

December 18th, 2008

Re: Lipid analysis of Seambiotic algal samples

Dear Dr. Ben-Amotz,

Further to your request for the analysis results of the biomass that you have sent in August, we are reporting on the first results on the total lipid content and fatty acid composition of the second batch of *Nannochloropsis sp.*, *Tetraselmis sp.* and *Nannochloris sp.* samples.

We have calculated the total lipid content, by gravimetrically measuring the solvent soluble fraction from lyophilized biomass, recovered after extraction by accelerated solvent extraction (ASE). We are aware of the fact that the gravimetric lipid content is highly dependent on the solvent system used, however, to stay in line with the literature, we have used a 2:1 Chloroform:Methanol and a 3:2 Hexane:Isopropanol mixture. As we have mentioned before, we are in the process of optimizing the lipid extraction protocol with regards to temperature, pressure and time of extraction. The results of the analysis and the extraction conditions used are shown in Table 1.

The necessity of optimizing this methodology is demonstrated in Table 1. The total gravimetric lipid yield of the same sample was determined after extraction at different temperatures and pressures and using two separate solvent mixtures. It is apparent that there are considerable differences, which makes drawing conclusions with regards to total lipid content difficult. The ‘lipid’ content increases with increased severity of the extraction conditions for all three species. For example for *Nannochloropsis sp.*, there is an increase of 25% in lipid yield when temperature was increased from 40 to 100°C and pressure from 500 to 1500 psi. This compares to an increase of 16% for *Tetraselmis sp.* These results indicate that *Nannochloropsis sp.* is more difficult to extract. At this point in time we don’t know whether the increase in gravimetric yield is solely due to an increase in lipids or whether other compounds (such as lipophilic proteins) contribute to the weight increase.

Lipid content (w/w biomass):	Chloroform:Methanol (2:1)			Hexane:Isopropanol (3:2)	
	Extraction conditions	40°C / 500 psi	70°C / 1500 psi	100°C / 1500 psi	70°C / 1500 psi
<i>Nannochloropsis sp.</i>		25.25 ± 0.15	29.83 ± 0.01	33.33 ± ND	15.15 ± 0.51
<i>Tetraselmis chuii</i>		14.28 ± 0.17	-	16.93 ± 0.055	-
<i>Nannochloris sp.</i>		9.81 ± 0.41	-	13.08 ± 0.20	-

Table 1: Gravimetric lipid yield of three species of microalgae. Two solvent mixtures were used (Chloroform:Methanol and Hexane:Isopropanol) under three different extraction conditions using an Accelerated Solvent Extractor (ASE, Dionex). The results presented are the mean ± SEM of three replicate extraction (or transesterification reactions) of the same biomass. ND = not determined

For the three species, we calculated the relative FAME composition by area integration after GC-FID of the peaks corresponding to the methyl esters of the constituent fatty acids. The identification of the peaks was done by GC-MS.

The relative composition of fatty acids is shown in Table 2 for the three species. There are marked differences between the species, in particular with regards to the EPA content.

Fatty Acid Methyl Esters (FAME)	<i>Nannochloropsis</i> sp.	<i>Tetraselmis chuii</i>	<i>Nannochloris</i> sp.
C14:0 methyl myristate	3.32 ± 0.83	2.48 ± 0.03	4.2 ± 0.1
C16:0 methyl palmitate	18.51 ± 0.27	38.17 ± 0.24	34.26 ± 0.73
C16:1 methyl palmitoleate	30.61 ± 0.38	10.37 ± 0.06	20.02 ± 0.13
C18:0 methyl stearate	ND	1.70 ± 0.01	0.65 ± 0.56
C18:1 methyl oleate (cis-9)	ND	ND	ND
C18:2 methyl linoleate (cis-9,12)	3.4 ± 0.15	6.88 ± 0.03	24.86 ± 0.49
C18:3 methyl linolenate	ND	33.80 ± 0.16	11.00 ± 0.16
C20:4 methyl arachidonate (cis-5,8,11,14)	5.1 ± 0.02	4.91 ± 0.1	4.73 ± 0.2
C20:5 methyl eicosapentanoate (cis-5,8,11,14,17) / EPA	39.05 ± 0.09	1.69 ± 0.03	0.28 ± 0.49

Table 2: Relative FAME composition of three species investigated. The results presented are the mean ± SEM of three replicate reactions using acid-catalyzed methanolic *trans*-esterification of lipids extracted using chloroform-methanol as a solvent mixture. ND = not determined.

We hope you find this information useful and look forward to further collaborations.

Yours sincerely,

Lieve Laurens, PhD
 Biomass Analysis, National Bioenergy Center
 National Renewable Energy Laboratory

Email: Lieve_Laurens@nrel.gov