Electronic Supplementary Material (ESI) for Catalysis Science & Technology

Increased hydrolysis by *Thermomyces lanuginosus* lipase for omega-3 fatty acids in the presence of a protic ionic liquid.

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Supporting information

Experimental procedure

Materials

Bleached anchovy fish oil was supplied by Ocean Nutrition Canada. The main fatty acids of the fish oil were: Myristic acid (C14:0) 8.4%, Palmitic acid (C16:0) 16.7%, Palmitoleic acid (C16:1n7) 9.8%, Oleic acid (C18:1n9 – cis + trans) 7.1%, EPA (C20:5n3) 17.0%, DHA (C22:6n3) 13%. Lipase from *Thermomyces lanuginosus* (TLL) was a generous gift from Novozymes Australia Pty Ltd. Gas and thin layer chromatography standards were purchased from Sigma chemical company Australia and *Nu-Check*-Prep. (Elysian, Minn. 56028, USA). All other chemicals used were of analytical grade.

Lipase concentration determination

Lipase concentration was determined at the Australian Proteome Analysis Facility (APAF) using high sensitivity amino acid quantification technique of Waters AccQTag Ultra chemistry on a Waters ACQUITY UPLC system by the combination of two previously reported techniques[1,2]

Ionic liquid preparation

The protic ionic liquid triethylammonium methanesulfonate (TeaMs) was prepared as previously reported[3].

Circular dichroism (CD) spectroscopy

CD spectra analysis of lipase (concentration: 215 and 645µg/mL for secondary and tertiary structures, respectively) was carried out under nitrogen atmosphere on a Jasco J-815 CD spectrophotometer (ATA Scientific, NSW, Australia) using a quartz cell of 0.1 cm path length (Starna Pty. Ltd., Atascadero, CA, USA). Wavelength scans were collected using a 1.0 nm bandwidth, 1.0 s averaging time and an average of 5

scans for each sample. Spectra were smoothed but not baseline corrected and signals were recorded in ellipticity (θ) given in millidegrees (mdeg)

Enzymatic hydrolysis of fish oil

Unless otherwise stated, 2g of anchovy oil, 150 µL of 0.1M-phosphate buffer (pH 8.0), were placed in a 50mL round bottom flask for 5 minutes under nitrogen and magnetic stirring at 200 rpm. After 5 minutes, 1200 U of TLL per gram of fish oil was then added and incubated at 40°C for 30/36 hours. During the hydrolysis, a Cyberscan 510 pH meter was used to monitor the pH. Glycerides and free fatty acids (FFA) portions of the hydrolysate were obtained by solvent extraction according to a previously reported method[4]. The percentage hydrolysis was determined from the acid and saponification values of the oil before and after hydrolysis as earlier reported[5]. The whole lipid, glycerides or FFA fractions as the case may be were kept under nitrogen and stored in a refrigerator until used.

Analysis of lipid classes by thin-layer chromatography

Both the hydrolyzed and unhydrolyzed portions of the fish oil were analysed by thin-layer chromatography with flame ionization detector (TLC-FID) (Iatroscan MK5, Iatron Laboratories Inc., Tokyo, Japan). The Iatroscan settings were: air flow rate; 200mL/min, hydrogen flow rate; 160mL/min and scan speed of 30secs/scan. Under these conditions, the chromarods were scanned twice for cleanliness before applying samples. One microliter of the lipid fractions contained in hexane were spotted on the rods with the aid of an auto pipette along the line indicating the origin on the rod holder and developed for 22 minutes in a solvent tank containing hexane/diethyl ether/acetic acid (60:17:0.2, vol/vol/vol). TLC standards purchased from Nu-check (Elysian, Minn. 56028, USA) were used to identify the peaks of each lipid class.

Analysis of fatty acid composition by gas chromatography

Prior to the determination of their composition by gas chromatography, fatty acids in both the unhydrolyzed and hydrolyzed fish oil were converted to methyl esters following the method of Christie and Han[5] with some modifications. Ten milligrams of oil was dissolved in 1 mL toluene, and 200 µL of internal standard (5 mg/mL methyl nonadecanoate (Sigma-Aldrich) in toluene) and 200 µL of antioxidant (1 mg/mL 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene; BHT, Sigma-Aldrich) in toluene) were added. To this, 2 mL of hydrogen chloride in methanol (prepared by adding 5 mL

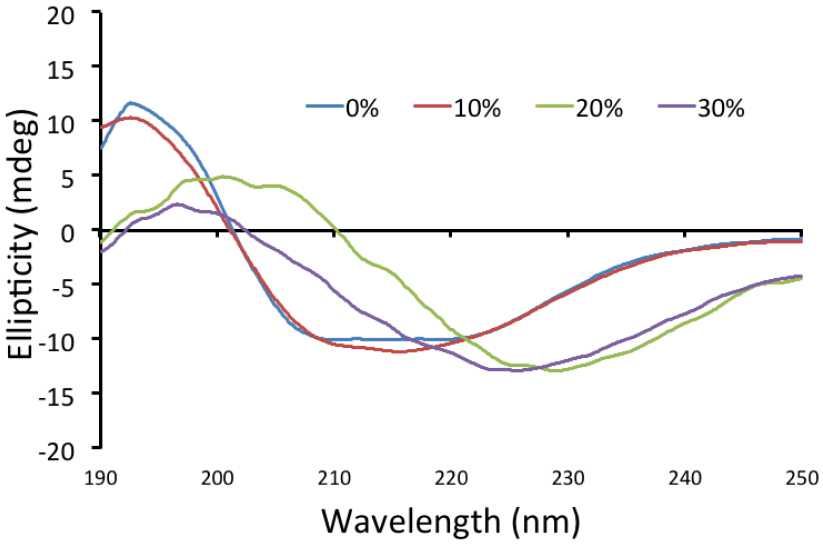
acetyl chloride (Sigma-Aldrich) drop wise to 50 mL methanol on ice) was added, the solutions mixed and left overnight at 50 °C in a sealed tube. The solution was cooled and 5 mL sodium chloride solution (5% m/v) was added. The fatty acid methyl esters were extracted twice with hexane (5 mL) and the hexane layer washed with 5 mL potassium bicarbonate solution (2% m/v). The hexane layer was dried over sodium sulphate, and most of the hexane was removed by rotary evaporation, leaving 1.5 mL for analysis.

Samples were analysed using an Agilent 6890 gas chromatograph with flame ionisation detector (FID), equipped with a Supelcowax 10 capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness; Supelco). The oven was programmed from 140°C (5 min hold) to 240°C (10 min hold) at a rate of 4°C/min for a total run time of 35 min. A volume of 1 µL of solution was injected with a split ratio of 50:1 (injector temperature, 250°C). Helium was used as the carrier gas (1.5 mL/min, constant flow). Detector gases were 30 mL/min hydrogen, 300 mL/min air and 30 mL/min nitrogen. Peak areas were integrated by ChemStation software and corrected using theoretical relative FID response factors[6].

Unless otherwise stated, all experiments in this study were carried out in triplicates with standard error below 5%.

Results and Discussion

a)



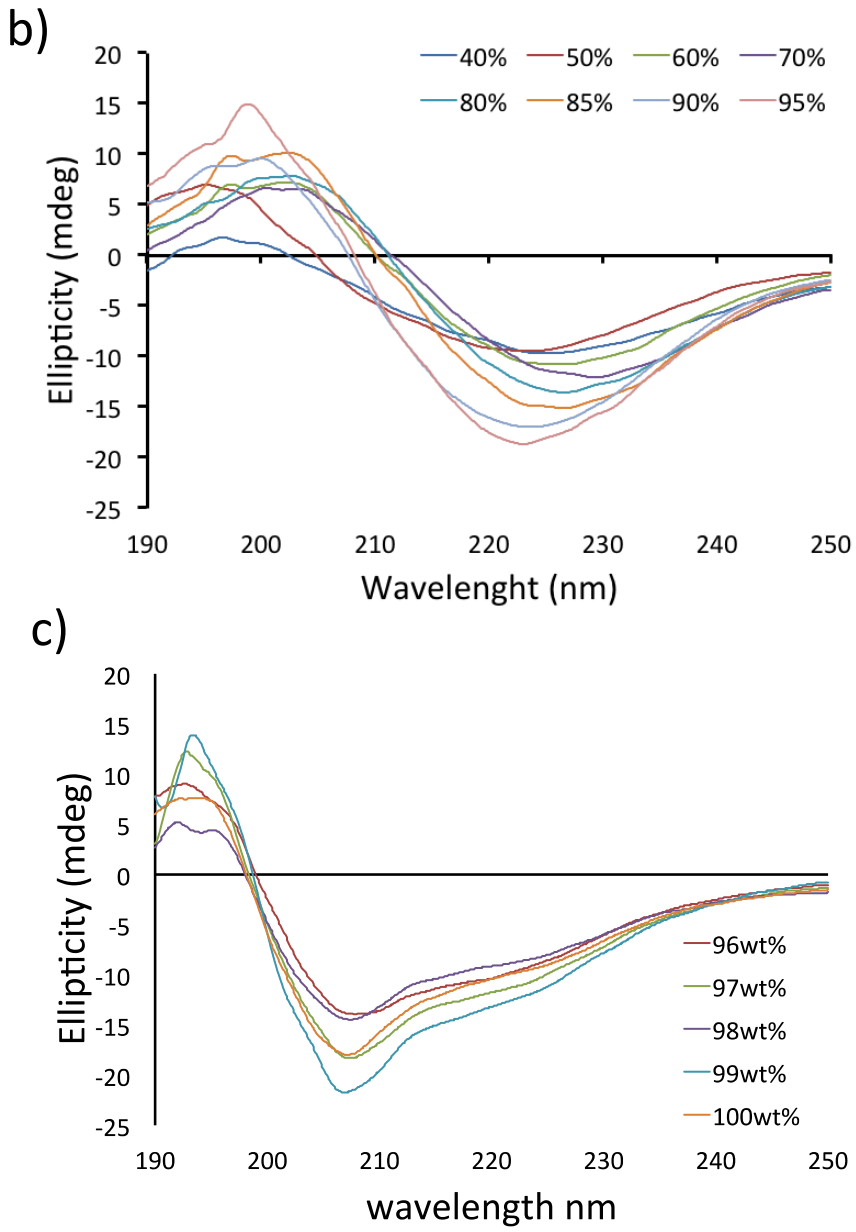


Figure S1: CD spectrum showing the changes in secondary structure of TLL as a function of TeaMs concentration where TeaMs concetrations are a) 0-30wt%, b) 40-95wt% and c) is 96-100wt%

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