

# Quantitative Analysis of Chlorophyll-a from *Spirulina Pacifica*

## Background:

*Spirulina* contains only Chlorophyll-a. Carotenoids and chlorophyll are acetone soluble but the carotenoids do not interfere in this spectrophotometric method as the absorbency for carotenoids range from 400-500 nm, and the absorbency for chlorophyll A is 666 nm.

The other major pigments in *Spirulina* are the water-soluble phycocyanins, these remain in an acetone-insoluble pellet during the assay.

## Equipment and instruments:

Analytical balance

Centrifuge

Drying pans

Drying oven at 110 degrees C.

85 % acetone in water

Spectrophotometer

35 ml round bottom glass centrifuge tubes with caps

50 ml volumetric flask with lid

Vortexer (Maxi Mix II)

Centrifuge

Glass beads (through 20 mesh)

Pipettes

## Method:

### 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.  
Record the weight of the powder.
- 4) Place pan and powder in the oven and dry for two hours.
- 5) Remove pan and powder from the oven and place in desiccator 15 minutes to cool.
- 6) Weigh and record the total weight of the pan and the dry powder.
- 7) Perform duplicates for each sample.

## Chlorophyll A Assay

- 1) Weigh approximately 50 mg of Spirulina into a 35 ml centrifuge tube. Record weight.
- 2) Add 5 grams of glass beads and 2.5 ml of 85 % acetone in water.
- 3) Vortex vigorously for 5 minutes.
- 4) Add 10 ml of 85% acetone in water, vortex briefly and centrifuge at 3200 RPM of 5 minutes.
- 5) Collect the supernate in a 50 ml volumetric flask .
- 6) Repeat steps 3-5 until supernate is clear. Four extractions should be sufficient.
- 7) Bring the flask up to volume with 85% acetone in water and cap the flask and invert gently to mix the contents.
- 8) Read the absorbency with the spectrophotometer at 666nm and 642 nm against an 85 % acetone/water blank.

### Calculations:

#### Dry weight

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

#### Chlorophyll A

$$\text{Chlorophyll A (\%)} = \frac{[(9.93 \times \text{Abs}_{666}) - (0.0777 \times \text{Abs}_{642})] \times 0.05 \text{ liter}}{\text{Sample weight (mg)} \times \% \text{ dry wt.}} \times 100$$

#### References

A.O.A.C. Official Methods of Analysis (1995); 940.03

Ind. Eng. Chem. Anal. Ed. 12:148 (1940) and 15:524 (1943).

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