Foods 2024 Conference

The 5th International Electronic Conference on Foods

28–30 October 2024 | Online

MDPI

Green and Scalable Process for the Production of High-purity C-phycocyanin from *Arthrospira maxima*

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INTRODUCTION & AIM

- C-phycocyanin (C-PC) is one of the most important proteins of *Spirulina* and it possesses several commercially interesting bioactivities, including antioxidant-, anti-inflammatory-, neuroprotective, and hepatoprotective properties.
- Extraction of C-PC in the first step is recommended due to possible losses in the lengthy extraction process, as it is highly sensitive to light, pH, temperature, and chemical reagents.
- Ammonium sulfate precipitation is a common method for further purification of C-Phycocyanin, but this process is time-consuming and expensive, as 50-60% of ammonium sulfate are required for the precipitation of C-PC and multiple steps are required for recovery, including separation, neutralization, and desalting, generating large volume of wastes.

METHOD

❖ Optimizing the process of disrupting the cell wall

Cell wall disruption to obtain crude protein extracts used ultrasound and the procedure was carried out on the ice. Briefly, 2 g dried *Arthrospira maxima* was mixed with 40 mL Milli-Q water, followed by sonication at 750 Watt at 20 kHz with a probe diameter of 25 mm (SON-ICS, Vibro-Cell). Disruption at an amplitude setting of the ultrasonicator of 80%, a treatment time of 16 min with pulses of 5 s on and 5 s off.

❖ Optimizing the purification method

The pH of the obtained slurry was adjusted to values 5.0 using 0.5 M acetic acid, to facilitate the separation of insoluble cell debris and fragments. Supernatants, defined as crude extracts, were obtained by centrifugation (Beckman, J2-MC) at 2,500 g at 4℃ for 30 min. The purity of C-PC in the crude ex-tracts was determined spectrophotometrically. The crude extract with the highest C-PC purity was selected for further purification.

❖ Characterizations of C-PC

Protein content was determined with the Lowry method using a commercial protein assay kit (TP0300-1KT, Total Protein Kit, Micro Lowry), C-PC concentrations and purities were determined by equations of (A620-0.474×A650)/5.34 and A620/A280, respectively. The UV-Visible spectra of samples were measured from 200 nm to 800 nm on a Cary 50 EST 70772 (VARIAN, Palo Alto, USA). C-PC samples from each step were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) on 4-20% Mini-PROTEAN TGX Stain-Free (Catalog No. 4568095, Bio-Rad, Hercules, USA) gradient gels using Precision Plus Protein Standards (Catalog No. 161-0374, Bio-Rad, Hercules, USA). For sample preparation, 15 µL samples were mixed well with 5 µL sample buffer (50% (v/v) 0.5 M Tris-HCl at pH 6.8, 40% (v/v) glycerol, 8% (w/v) SDS, 6.2% (w/v) DTT, and 0.04% (w/v) bromophenol blue), followed by heating at 95℃ for 2 min. Electrophoresis settings were 200 V for 30 min. Images were captured using a Bio-Rad Gel Doc EZ system and Image Lab software.

FUTURE WORK / REFERENCES

- Scaling up the method to a pilot scale to test its robustness.
- Exploring ways of reusing the spent activated charcoals .

RESULTS & DISCUSSION

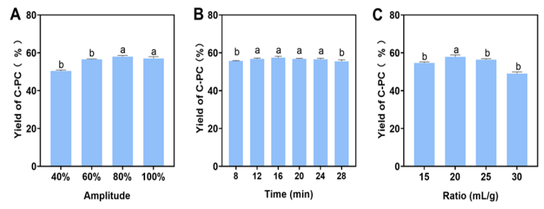

Figure 1. Effect of (A) amplitude, (B) time, and (C) ratio of the solid to liquid on the yield of C-PC in the slurry. (a, b stand for $p < 0.05$).

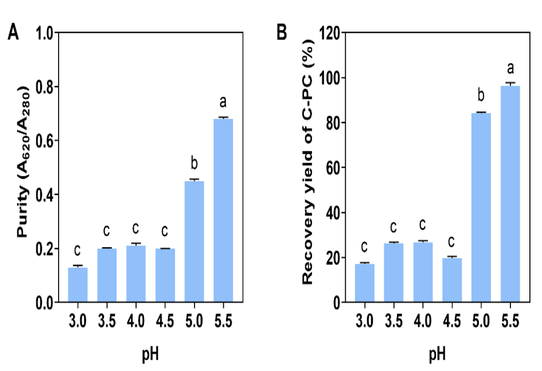

Figure 2. Effect of pH on (A) purity of C-PC in the crude extracts and (B) yield of the extracted C-PC. (a, b, c indicate significant difference ($p < 0.05$)).

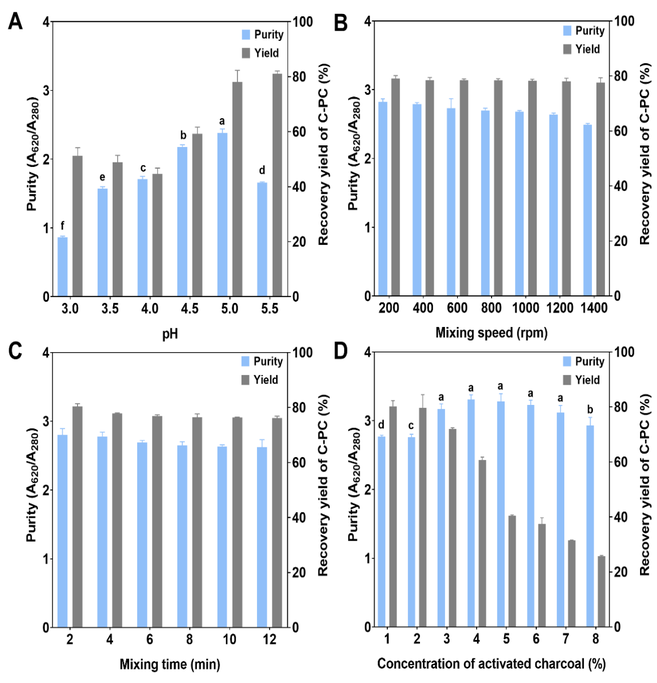

Figure 3. Effect of pH (A), mixing speed (B), mixing time (C), and AC concentration (D) on the purity and recovery yields of C-PC. (a, b, c, d, e, f determine statistically significant differences ($p<0.05$)).

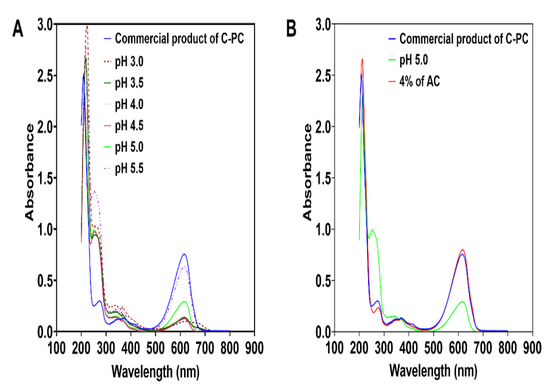

Figure 4. Absorption scans of fractions obtained from different purification conditions for determining purity in comparison with commercial C-PC. (A) Absorption spectrum of samples collected at different pHs. (B) Absorption spectrum of samples collected from optimal parameter processing steps for pH 5.0, and AC purification. Commercial product of C-PC was used as the control.

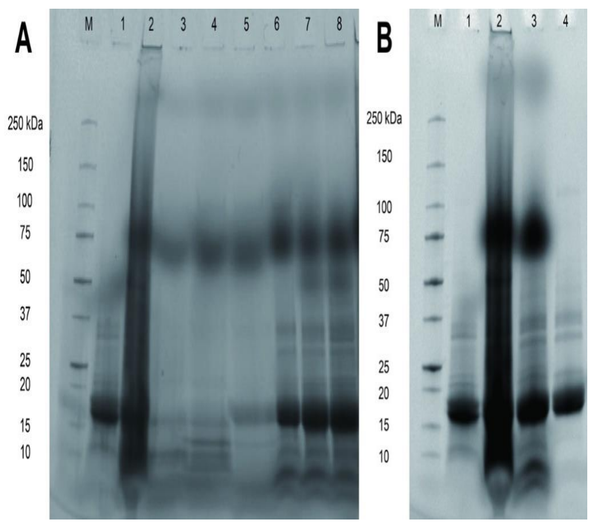

Figure 5. SDS-PAGE of fractions obtained from different purification conditions for determining purity in comparison with commercial C-PC. (A) SDS-PAGE gel of pH shift samples in comparison to crude extracts and commercial C-PC, lane M: molecular weight markers. Lane 1: Commercial C-PC. Lane 2: Extract obtained after ultrasonic treatment. Lane 3: pH 3.0. Lane 4: pH 3.5. Lane 5: pH 4.0. Lane 6: pH 4.5. Lane 7: pH 5.0. Lane 8: pH 5.5. (B) SDS-PAGE gel of samples for optimized separation (pH) after purification with AC in comparison to crude extracts and commercial C-PC, lane M: molecular weight markers. Lane 1: commercial C-PC. Lane 2: extract obtained after ultrasonic treatment. Lane 3: C-PC obtained from extracts at pH 5.0. Lane 4: activated charcoal purified C-PC at pH 5.0.

CONCLUSION

- In summary, a simple and eco-friendly process has been developed for cosmetic grade production of C-PC from *A. maxima*.
- The most important breakthrough was achieved by applying pH-adjustments after disruption of cell walls immediately followed by further purified with activated charcoal, improving the purity of C-PC from 0.45 to 3.31 in a single step.

❖ **Acknowledgments:** The authors acknowledge the support from the Centre for Marine Bioproducts Development (CMBD) in Flinders University.