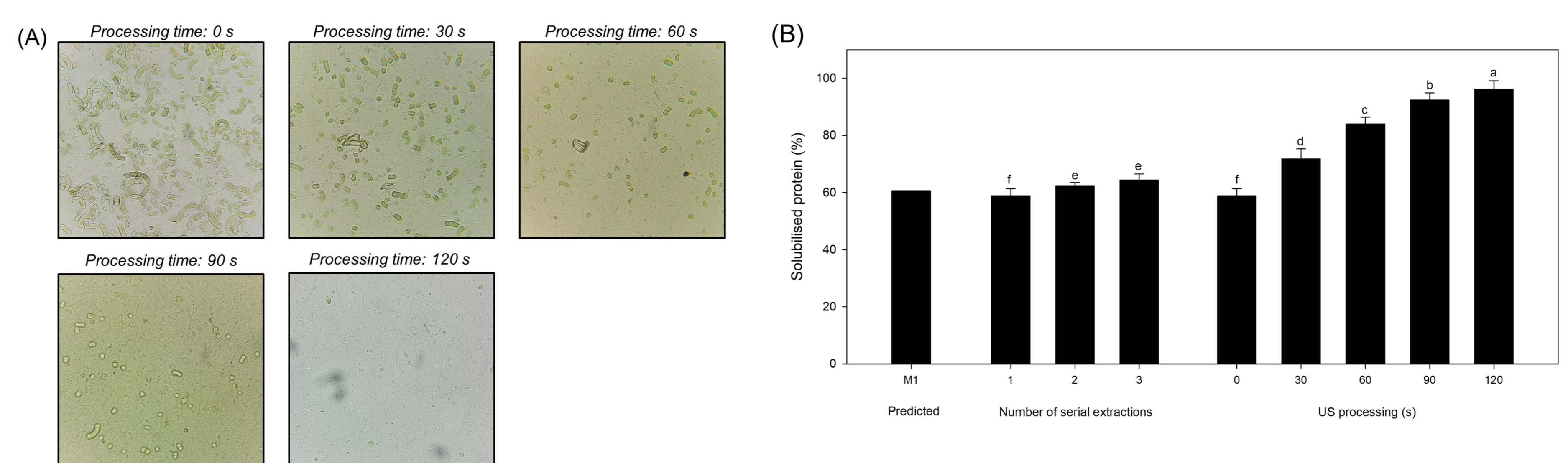
Two separate experiments were designed and conducted using Design Expert Version 7.0.0 (Stat-Ease Inc., MN, USA) in a sequential manner. Design model 1 (M1) was conducted to optimise protein solubilisation and design model 2 (M2) was conducted to optimise the recovery of the solubilised protein by precipitation using the independent variables time and pH. Experimental data were fitted to a polynomial response surface. Non-significant terms ($p < 0.05$) were deleted from the second order polynomial model after ANOVA analysis and a new ANOVA was performed to obtain the coefficients of the final equation. Both models M1 and M2 were validated in duplicate and optimised.

CONCLUSIONS

Although spray-drying and the osmotic shock suffered when resuspending the biomass into distilled water led to a certain degree of cell wall disruption, the amount of protein that could be solubilised was relatively low. Sonication led to a high amount of proteins solubilised (over 95%). At the optimised conditions, almost 75% of the total proteins could be recovered with a purity of approximately 80%, which could be further increased by dialysis and other purification steps.

RESULTS & DISCUSSION

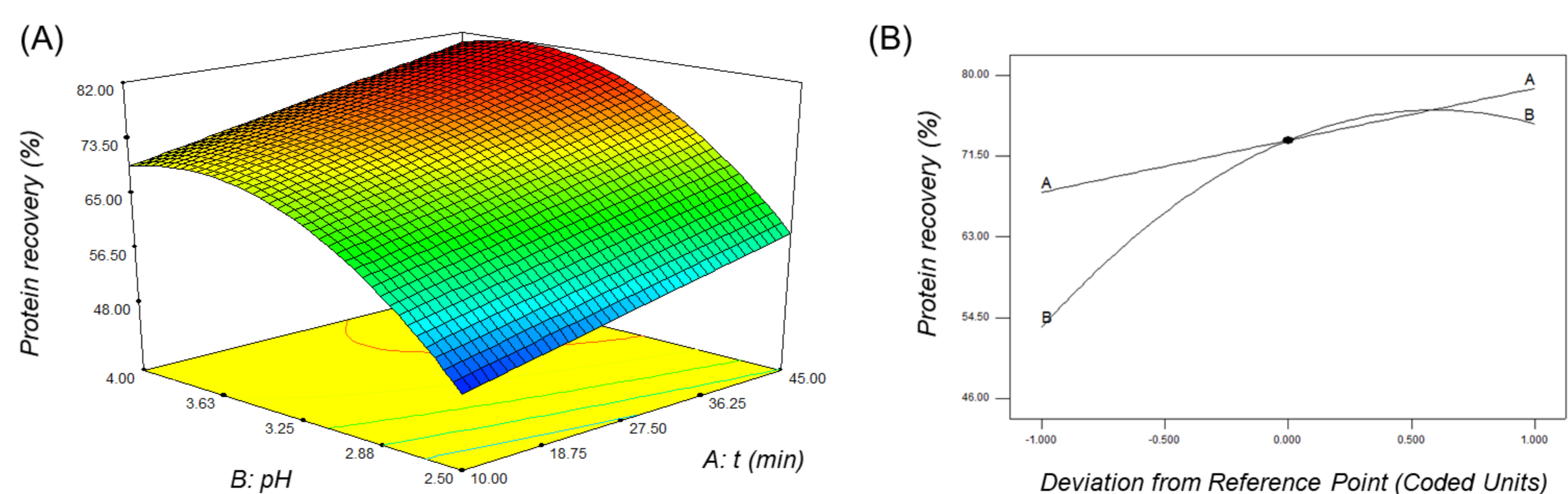
Protein solubilisation from *A. platensis* was also influenced by pH ($p < 0.001$) extraction time ($p < 0.0001$) and biomass concentration ($p < 0.0001$). Different strategies were assessed to improve solubilisation (Figure 1) achieving an almost complete solubilisation after sonication at 400 W and 24 kHz for 2 min.



Design model 2 led to the following quadratic equation in terms of actual factors, which represents an empirical relationship between the percentage of protein from *A. platensis* that could be recovered by isoelectric solubilisation/precipitation and the independent variables:

$$\% \text{ Protein recovery} = -150.43 + 0.31 \cdot t + 118.05 \cdot pH - 15.96 \cdot pH^2$$

Where t is extraction time expressed in min and pH is the pH value at which precipitation was conducted. Figure 2 represents the 3D contour plot model graph. Protein recovery was more sensitive to the pH value at which the protein precipitation occurred than to the extraction time, although both variables were significant.



Conditions needed to maximise protein recovery were predicted to be a pH value of 3.89 during 45 min. The software predicted that using these conditions, a predicted protein recovery of approximately 80% could be achieved. These conditions were validated in the lab and a protein recovery of $75.2 \pm 1.5\%$ was achieved, demonstrating the validity of the model.

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