# Effect of Culture Media on Growth and Chemical Composition of *Spirulina* sp.

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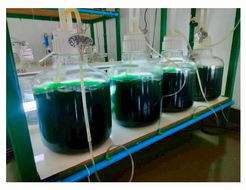
## ABSTRACT

*Spirulina* sp. is a raw material that has attracted considerable attention in the food industry due to its richness in bioactive compounds with functional properties, as well as its safety and non-toxic nature. In order to obtain desirable biomass and bioactive compounds from *Spirulina* sp., the cultivation medium plays a crucial role in algal growth and metabolite production, particularly nitrogen, which is an essential nutrient for algal metabolism. Therefore, this study aimed to investigate the effects of five modified Zarrouk media with different sodium nitrate ($NaNO_3$) concentrations (1.5–3.5 g/L) on the growth performance and chemical composition of *Spirulina* sp. *Spirulina* sp. was cultivated under laboratory conditions for 21 days, and algal growth was monitored every 6 days. At the end of the cultivation period, the algal biomass was harvested and analyzed for chemical composition, including total lipid, total carbohydrate, total fatty acids, total phenolic content, and C-phycocyanin content. The results revealed that medium formulation 2 yielded the highest biomass production, with a value of 1.25 ± 0.05 g/L. In terms of chemical composition, medium formulation 1 resulted in the highest total lipid and total carbohydrate contents, at 17.80 ± 0.44% and 12.28 ± 1.69%, respectively, whereas medium formulation 2 exhibited the highest total fatty acid content (2.17 ± 0.00%). Palmitic acid was identified as the predominant fatty acid in all media formulations. Regarding bioactive properties, medium formulation 1 showed the highest total phenolic content (7.05 ± 0.08 mg GAE/g), while medium formulation 2 produced the highest C-phycocyanin content (10.28 ± 0.27%). The C-phycocyanin obtained from all media formulations exhibited food-grade purity. These findings demonstrate that varying $NaNO_3$ concentrations in modified Zarrouk media significantly affect the growth and chemical composition of *Spirulina* sp., and the results may serve as a guideline for optimizing cultivation media to enhance biomass yield and bioactive compound production for food industry applications.

## METHODOLOGY

### *Spirulina* sp. cultivation

| Medium formulation | [$NaNO_3$] (g/L) |
|---|---|
| MF 1 (control) | 2.5 |
| MF 2 | 1.5 |
| MF 3 | 2.0 |
| MF 4 | 3.0 |
| MF 5 | 3.5 |



Cultivated for 21 days → Measured growth by dry weight

### Chemical composition analysis

**1. Total lipid (Bligh & Dryer, 1959)**

4% $H_2SO_4$ in EtOH 3 mL → mixed for 1 min → $CHCl_3$:MtOH (1:2 v/v) 3.75 mL → Incubated at 50 ºC, 3 h → $CHCl_3$ 3 mL, $H_2O$ 2 mL → 50 ºC, 12 hr → Measured weight

**2. Total carbohydrate (Kochert, 1978)**

2 M $H_2SO_4$ 7 mL → △ 100 ºC, 2 hr → sample extract 0.1 mL + $H_2O$ 1.9 mL → 5% Phenol 1 mL, Conc. $H_2SO_4$ 5 mL → Measured the absorbance at 485 nm

**3. Total fatty acid**

MtOH:HCl (95:5 v/v) 2 mL, C19:0 (IS) 0.1 mL → Incubated at 85 ºC, 90 min → $H_2O$ 1 mL, 0.1% BHT in hexane 1 mL → Centrifuged 4000 rpm, 8 min → extract → Gas chromatography

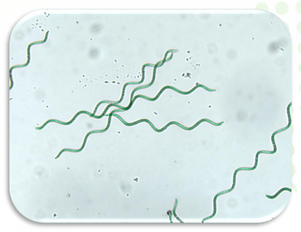
**4. Total phenol (Adapted from Goiris *et al*. (2012))**

sample extract 0.2 mL, 10% Folin ciocalteu reagent 1.5 mL → 5 min → 6% $Na_2CO_3$ 1.5 mL → Incubated in the dark for 90 min → Measured the absorbance at 750 nm

**5. C-phycocyanin (Adapted from United States Pharmacopeia (2017))**

0.1 M potassium phosphate buffer 5 mL → Freeze-Thawing → Stored at 4 °C for 16–24 h → Centrifuged 4000 rpm, 7 min → Measured the absorbance at 280, 620, and 650 nm

## BACKGROUND



*Spirulina* sp.

**Media formulation** → 7 N Nitrogen 14.0067 → Amino acid → Protein

- ✓ Protein
- ✓ Carbohydrates
- ✓ Lipids
- ✓ Pigments
- ✓ Vitamins
- ✓ Phenolic compounds

## RESULTS

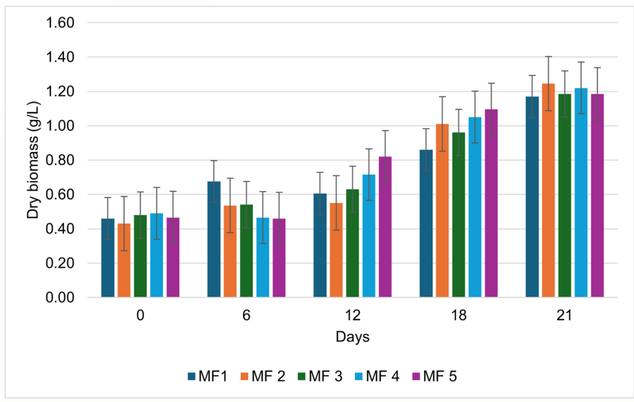



**Figure 1** Effect of different $NaNO_3$ concentrations on growth of *Spirulina* sp.

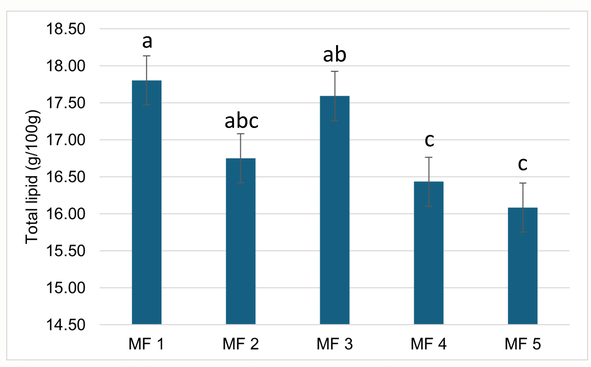



**Figure 2** Effect of different $NaNO_3$ concentrations on lipid content.

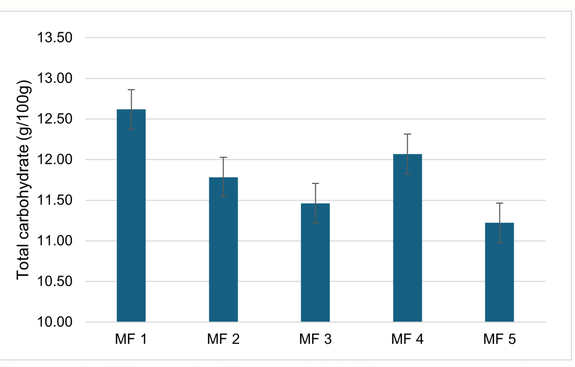



**Figure 3** Effect of different $NaNO_3$ concentrations on total carbohydrate content.

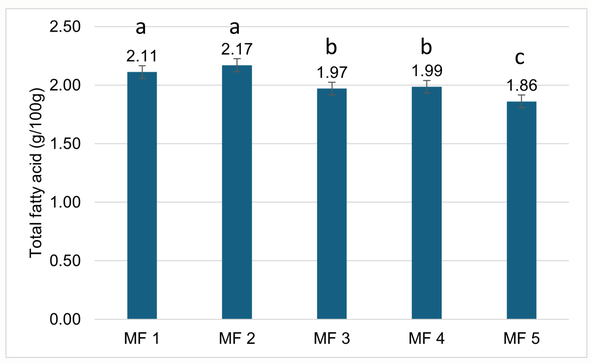



**Figure 4** Effect of different $NaNO_3$ concentrations on total fatty acid content.

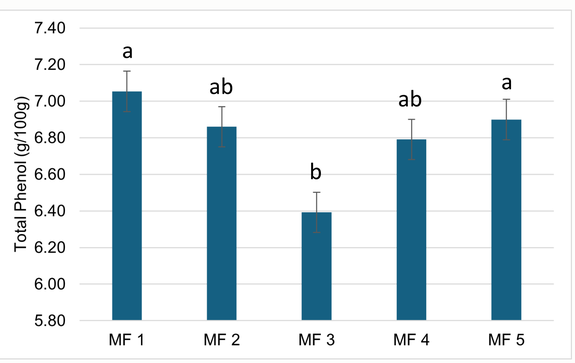



**Figure 5** Effect of different $NaNO_3$ concentrations on total phenol content.

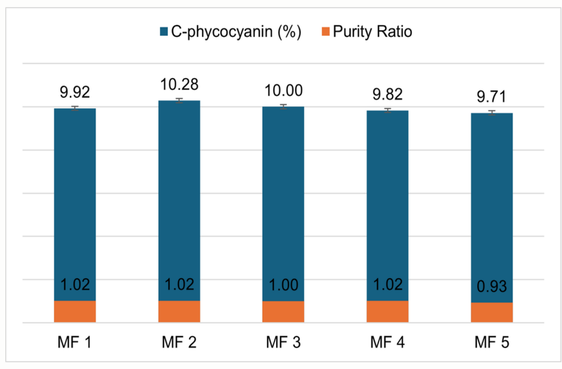



**Figure 6** Effect of different $NaNO_3$ concentrations on C-phycocyanin contents.

## CONCLUSION

- Sodium nitrate concentrations in modified Zarrouk medium ranging from 1.5 to 3.5 g/L did not significantly affect the growth of *Spirulina* sp.
- Sodium nitrate concentrations in modified Zarrouk medium ranging from 1.5 to 3.5 g/L significantly affected certain chemical compositions, including lipids, fatty acids, and phenolic compounds.

## ACKNOWLEDGEMENT

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- Algal Excellent Center, Thailand Institute of Scientific and Technological Research

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