Reaction of Sulfur and Sustainable Algae Oil for Polymer Synthesis and Enrichment of Saturated Triglycerides

Adarsha Gupta,a,‡ Max J. H. Worthington,b,‡ Munish Puri,a,* and Justin M. Chalkerb,*

a) Medical Biotechnology, Centre for Marine Bioproducts Development, College of Medicine and Public Health, and Flinders Health and Medical Research Institute, Flinders University, Bedford Park, South Australia, 5042, Australia

b) Institute for Nanoscale Science and Technology, College of Science and Engineering, Flinders University, Bedford Park, South Australia, 5042, Australia

*To whom correspondence should be addressed: munish.puri@flinders.edu.au
justin.chalker@flinders.edu.au

‡ These authors contributed equally to this publication

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GENERAL EXPERIMENTAL CONSIDERATIONS

NMR Spectroscopy: Proton nuclear magnetic resonance (\(^1\)H NMR) spectra were recorded on a 600 MHz spectrometer or 400 MHz spectrometer where noted. All chemical shifts are quoted on the \(\delta\) scale in ppm using residual solvent as the internal standard (\(^1\)H NMR: CDCl\(_3\) \(\delta = 7.26\), methanol-d\(_4\) \(\delta = 4.87\) and pyridine-d\(_5\) \(\delta = 8.74\)).

SEM and EDS: Scanning Electron Microscopy (SEM) images were obtained using an FEI F50 Inspetct system, while corresponding EDS spectra were obtained using an EDAX Octane Pro detector.

Thermogravimetric Analysis (TGA): Simultaneous Thermal Analysis (STA) was carried out on a Perkin Elmer STA8000 simultaneous thermal analyzer. A sample size between 10 and 15 mg was used in each run. The furnace was purged at 20 mL/min with nitrogen, and equilibrated for 1 minute at 30 °C before each run. Heating was carried out up to 700 °C using a 20 °C/min heating rate. The temperature was held isothermally at 700 °C at the end of each experiment to oxidize remaining organic matter.

Differential Scanning Calorimetry (DSC): In order to determine glass transition temperatures, thermal analysis was performed on a Perkin Elmer DSC8000. A sample size between 5 and 10 mg was used in each run. The furnace was purged at 20 mL/min with nitrogen, and equilibrated for 1 minute at 30 °C before each run. Heating was carried out up to 140 °C using a 10 °C/min heating rate, followed by a cooling step down to -60 °C at the same rate. Heating and cooling from -60 °C to 140 °C and back was repeated twice more to monitor any changes in the DSC profile over multiple cycles.

X-ray Diffraction: Powder X-ray diffraction (XRD) patterns were recorded on a Bruker D8 Advance Eco diffractometer (Bragg-Brentano geometry) using Co-K\(\alpha\) radiation (\(\lambda = 1.78897\) Å). The Bragg angle (2\(\theta\)) was varied from 15° to 90° with a step size of 0.019°, measurement time of 0.45 s per step and sample rotation at 15 rpm. The XRD patterns were collected on a silicon low background sample holder, where powder samples were deposited onto the surface of the holder and spread evenly using a drop of acetone.

EXPERIMENTAL DETAILS

Algal Biomass Production
An in-house algae strain (\textit{Schizochytrium} sp. MASA#4) was grown in a 6 L bioreactor (Infors HT, Invitrogen Technologies) to produce the biomass required for this study. The inoculum was prepared in medium containing glycerol (10 g/L), yeast extract (1 g/L), peptone (1 g/L) and incubated for 48 h at 25 °C and 150 rpm. Inoculum (10%) was used in the production medium containing glycerol (120 g/L), yeast extract (10 g/L), peptone (1 g/L), MgSO\(_4\) (10 g/L), and sodium acetate (4 g/L) in a bioreactor and cultivated at 25 °C for 5 days. The pH of the medium was maintained at 6.5 using potassium hydroxide solution. The culture medium was harvested after 5 days and subjected to freeze drying to obtain dried biomass.

Lipid Extraction
Freeze dried biomass (2.0 g) was used for lipid extraction using homogenization in a chloroform: methanol mixture (2:1, v/v). The homogenization was repeated three times and the extracts were combined and filtered through a 0.22 \(\mu\)m filter. The solvent was then removed using a rotary evaporator (bath temperature = 50 °C) and lipid weight was determined gravimetrically.
**FAME Analysis by GC:** Lipids were transesterified and fatty acid methyl esters (FAMEs) were analysed using a previously described method.\(^1\) FAMEs analysis was performed on a Shimadzu Gas chromatography (GC, 2090N) equipped with flame ionisation detector (FID) and connected to a BID 2030 unit using FAMEWAX column (30 m x 0.32 mm ID (inner diameter)). The inlet was held at 25 °C with a constant column flow rate at 5 mL/min with split injection (1/150). The oven program was held at 150 °C (5 min. hold), ramped to 250 °C at a rate of 10 °C per min. and held at 250 °C for 1 min. Fatty acid esters were quantified by comparison of peak areas of authentic FAMEs standards (Sigma Aldrich CRM47885).

**Hydrogenation of Algae Oil**
Algae oil (409 mg) was dissolved in 15 mL ethanol in a 25 mL round bottom flask. Palladium on carbon (20 mg, 10 wt.% Pd) was added, the flask sealed with a septum and sparged with hydrogen gas for 20 minutes. The mixture was left to react at room temperature with magnetic stirring for 3 days under an atmosphere of hydrogen (1 atm, balloon). The mixture was then filtered over celite (SiO\(_2\)) under vacuum to remove the catalyst and dried to isolate the oil. The average yield of isolated hydrogenated oil from three trials was 261 mg (72% yield).

**Copolymerization of Algae Oil with Sulfur**
Algae oil (409 mg) was heated to 170 °C with stirring in a 25 mL round bottom flask. After 5 minutes, the algae oil turned from a viscous orange liquid to dark brown and free flowing liquid. 409.5 mg Elemental sulfur (409 mg) was added slowly over 5 minutes to keep the mixture at approximately 170 °C. A heat gun was used to melt sulfur adhering to the upper walls of the flask. After 30 minutes the viscosity increased and stirring was slowed to ensure continual mixing. Approximately 5 minutes after the increase in viscosity, the reaction mixture could not be stirred. After a further 10 minutes at 170 °C, the flask was removed from the oil bath and allowed to cool to room temperature. After 1 hour of cooling the material had vitrified into a dark brown rubber. The synthesis was carried out in triplicate. The average amount of product formed in these reactions was 458 mg of solid product and 146 mg of unreacted oil (obtained by extraction into ethanol). Some unreacted sulfur was embedded in the polymer (see below) and some was lost to sublimation during the reaction.

**Analysis of algae oil recovered after hydrogenation or after copolymerization with sulfur**
After the hydrogenation process, 10 mg of the oil sample was directly used for transesterification and GC analysis following the FAME analysis cited above. However, after the sulphur polymerization process, the polymer containing oil was subjected to ethanol extraction (20 mL) at 50 °C for 2 hours. The ethanol in the solvent extract was evaporated using rotavap at 50 °C (R-300, Buchi) and lipid weight was determined gravimetrically. 10 mg of this dried lipid was used for transesterification and GC analysis.
Characterisation of Hydrogenated Algae Oils

**FAME analysis by GC**

Approximately 50 wt% of the dried algae mass was lipid, which was extracted with a 2:1 chloroform: methanol mixture, as described above. These lipid samples were used for the hydrogenation and sulfur copolymerization processes. The recovered oils (either hydrogenated or the oil fraction that did not react with sulfur) were then subjected to the standard FAME analysis by GC after conversion to their corresponding methyl esters.

**Distribution of fatty acids before and after hydrogenation**

The distribution of fatty acids before and after the hydrogenation process are shown in Figure S1 and S2. Catalytic hydrogenation reduced all polyunsaturated fatty acids (PUFAs) in the algae triglycerides and only relatively small amounts of monounsaturated fatty acids were detected (e.g. C16:1). This experiment illustrates that catalytic hydrogenation is a straightforward way to convert polyunsaturated triglycerides in algae oil to mono- and unsaturated triglycerides.

![Figure S1](image1.png)

**Figure S1** | Fatty acid profile of the oil obtained after hydrogenation process. The green and red bars show the FAMEs from the extracted algae oil and the hydrogenated oil, respectively. No polyunsaturated fatty acids (PUFAs) were detected in the hydrogenated oil sample (red bars).

![Figure S2](image2.png)

**Figure S2** | Catalytic hydrogenation converts polyunsaturated triglycerides of algae oil into saturated and monounsaturated triglycerides.
$^1$H NMR spectroscopy of algae oil before and after catalytic hydrogenation

**Figure S3** | $^1$H NMR spectra of algae oil before and after hydrogenation. The alkene signals (C) decrease as expected following hydrogenation.

**Figure S4** | $^1$H NMR spectrum of algae oil in methanol-d$_4$ before hydrogenation. The spectrum indicates an average of 2.60 alkenes (shown as 5.20 alkene protons, $\delta = 5.39$ ppm) per triglyceride molecule. Methyl end-group protons were used as a reference (9H per triglyceride, $\delta = 0.92$ ppm).
Figure S5 | $^1$H NMR spectrum of algae oil in methanol-d$_4$ after hydrogenation. The spectrum indicates an average of 0.98 alkenes (shown as 1.96 alkene protons, sum of $\delta = 5.36$ and 5.41 ppm) per triglyceride molecule. Methyl end-group protons were used as a reference (9H per molecule, $\delta = 0.92$ ppm).
Characterisation of unreacted algae oil after processing with sulfur

FAME analysis by GC-FID

Figure S6 | Fatty acid profile of the oil fraction obtained after reaction of the algae oil with sulfur. The green and yellow bars show the FAMEs from the algae oil before and after the reaction with sulfur, respectively. No polyunsaturated fatty acids (PUFAs) were detected in the FA profile of the inverse vulcanised oil sample (yellow bars). Less than 10% of the fatty acid esters were monosaturated in the sulfur processing method. This is less than that observed in the catalytic hydrogenation method (21% monounsaturated fatty acid ester products). This experiment demonstrates that sulfur is effective at removing unsaturated fatty acids from the algae oil mixture by conversion to a polymer material.

Figure S7 | After the algae oil is reacted with sulfur, the extractable unreacted oil contains >90% saturated fatty acids. This result indicates that sulfur is effective at reacting and removing unsaturated fatty acid triglycerides from the algae oil mixture.
\(^1\)H NMR spectroscopy of algae oil extract after reaction with sulfur

**Figure S8** \(^1\)H NMR spectrum of soluble fraction of algae oil in CDCl\(_3\) after copolymerization with sulfur, acquired on a 400 MHz spectrometer. The spectrum indicates an average of 0.52 alkenes (shown as 1.04 alkene protons, sum of \(\delta = 5.35\) and 5.39 ppm) per triglyceride molecule, methyl end-group protons were used as a reference (9H per triglyceride, \(\delta = 0.89\) ppm).
Characterisation of Algae Oil Sulfur Copolymer

Physical description
The solid product formed after the reaction of the algae oil and sulfur is a dark brown and brittle. This is a contrast to other polymers made from unsaturated vegetable oils and sulfur that are soft, rubbery, and friable.\textsuperscript{2,3}

\textbf{Figure S9} | Algae oil and sulfur copolymer
SEM and EDS

Figure S10 | Top: SEM micrographs of algae oil polymer showing a whole particle (roughly 6 mm in diameter) and a high magnification image of the surface. Bottom: SEM micrograph and corresponding EDX elemental map of algae oil polymer demonstrating the distribution of sulfur, carbon, and oxygen on the surface. The material is a composite of unreacted sulfur crystals embedded in a polysulfide polymer.
Figure S11 | Elemental map of algae oil polymer by EDX. The top left shows the corresponding SEM micrograph with an overlay of where the EDX signal was concentrated on the sample.
Simultaneous Thermal Analysis

Figure S12 | STA analysis the polymer product formed by the reaction of sulfur with the algae oil. DSC trace in orange, TGA in blue. The exotherm at 126 °C coincides with the melting point of sulfur and indicates the presence of unreacted S\textsubscript{8} within the polymer. Based on a calibration curve of sulfur melting,\textsuperscript{2} this exotherm indicates 50% of the mass of the polymer is free sulfur.

Differential Scanning Calorimetry for Glass Transition Temperature

Figure S13 | DSC trace of algae oil polymer from -60 to 140 °C. Glass transition temperature could not be derived from this data. The endotherms at 100–120 °C in the heating step and exotherms at 10–50 °C in the cooling step are due to melting and crystallising events of the free sulfur trapped within the polymer composite.
\[ ^1H \text{NMR spectroscopy of polymer formed from reaction of sulfur with algae oil} \]

**Figure S14** | \(^1H\) NMR spectrum of algae oil polymer in pyridine-d\(_5\). The spectrum indicates an average of 0.18 alkenes (shown as 0.36 alkene protons, \(\delta = 5.72\)) per triglyceride molecule. Methyl end-group protons were used as a reference (9H per molecule, \(\delta = 0.90\) ppm). This indicates that this polymer vitrifies and can be isolated with some unreacted alkene in its structure.

**Scanning Electron Microscopy and Electron Dispersive X-Ray Spectroscopy**
Figure S15 | Above: XRD spectrum of algae oil polymer. Below: XRD spectrum of algae oil polymer, sulfur ($S_8$) reference spectra overlayed in red. The spectrum appears to be an identical match for crystal sulfur. This result confirms that the polymer product is co-isolated with unreacted elemental sulfur presenting as crystalline $S_8$.

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