Liquid and Supercritical CO_2 Extraction of Pigments from Microalgae

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Abstract

Microalgae are a good source of pigments that are of interest to the food, cosmetic and nutraceutical industries. In this study, to reduce the use of petrochemically derived solvents, liquid (L) and supercritical (SC) CO_2 are used as solvents in the extraction of pigments from four samples of microalgae. 12 extracts are produced from the four algae samples and are subsequently analysed using HPLC-DAD. A total of 23 pigments are identified from the extracts including fucoxanthin, zeaxanthin, canthaxanthin and β-carotene.

Introduction

The use of SC fluids as an extraction solvent can be considered a green technology as it can eliminate the use of petrochemical derived solvents. SC CO_2 has a similar polarity to heptane and can be modified by changing pressure, temperature and use of co-solvents.

Microalgae are known to be rich in carotenoids which are of interest to nutraceutical and cosmetic industries due to free radical scavenging activity and as natural colourings.

Here, three microalgae species (*Nannochloropsis sp* (fig 1.) (dye rich and peak growth samples), *Chlorella vulgaris* and *Phaeodactylum tricornutum* (fig 2.)) are subjected to L and SC CO_2. Extracts were analysed using HPLC-DAD.

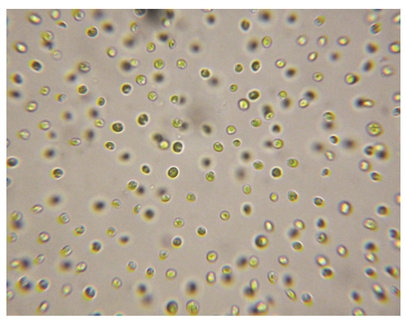

Figure 1. (Left), *Nannochloropsis sp.*

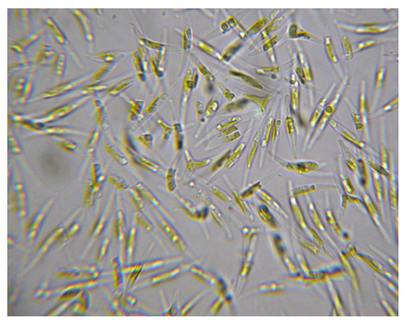

Figure 2. (Right), *Phaeodactylum tricornutum*

Method

Algae samples, provided by Merlin Bioproducts, were lyophilised and stored at -20°C.

Extraction: Extraction carried out on a Thar CO_2 extraction unit (fig. 3). Algae were sequentially extracted using L CO_2, SC and finally SC with 20% industrial methylated spirit (IMS) using the following conditions: L CO_2 pressure 60 bar, extractor temperature 5°C, CO_2 flow 5 g min^{-1}: SC, pressure 300 bar, extractor temperature 50°C. IMS was introduced at 1 g min^{-1} and CO_2 flow at 4 g min^{-1}.

HPLC Analysis[1]: Solvent A: 70/30 methanol/28mM TBA, solvent B: methanol. 0 min: 95% A, 5% B. 22 mins: 5% A 95% B, 29 mins: 5% A, 95% B, 31 mins: 95% A, 5% B, 36 mins: 95% A , 5% B. Oven temp, 60 °C. Injection volume, 500 µl. Column, Eclipse XDB-C8, 150x46 mm, 3.5um.

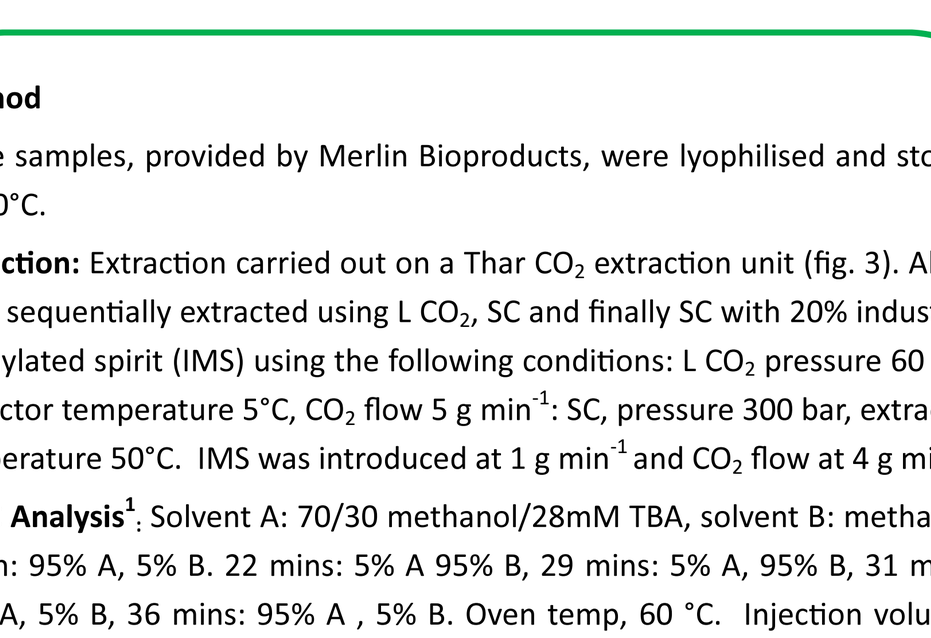

Figure 3. Thar CO_2 extraction unit

Results

23 pigments were identified from 12 extracts (Table 1). For examples of extraction products see figure 4. Only fucoxanthin and β-carotene were common pigments in all algae. L CO_2 extraction yielded one pigment from each *C. vulgaris* and *P. tricornutum* (alloxanthin and fucoxanthin), with more being isolated from *N. sp*. Neoxanthin, lutein, chlorphyll b and α-carotene are only found in *C. vulgaris*, with peridin isomer, violaxanthin, astaxanthin, antheraxanthin and divinyl chlorophyll a are only found in the *N. sps*.

	C. vol liq	*C. vol scCO₂*	*C. vol scCO₂ IMS*	*P. tri liq*	*P. tri scCO₂*	*P. tri scCO₂ IMS*	*N.sp PG Liq*	*N. sp PG scCO₂*	*N. sp PG scCO₂ IMS*	*N.sp DR Liq*	*N. sp DR scCO₂*	*N. sp DR scCO₂ IMS*
% Yeild	0.05	0.25	7.05	0.02	0.6	13.94	0.33	0.36	13.38	0.08	1.05	6.48
Chlorophyll C2						x			x			
Mg 3,8-divinyl pheoporphyrin a5 monomethyl ester						x			x			
Chlorophyllide a						x			x			
Peridinin isomer									x			x
19-Butanoyloxy-fucoxanthin		x	x						x			x
Fucoxanthin		x	x	x	x	x	x	x	x		x	x
Neoxanthin		x	x									
Violaxanthin								x	x		x	x
Astaxanthin									x		x	x
Diadinoxanthin					x	x		x	x		x	x
Antheraxanthin								x			x	x
Alloxanthin	x											x
Diatoxanthin					x	x		x	x			
Zeaxanthin			x				x	x	x		x	x
Lutein		x	x									
Canthaxanthin					x	x	x	x	x		x	x
Chlorophyll b			x									
Crocoxanthin		x									x	x
Divinyl Chlorophyll a							x			x	x	
Chlorophyll a			x			x			x			
Pheophytin a		x	x					x			x	x
α-Carotene		x	x									
β-Carotene		x	x		x	x	x	x	x	x	x	x

Table 1. Pigments extracted from algae using L and SC CO_2

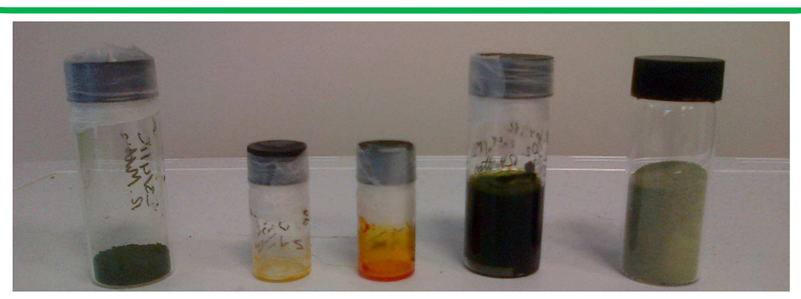

Figure 4. *Nannochloropsis sp* and extracts (from left to right: freeze dried algae, L CO2 extract, SC CO2 extract, SC CO2 20 %IMS extract, used algae

Discussion

L CO_2 extractions showed good selectivity with only one compound being identified in each *P. tricornutum* and *C. vulgaris* and two in the dye rich *N. sp* sample. The pressure during the L CO_2 extraction of *N. sp* (PGP) did rise above the SC point which sees more compounds being extracted and a great yield than other liquid extractions. The effect of introducing a co-solvent to the system is more polar compounds are extracted (e.g chlorophyll derivatives). An extended extraction period under SC conditions would predominantly favour the isolation of higher levels of carotenoids.

Conclusion

The use of L and SC CO_2 to extract pigments from microalgae is an effective method of extraction. Controlling pressure, temperature and co-solvent allows for selective compound targeting.

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Reference:

1. Van Heukelem, L. and C. S. Thomas, 2001, Computer-assisted high-performance liquid chromatography meth development with applications to the isolation and analysis of phytoplankton pigments. Journal of Chromatography A. 910: 31-49

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