Comparison of extraction and derivatization methods for sample preparation of algal oils prior to FAME determination

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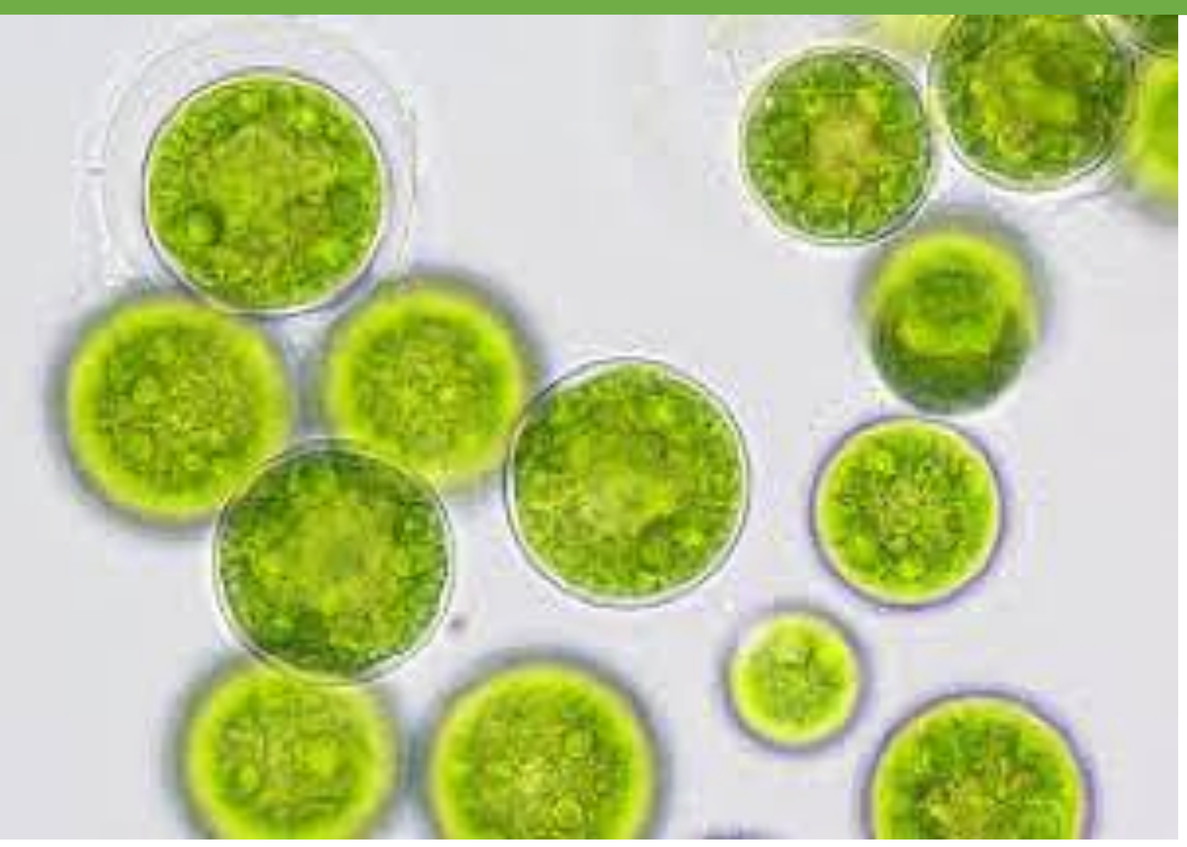
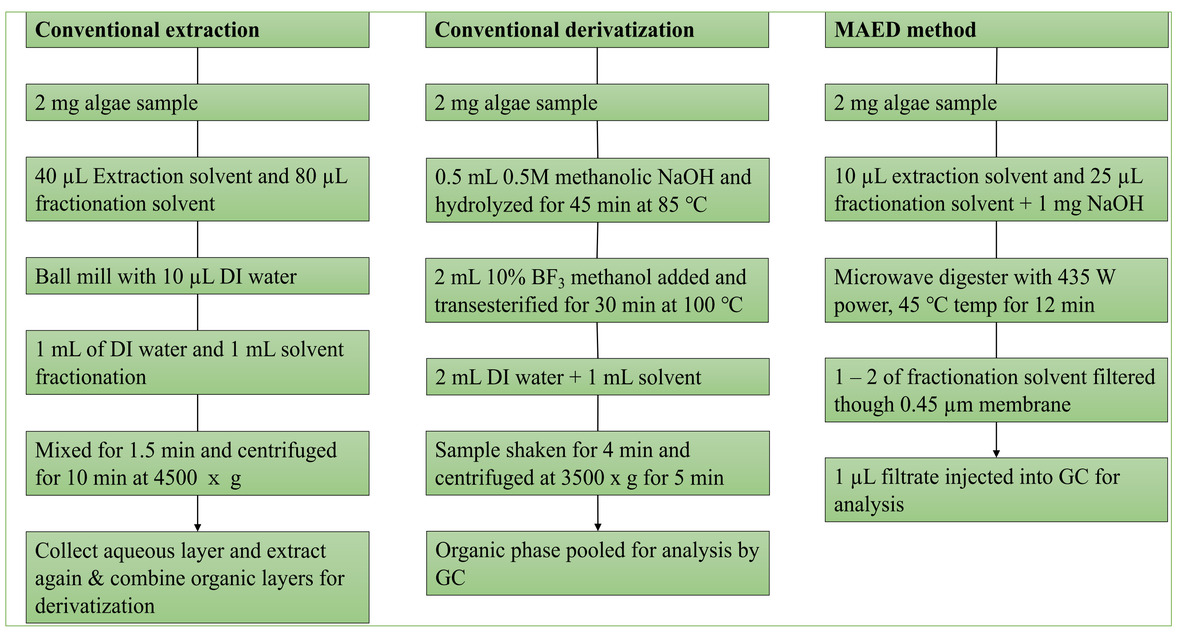


Introduction

Nannochloropsis is a species of algae most commonly found in the marine environment (1). *Nannochloropsis Sp.* provide an important source of C20:5w3 (2) which provide treatment and prevention of diseases such as cardio vascular, diabetes, arthritis and to control cholesterol and blood pressure (1). This marine species is also considered promising for mass cultivation for biodiesel production due to its high ability to produce storage triglycerides (TAG) (3). Fatty acids (FA) which contribute to the production of biodiesels include C14-18 (2).

In order to analyse the suitability of this complex species for the mentioned functions, their composition must be investigated. However, in order to investigate the FA present in algae by GC-MS, the sample must undergo derivatization to synthesise the FA into Fatty Acid Methyl Esters (FAME) which can easily be detected by GC-MS. This investigation focuses on the most optimum method to carry out sample preparation on the algae sample to investigate the composition of the sample.

Methods



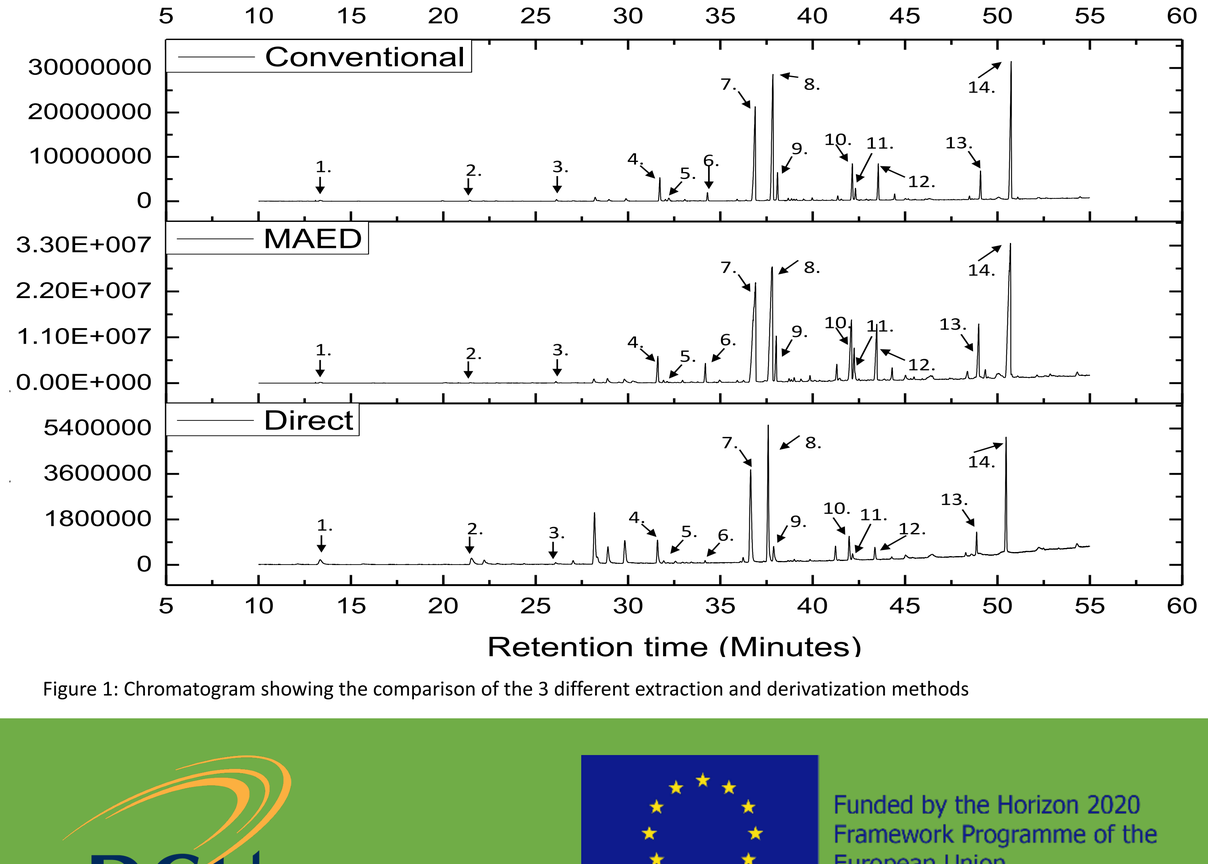


Figure 1: Chromatogram showing the comparison of the 3 different extraction and derivatization methods

		Direct synthesis (n=3)	Conventional method (n=3)	MAED method (n=3)
FA	m/z	FA content (%)	FA content (%)	FA content (%)
C8:0	74.10	0.01	0.04	0.67
C10:0	74.10	0.04	0.06	0.05
C12:0	74.10	0.06	0.16	0.20
C13:0	74.10	1.20	2.07	2.08
C14:0	74.10	0.10	0.38	0.24
C16:0	74.10	8.38	9.83	9.16
C16:1w7t	55.10	9.76	14.76	10.20
C16:1w7	74.10	1.51	1.97	1.34
C18:0	74.10	0.49	0.28	0.85
C18:1w9	125.00	2.47	2.17	1.29
C18:1w7	55.10	1.16	0.82	0.86
C18:2w6	125.00	2.69	2.75	4.77
C20:4w6	74.10	1.19	1.09	1.39
C20:5w3	125.00	5.07	6.17	18.68

	Direct synthesis	Conventional method	MAED method
∑TFA	34.130333	42.5585954	51.7594336
∑SFA	10.2770498	12.82841	13.2410457
∑MUFA	5.63186349	4.96388545	3.49005299
∑PUFA	18.7124422	24.7663	35.028335
∑TUFA	23.8532832	29.7301854	38.5183879

Discussion

The table above shows a statistical analysis of the FA present in the algae sample. 14 different FA species were detected in the *Nannochloropsis Sp.* $C_{20:5w3}$ was found to have the highest levels of FA in the sample at 18%. This is in agreement with other studies focusing on this species (1). Other FA present in this species were C_{14}, $C_{16:1}$, $C_{18:1}$ and $C_{18:2}$ which are all characteristic FA in the production of biodiesel (3).

The chromatograms below shows a comparison of the 3 methods used for sample preparation (MAED, conventional and direct derivatization method). It can be seen that all FA eluted at the same retention times with different intensities. The MAED method was found to have the highest TFA recovery at 51% FA content in the sample. Direct derivatization had 34% FA content in the sample and the conventional method had 42% FA content in the sample. Saturated FA (SFA) and unsaturated FA (UFA) were also analysed and the results show that MAED recovered a higher quantity of SFA at 13.2% compared with 10.2 and 12.8% for the direct and conventional method, respectively. Of the UFA investigated it was noted that MAED also recovered the highest quantity at 39% compared to 24 and 30% for the direct and conventional method, respectively. The MAED method may have produced such a large quantity of UFA as MAED reduces the oxidation and isomerization of UFA compared with the other methods. Overall, these results depict that the MAED method produced higher efficiencies of extraction and derivatization than that of the direct and conventional methods. The difference between the conventional and direct derivatization method also suggest that the extraction step is required prior to sample derivatization.

Conclusion

This research demonstrated that a number of methods can be carried out in order to prepare algae oils for FAME analysis. The FAME recovery was found to be highest when samples were prepared using the MAED method with a TFA recovery of 51% compared to 34 and 42% for direct derivatization and conventional method, respectively. The MAED method was also found to have a quicker retention time at 15 min compared to 60 min + for the other two methods. From these findings, it was also concluded that the extraction step is required prior to sample derivatization in order to recover the optimum amount of FAs if using the conventional method.

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