

SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF MICROALGAE OILS FOR BIODIESEL PRODUCTION

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INTRODUCTION

As traditional oil reserves become limited, investigation into alternative energy sources becomes increasingly important. Microalgae are a versatile, renewable resource. Certain species can produce large amounts of lipids that can be converted to fatty acid methyl ethers (FAME) for biodiesel.

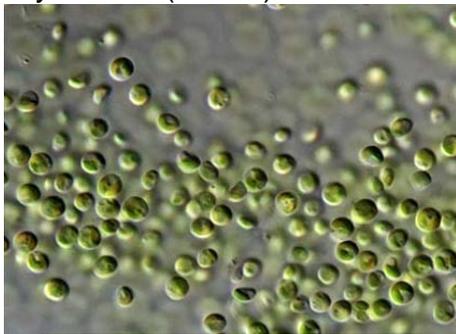


Figure 1. *Nannochloris oculata*

Supercritical fluid extraction (SFE) holds greater potential for the extraction of algae lipids in higher concentration and purities than traditional organic separation methods. SFT Inc. has developed a method utilizing the lab-scale SFT-110 SFE to extract algae oil utilizing supercritical carbon dioxide.

The SFT-110 utilizes pressurized carbon dioxide, allowing extraction to take place in room temperature environments, so purer, less thermally decomposed extract is generated. The

carbon dioxide is tunable, meaning certain conditions such as flow rate, temperature, and pressure can yield products that are lipid specific. Supercritical carbon dioxide also separates the oils in a clean, energy efficient fashion. From a large scale perspective, the carbon dioxide can be recycled from supercritical extraction into a necessary component for algal growth.

To determine which parameters would be most advantageous for future production, several varieties were extracted via supercritical carbon dioxide. GC/MS analysis is used to determine which alga produces the highest and purest yield of oil.

Three types of algae samples were utilized in the experiment: untreated fresh *Schizochytrium*, spray dried *Nannochloropsis*, and freeze dried *Nannochloropsis*. The fresh algae sample was newly cultivated algae frozen and stored in the dark. Freeze dried algae was frozen in the first step of storage. Following the freeze, the algae are depressurized to 0.01 psi. The ice is removed via a cold condenser and vacuum chamber. The final algae product is left dry with its original components except for water. Spray dried algae is prepared by flash drying the cultivated sample. Air is thrust into a combustion chamber of a drying unit with fuel until it explodes. This controlled explosion creates 3 psi pressurized air. The dryer controls the explosion with air to control the heat that comes into the presence of the algae. Combustion occurs in a controlled series to yield algae with original nutrients.



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Supercritical extracted fats and algal oil can be converted into crude biodiesel precursor ingredients via a transesterification process. Triglycerides and alcohol are mixed with an acid or base catalyst with to yield fatty acid methyl ethers (FAME).



Figure 2. SFT-110 SFE Unit

EQUIPMENT

- Analytical Balance
- SFT-110 SFE Unit
- SFT 50 cc Sample Vessel
- SFT 50 cc Sample Bag
- Heating/Stir Plate
- Centrifuge
- Thermometer
- pH Meter
- Stir Rods
- SFT Collection Vials
- Vent Tube
- Weigh Boats
- Falcon Tubes

MATERIALS

- Microalgae samples
- Hexane

- Sulfuric Acid
- Potassium Methoxide

EXPERIMENTAL PROCEDURE

A simple one step SFE extraction method using the SFT-110 was employed to isolate algae oil and triacylglycerides in three algae samples: untreated *Schizochytrium*, spray dried *Nannochloris*, and freeze dried *Nannochloris*. All samples are from Israel and harvested in 2012.

Weigh 20 grams of spray or freeze dried microalgae sample on an analytical balance. Grind up pellets to 1mm x 1mm.

For fresh samples, weigh 20 gram samples and place into falcon tubes with 1 mm glass beads. Bead beat samples at 12,000 rpm for 20 minutes at 4°C.

Load each variety of microalgae into a SFT-50 cc Sample Bag. Situate the SFT 50 cc Sample Bag into a SFT-50 cc sample vessel (10kpsi, 200°C operation). Seal the vessel and set into a SFT-110 SFE unit. One fraction of oil will be collected via multiple (10) soak and dynamic flow steps. Place pre-weighed SFT collection vial on the flow line. Extract the samples according to the following parameters:

EXTRACTION PARAMETERS

One fraction of oil will be collected via multiple soak and dynamic flow cycles according to the guidelines below.

FRACTION 1: ESSENTIAL OIL EXTRACT

- Pressure: 6000 psi
- Temperature: 100°C
- CO₂ Flow Rate: 8-10 mL/min



- 10 static and dynamic steps for 10 minutes apiece

Store samples in 4°C environment for up to 30 days in 20 mL hexane to preserve lipid extract.

FAME CONVERSION

Add 20 mL methanol and 10% sulfuric acid in 1 mL methanol to the lipid extract in a flask. Heat the sample to 50°C and stir for 2 hours. As methanol evaporates, add more. Following the 2 hours, add 25% potassium methoxide until the solution reaches a pH of 13. Heat the solution to 60°C in the oven until the dried post-methylated lipid is all that remains. Redissolve in 20 mL of hexane for FAME GC/MS analysis.

GC/MS PARAMETERS

- Injection size: 1 µL sample
- Carrier Gas: Helium 1.5 mL/min
- Split Ratio: 20:1
- Injector temperature: 150°C
- Oven: 140°C at 5°C/min to 240°C and maintained for 5 min.
- Solvent Delay: 5 min.

RESULTS

Table 1 is the extraction yield results for the algae varieties at 6000 psi. As you can see freeze dried *Nannochloris*, has the highest total yield of products. Fresh *Schizochytrium* and spray dried *Nannochloris* respectively followed in yield product. The extracts will yield a light yellow to medium green color product. Ideally very little color would be present, as the presence of chlorophyll indicates artifacts in the extracted product.

Time (min.)	Fresh <i>Schizochytrium</i> (%)	Spray Dried <i>Nannochloris</i> (%)	Freeze Dried <i>Nannochloris</i> (%)
0	0.000	0.000	0.000
10	0.116	0.462	0.497
20	1.124	0.881	0.962
30	1.808	1.089	1.096
40	1.997	1.209	1.491
50	2.139	1.375	1.520
60	2.336	1.442	1.549
70	2.832	1.561	2.002
80	3.056	1.595	2.993
90	3.430	1.797	3.687
100	3.550	1.867	3.712

Table 1. SFE-CO₂ Algae Variety Extraction Yields

Figure 3 is the extraction yield results for the algae samples. Basically this table is serving to indicate the yield of SFE CO₂ algae extraction at specific time intervals.

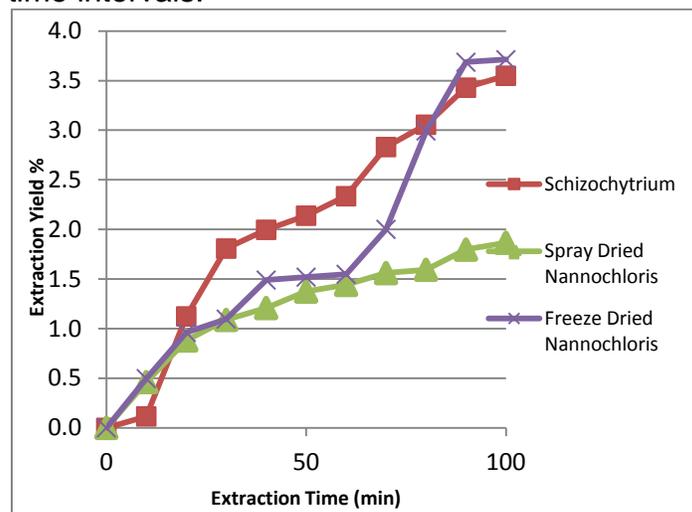


Figure 3. SFE-CO₂ Extraction of Algae Varieties @6000 psi

Figure 4 and 5 are the GC/MS analysis of the highest yielding samples, freeze dried *Nannochloris* and fresh *Schizochytrium*. Freeze dried *Nannochloris* contains the FAME



components: methyl myristate (C14:0), methyl stearate (C18:0), methyl oleate (C18:1), methyl eicosadienoate (C20:1), methyl arachidonate (C20:4), methyl eicosatrienoate (C20:3), and methyl docosahexaenoate (C22:6).

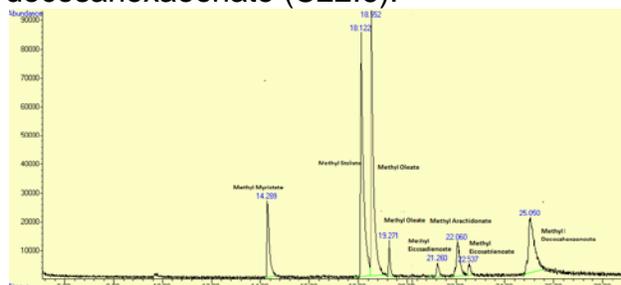


Figure 4. GC/MS *Nannochloris* Freeze Dried

Fresh *Schizocytrium* includes the FAME components: methyl myristate (C14:0), methyl isomyristate (C14:1), methyl

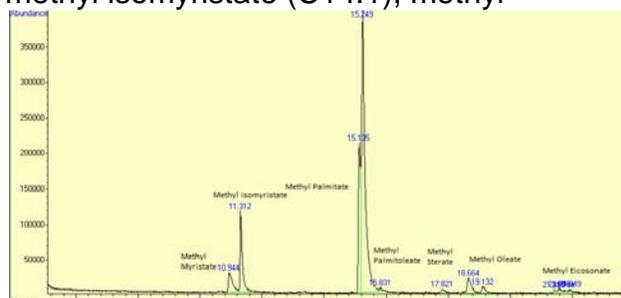


Figure 5. GC/MS *Schizocytrium*

palmitate (C16:1), methyl state (C18:0), methyl oleate (C18:1), and methyl eicosonate (C20:1).

CONCLUSIONS

Oil components of algae can be extracted using the lab-scale SFT-110 SFE Unit. Supercritical CO₂ extraction can isolate specific components with slight adjustments to temperature or pressure.

Freeze dried *Nannochloris* yielded the highest oil fractions followed by *Schizocytrium*. GC/MS analysis of

Nannochloris and *Schizocytrium* fractions yielded a variety of TAG constituents indicating that the extraction and conversion was indeed successful for some biodiesel precursors. Further manipulating the temperature and pressure parameters specifically based on Table 3 extraction rates could yield similar and higher amounts of TAG components between the different algae types.

Further investigation could be performed to determine what parameters would yield the ideal amount of product and least presence of artifacts. Ideally there would be dry oil content closer to 35-54% of *Nannochloris* and a dry oil content of 50-77% *Schizocytrium*. The previous percentages include the entire oil content of the algae, not limited to lipids, hydrocarbons, and associated oils. This percentage is not representative of the specific oils for biodiesel production. Increasing the flow rate from 24 mL/min could yield higher amounts of algae oil and important biodiesel precursors.

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