Quantitative Analysis of C-Phycocyanin from Spirulina Pacifica (Low Temperature Method)

Background:

Spirulina **and other blue-green algae contain c-phycocyanin, which acts as an accessory pigment when light energy is captured and transferred to chlorophyll a. This is a spectrophotometry method adapted to extract and quantify a relatively pure c-phycocyanin fraction from Spirulina Pacifica.**

Equipment and instruments:

Spectrophotometer at 620nm
Refrigerator (4 C)
Phosphate buffer (pH 7.0)*
10 ml centrifuge tubes
Cooled centrifuge (10 C @ 3500 RPM)
Dessicator
Weigh pans
Analytical balance

Methods:

Dry Weight

1) **Place drying pans in oven for 30 minutes place in desiccator to remove excess moisture.**
2) **When pans are cool, weigh and record weight of pan.**
3) **Tare the balance with the pan on it and place about two grams of powder in the pan.**
4) **Record the weight of the powder.**
5) **Place pan and powder in the oven to dry for six hours.**
6) **Remove pan and powder from the oven and place in desiccator 15 minutes to cool.**
7) **Weigh and record the total weight of the pan and the dry powder.**
8) **Perform duplicates for each sample.**

Dry Weight Calculations

$$\text{Percent dry wt} = \frac{\text{(pan (g)+ dried powder (g)) - pan wt (g)}}{\text{powder wt (not dried) (g)}}$$

Phycocyanin Assay

1) **Weigh accurately 40 mg. Spirulina powder into a 10-ml. centrifuge tube.**

2) **Add 10 mls. of the 100 mM phosphate buffer (100-mM Phosphate buffer contains 10.64 g. K_2HPO_4 and 5.29g. KH_2PO_4 per liter, pH 7.).**
3) **Vortex to mix well.**
4) **Store in refrigerator overnight.**
5) **Vortex to mix well.**
6) **Centrifuge 5 minutes at 10 C at 3500 RPM.**
7) **Read absorbency of each replicate at 620 nm, using phosphate buffer as blank.**
8) **Average absorbency readings for dilution replicates.**
9) **Calculate percent C-Phycocyanin:**

Derivation of pure C-Phycocyanin:

$$\% \text{ pure CPC} = \frac{A_{620} \times (10) \times (100)}{7.3 \times (\text{mg. sample}). \times (\% \text{ dry wt.})}$$

where 7.3 is Extinction coefficient of CPC at 620 nm
10 is total volume;
100 represents 100%.

Derivation of crude C-Phycocyanin:

$$\% \text{ crude CPC} = \frac{A_{620} \times (10) \times (100)}{3.39 \times (\text{mg. sample}). \times (\% \text{ dry wt.})}$$

where 3.39 is Extinction coefficient of CPC at 620 nm
10 is total volume;
100 represents 100%.

References

Boussiba S. and A. Richmond. 1979. Isolation and purification of phycocyanins from the blue-green alga *Spirulina platensis*. Arch. Microbiol. 120:155-159.

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