Lipid profiles, in vitro digestion and oxidative stability of mutton bird oil

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Abstract The lipid profile, in vitro digestion and oxidative stability of mutton bird oil were investigated. Wax ester, triacylglycerol and sterol were the major lipids present as determined using capillary chromatography with flame ionisation detector (Iatroscan). Fatty acid analysis by gas chromatography (GC) showed that wax esters had a higher total omega-3 fatty acids content including EPA, DPA and DHA than TAGs (31 % and 24 %, respectively). In TAGs, ¹³C nuclear magnetic resonance (NMR) data showed that EPA was statistically positioned at *sn*-1,3 and *sn*-2, while DHA was preferentially at *sn*-2. *In vitro* digestion using porcine pancreatic lipase resulted in 75 % of TAG and 10 % wax ester hydrolysis in 120 min. As reflected in the measured conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) values during accelerated oxidation at 60 °C for 5 days, the oil was relatively stable against oxidation considering its high omega-3 content.

Keywords Mutton bird oil · Triacylglycerol · Wax ester · Carotenoids · Omega-3

Introduction

Mutton birds found mainly on the oceanic islands of Australia and New Zealand were a major part of the diets of some indigenous people (Anderson 1996). These birds belong to the petrel group in the family *Procellariidae* (Anderson 1996). Australian species, also known as Tasman mutton birds

(*Puffinus tenuirostris*), are found mainly on the Furneaux Island of the eastern Bass Strait (Anderson 1996). Aboriginal communities in Tasmania use mutton birds and their eggs as food (Skira 1990). The chicks are harvested and the meat sold for human consumption, feathers are also used for bedding and the oil has been used as liniment (Skira 1990). Apart from being a good source of meat, mutton birds are also rich in omega-3 oils, including appreciable quantities of oil found in their stomach (Warham 1977).

The reddish-brown colour of the stomach oil of mutton bird has been associated with their feeding on Australian krill (Virtue et al. 1995). While this may be true to some extent, the high wax ester content of mutton bird oil, as compared to none in krill oil (Weimerskirch and Cherel 1998), indicates the complex nature of the mutton bird's diet. Studies have shown that oily fish, such as anchovy and mackerel, squids and crustaceans that are very rich in omega-3 fatty acids can form a significant part of the mutton bird's diet (Einoder et al. 2013; Weimerskirch and Cherel 1998). Also, the unidentified fish species found in the diets of mutton birds (Einoder et al. 2013) can come from a combination of 22 species earlier identified in the Bass strait region where these birds exclusively feed (Dunstan et al. 1988). Therefore, profiling the lipid classes of the oil may provide information on food sources, as well as indicate the potential health benefits of this oil as a food or nutritional supplement.

Mutton bird oil possesses an array of therapeutic benefits. Based on its high omega-3 fatty acid contents, including EPA and DHA, it was concluded that the consumption of 1 g oil per day would provide approximately 160 mg omega-3 fatty acids per day, thereby doubling a typical Australian's omega-3 fatty acid intake (Woodward et al. 1995). The atherogenicity (IA) and thrombogenicity (IT) indexes of mutton bird oil have been reported to be 0.21 and 0.08, respectively, which suggest that this oil may be helpful to individuals with atherosclerotic or

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thrombotic conditions (Woodward et al. 1995). The smaller the IA and IT values, the greater the protective potential for coronary artery disease (Fernandes et al. 2014). Mutton bird oil is a healthy oil and is currently used as dietary supplements in Australia.

There is considerable interest in the triacylglycerol, ethyl esters and free fatty acids forms of omega-3 fatty acid supplements (Kralovec et al. 2010) and the relative digestion of these preparations have been widely studied (Yang et al. 1990a, 1990b). For instance, during the *in-vitro* hydrolysis of menhaden oil and its ethyl ester form by pancreatic lipase, the cleavage of the alkyl esters in ethyl ester and primary ester bonds in the triacylglycerol were observed (Yang et al. 1990b) suggesting that none of these was completely resistant to hydrolysis by pancreatic lipase. However, there is limited information on the hydrolysis of naturally occurring omega-3 rich wax esters, probably because these lipids are less abundant and do not form major parts of human diets. Since, much of mutton bird oil comprises wax esters (Woodward et al. 1995), *in-vitro* digestion of the oil may provide useful information on the digestion and absorption of this complex and nutrient-dense oil.

Carotenoids have been reported to act as antioxidants in oils by quenching singlet oxygen or free radicals (Conn et al. 1992). For instance, β -carotene is capable of scavenging oxygen and peroxy radicals (Conn et al. 1992), thereby protecting oil against light-induced oxidation. However, because of their high level of unsaturation, carotenoids may be susceptible to degradation during prolonged storage (Hidalgo and Brandolini 2008). Although mutton bird has been reported to be a rich source of carotenoids such as β -carotene and astaxanthin (Woodward et al. 1995), the specific roles of these carotenoids in stabilising the oil is unknown.

It has been reported that the positional distribution of EPA and DHA in the triacylglycerol (TAG) of oil impacts their bioavailability (Christensen et al. 1998). For instance, higher levels of DHA have been found in the brain when administered orally in oil with DHA located in position 2 (*sn*-2), whereas in oil where DHA is randomised in all the three positions (i.e. *sn*-1, *sn*-2 and *sn*-3), more DHA is found in the liver (Christensen et al. 1998). DHA located in the *sn*-2 position is also more stable to oxidation than when it is located in the *sn*-1,3 positions (Wijesundera et al. 2008). Therefore, information on the positional distribution of omega-3 fatty acids in the TAG of mutton bird oil could indicate information on potential health benefits, in addition to enable comparison with dietary oil sources for mutton bird.

Therefore, this study focuses on the regiospecific analysis of the omega-3 fatty acids in TAG portion of mutton bird oil using ^{13}C NMR as well as *in vitro* digestion of the oil using pancreatic lipase. Also, the oxidative stability of mutton bird oil was investigated by comparing its conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) values at

different storage times and these results were validated using Fourier transform infrared (FTIR) spectroscopy.

Materials and methods

Materials

Mutton bird oil was a gift from Furneaux oil, Flinders Island, Tasmania and all fish oils were provided by Ocean Nutrition Canada (now DSM). Gas and thin layer chromatography (TLC) standards were purchased from Nu-Chek Prep (Elysian, MN, USA). Porcine pancreatic lipase was purchased from Sigma–Aldrich (Castle Hill, Australia). Deuterated chloroform (CDCl_3 ; 99.8 % pure) was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). All other chemicals were of analytical grade.

Analysis and separation of lipid classes

Lipid classes were analysed by capillary chromatography with flame ionisation detector (Iatroscan MK5, Iatron Laboratories Inc., Tokyo, Japan). The Iatroscan settings were as previously reported (Akanbi et al. 2013). Ten milligrams (10 mg) of oil was dissolved in 2 mL of chloroform/methanol mix (2:1 v/v) and 1 μL of the sample was spotted onto the Iatroscan chromarods along the origin on the rod holder and developed in three different solvent systems for different time periods. The rods were first developed for 22 min in hexane/acetic acid (99.9:0.1, v/v), followed by another 22 min development in 100 % toluene and finally in hexane/diethyl ether/acetic acid (60:17:0.2) for 10 min. The relative amount (in percentage) of each lipid class was determined using the SIC-480 II software for multiple chromatogram processing.

For lipid class separation, 750 mg of oil was suspended in 5 mL of chloroform/methanol mix (2:1 v/v) and carefully spotted onto thin layer chromatographic plates (TLC; 20×20 cm) (Merck). Plates were developed under the same conditions described above and spots were visualized under UV. Once the lipid classes have been identified using standards, spot representing each lipid class was scraped off, re-suspended in the chloroform/methanol mix (2:1 v/v) and kept in sealed dark bottles under nitrogen at 4°C for no more than one week before use.

Analysis of fatty acids composition by gas chromatography

Fatty acid compositions of the lipid classes of mutton bird oil were determined by gas chromatography (GC) after drying off the chloroform/methanol solvent with nitrogen. Fatty acid methyl esters (FAMES) of samples were analysed by an Agilent 6890 gas chromatography with flame ionisation

detector (FID) equipped with a BPX70 SGE column (30 m, 0.25 mm i.d., 0.25 μ m film thickness). The oven was programmed from 140 °C (5 min hold) to 220 °C (5 min hold) at a rate of 4 °C/min for a total run time of 30 min. A volume of 1 μ L 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. Theoretical relative FID response correction factors (Ackman 2002) were used when calculating the content of individual fatty acid.

¹³C NMR spectroscopy

Positional distribution of fatty acid in the TAG of mutton bird oil and other fish oils was analysed using carbon NMR. Quantitative ¹³C NMR spectra were recorded under continuous ¹H decoupling at 24 °C in a Bruker Avance 500 MHz and spectra were collected on 100 mg of each sample dissolved in 700 μ L 99.8 % CDCl₃ (Cambridge Isotope Laboratories Inc., MA, USA).

In vitro digestion with pancreatic lipase

Enzymatic hydrolysis of mutton bird oil was carried out using porcine pancreatic lipase as previously reported (Akanbi et al. 2014). Briefly, 300 mg of oil, 100 μ L of 22 % calcium chloride (CaCl₂) solution, 200 μ L of 1 M tris(hydroxymethyl)aminomethane at pH 7.7 and 320 μ L of 0.1 % bile salts solution were placed in a 5 mL flask and the mixtures were flushed with nitrogen. Hydrolysis was carried out in the dark at 37 °C under nitrogen with magnetic stirring at 200 rpm for 2 h and aliquots of sample were withdrawn at different time intervals. Lipid classes were analysed by Iatroscan and the relative amount of each lipid class was determined using the SIC-480 II software for multiple chromatogram processing.

Oxidative stability tests

The oxidative stability of mutton bird oil was studied by heating the oil for 96 h at 60 °C in the dark and under a stream of air at a flow rate of 10 L/g as previously described (Akanbi and Barrow 2014). Five hundred milligrams (500 mg) of oil sample was placed in a reaction vessel fitted with a screw cap containing air inlet and outlet valves. An air tube, made of borosilicate glass was inserted to ensure a steady airflow distribution and effluent air was channelled out using PVC tubing with light and kink protection. Under these conditions (60 °C and 10 L/g air flow rate), samples were withdrawn at 0, 6, 12, 24, 48, 72, 96 and 120 h. Samples were cooled to room

temperature, flushed with nitrogen, tightly capped and stored at –20 °C until analysed.

The conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) values of the oil samples were measured as previously reported (Hamam and Shahidi 2004).

ATR-FTIR spectroscopy

Attenuated total reflectance fourier transform infrared (ATR-FTIR) measurements of the native and oxidized portions of mutton bird oil were conducted using an Alpha FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) following a previously reported method (Vongsivut et al. 2014) with slight modifications. Briefly, 1 μ L of oil sample was placed onto the 2 × 2 mm² sensing surface of the diamond ATR crystal, and 3 high-quality spectra were collected from each sample. The ATR-FTIR spectra were acquired at 4 cm^{–1} spectral resolution with 64 co-added scans. Blackman-Harris 3-Term apodization, Power-Spectrum phase correction, and zero-filling factor of 2 were set at default acquisition parameters using OPUS 6.0 software suite (Bruker). Background spectra of a clean ATR surface were acquired prior to each sample measurement using the same acquisition parameters.

Statistical analysis

Unless otherwise stated, experiments were carried out in triplicates and data are presented as the mean $\pm$ standard deviation (SD). For fatty acid analysis of lipid classes, a Levene's test of one-way analysis of variance (ANOVA) was used to verify the homogeneity of variance and unpaired t-test was used to compare between their means. Multiple comparisons were achieved by Tukey-Kramer HSD (honestly significant difference). Mean differences were considered to be significant when probability is less than 0.05 ($P < 0.05$). Statistical analysis was performed using SPSS® statistics 22.0 software.

Results and discussion

Lipid class and fatty acid analyses of mutton bird oil

Results of the lipid class analysis of mutton bird oil, as measured by TLC-FID (Iatroscan) show a high amount of wax esters (66.0 $\pm$ 1.8 %), followed by triacylglycerols (TAG) (32.9 $\pm$ 0.9 %) and a small amount of sterols (1.1 $\pm$ 0.1 %). These lipid class profiles are similar to those reported earlier (Connan et al. 2005).

The fatty acid profiles of the TAG and wax ester portions of mutton bird oil after separation by thin layer chromatography are presented in Fig. 1. As shown, there are significant differences ($P < 0.05$) between the fatty acid compositions of both

Fig. 1 Major fatty acids associated with the triacylglycerol (TAG) and wax esters of mutton bird oil. Results are mean $\pm$ SD of triplicate replications. Bars not sharing a common letter are significantly different ($P < 0.05$) between mean values within the fatty acids. Total n-3 includes α -linolenic acid, ALA (C18:3n3) stearidonic acid, STA (C18:4n3), eicosapentaenoic acid, EPA (C20:5n3); docosapentaenoic acid, DPA (C22:5n3), docosahexaenoic acid DHA (C22:6n3). Total n-6 includes linolenic acid, LA (C18:2n6); γ -linolenic acid, GLA (C18:3n6); Dihomo- γ -linolenic acid, DGLA (C20:3n6); arachidonic acid, AA (C20:4n6)

lipid classes. Although, both lipid classes have high total omega-3 (*n*-3) fatty acids including EPA and DHA, those in TAG were lower (24.1 ± 0.6 %) than those in wax esters (30.7 ± 1.4 %) (Fig. 1a). For total omega-6 (*n*-6) fatty acids, slightly lower amounts (4.0 ± 3.0 %) were found in the TAG when compared with the wax esters (5.2 ± 0.2 %) (Fig. 1a). The TAG had a higher ($P < 0.05$) content of myristic and palmitic acids while wax esters had higher ($P < 0.05$) palmitoleic and oleic acids (Fig. 1b).

Mutton bird oil has an EPA and DHA content similar to omega-3 rich fish oils such as tuna and anchovy oils, but has higher levels of wax esters and free fatty acids. Free fatty acids are generally more rapidly bioavailable than acylglycerols, while wax esters in mutton bird oil may also assist in maintaining healthy cholesterol, since wax esters have been found to lower the concentration of liver cholesterol (Hargrove et al. 2004).

Regiospecific analysis of triacylglycerol in mutton bird oil using ^{13}C -NMR spectroscopy

Quantitative ^{13}C NMR spectra of the TAG portion of mutton bird oil were recorded using Bruker Avance 500 MHz spectrometer in order to understand the fatty acid positional distribution. Figure 2 shows the ^{13}C NMR spectra of the major fatty acids in the isolated TAG (Fig. 2a). Each peak was assigned as previously reported (Akanbi et al. 2013). The molar percentage of each fatty acid at positions 1 and 3 (*sn*-1,3) and position 2 (*sn*-2) are presented in Fig. 2b. As shown in Fig. 2b, the TAG has saturated and monounsaturated fatty acids predominantly located in *sn*-1,3 positions, while stearidonic acid (STA; C18:4, Δ 6) is distributed in an approximately statistical 1:1 ratio over *sn*-2 and *sn*-1,3. EPA is nearly randomised, with 62 mol% of the total EPA at *sn*-1,3 and 38 mol% at *sn*-2 (Fig. 2b insert). DHA is preferentially located at the *sn*-2 position, with 64 % at *sn*-2 and 36 % at *sn*-1,3.

It is not possible to make direct comparison between the positional distribution of omega-3 fatty acids in the TAG of mutton bird oil and the oils of many Australian marine organisms that form parts of mutton bird's diet, as they are complex and numerous. However, it is possible to compare the distribution of its omega-3 fatty acids especially EPA and DHA with those of fish oils, since their distributions are somewhat similar (Table 1). For instance, DHA was preferentially located in *sn*-2 position of salmon, mackerel and herring TAGs, while EPA was more evenly distributed across all three positions (Standal et al. 2009). Similar distributions have been observed for anchovy (Akanbi et al. 2013), squid (Ikeda et al. 1998), tuna (Sacchi et al. 1993) and hoki (Miller et al. 2011) oil TAGs. Since the positional distribution of EPA and DHA in the TAG of mutton bird oil is similar with these fish oil TAGs, it is likely that fish form a major part of the mutton birds' diet (Connan et al. 2005; Einoder et al. 2013; Weimerskirch and Cherel 1998).

In vitro digestion with pancreatic lipase

The result of the *in vitro* digestion of mutton bird oil by porcine pancreatic lipase (PPL), a lipase that shows a very high similarity index to human pancreatic lipase (Wishart et al. 1993) is presented in Table 2. As measured by Iatroscan, a combination of triacylglycerols (TAGs), free fatty acids (FFAs), diacylglycerols (DAGs) and monoacylglycerols (MAGs) were observed after hydrolysis. Results showed a rapid decrease in TAG from 33 to 8.4 % after 120 min, resulting in 75 % hydrolysis, while wax esters were poorer substrates for PPL, with approximately 10 % hydrolysis after 120 min. These results indicate that significant proportion of FFA, DAG and MAG released during hydrolysis were from TAG. Increases in the amounts of FFA and MAG during hydrolysis are characteristics of pancreatic lipase (Akanbi et al.

Fig. 2 a ^{13}C NMR spectra of major fatty acids in the triacylglycerol (TAG) of mutton bird oil. b Positional distribution of major fatty acids in the TAG as measured by ^{13}C NMR. Insert: positional distribution of EPA and DHA

2014). Since it has been shown that EPA and DHA are highly resistant to hydrolysis by pancreatic lipase (Akanbi et al. 2014; Yang et al. 1990b), the remaining 8.4 % TAG after hydrolysis should be highly enriched in EPA and DHA.

Although, there is very limited information on the hydrolysis of naturally occurring omega-3 rich wax esters by pancreatic lipase, the poor hydrolysis rate of wax esters as compared with TAG by PPL in this study is consistent with other

Table 1 Positional distribution of EPA and DHA in the TAG of fish oils and mutton bird oil

Position	Fatty acid	Molar percentage (%) ^a			
		Anchovy oil	Tuna oil	Benito oil	Mutton bird oil
sn-1,3	EPA	75	67	66	62
	DHA	39	46	48	36
sn-2	EPA	25	33	34	38
	DHA	61	54	52	64

^a Values are means of three (^{13}C NMR) spectral fittings. For each spectrum, 12,000 scans were collected

results showing that pancreatic lipase hydrolyses TAG faster than wax esters (Hargrove et al. 2004). A much less specific pancreatic enzyme, carboxyl ester lipase (CEL) (EC 3.1.1.13), has been reported to hydrolyse a broad range of substrates, including wax esters, cholesterol esters, phospholipids and lysophospholipids and others (Hargrove et al. 2004; Lindström et al. 1988). Under normal physiological functions, CEL has been shown to hydrolyse ester in oils after TAG has been hydrolysed by pancreatic lipase (Lindström et al. 1988). Hydrolysis of wax esters releases FFA and alcohol, which are both readily absorbed by the intestinal epithelium (Hargrove et al. 2004). It has been shown that despite a low hydrolysis rate of omega-3 rich ethyl esters by pancreatic lipase, their polyunsaturated fatty acids are completely absorbed in the mammalian digestive tract since they are incorporated into the chylomicrons and very low-density lipoprotein (VLDL) in the enterocyte (Nelson and Ackman 1988). Besides, the presence of omega-3 rich wax esters in mutton bird oil may help in lowering cholesterol levels, since studies have shown that PUFA-rich wax diets aided in lowering liver cholesterol (Hargrove et al. 2004). Therefore, pancreatic enzymes can digest the omega-3 fatty acids from mutton bird oil in both TAG and wax ester forms, meaning that all EPA and DHA in mutton bird oil will be bioavailable.

Oxidative stability of mutton bird oil

The appearance of conjugated dienes (CD) that absorbs at 234 nm indicates the rearrangement of non-conjugated double bonds of lipids that contain dienes or polyenes upon oxidation (Logani and Davies 1980). Accumulation of conjugated dienes is an indication of an increase in the formation of hydroperoxides, a primary oxidation product of fats (Hamam and Shahidi 2004). Figure 3 shows the CD contents of mutton bird oil under accelerated oxidation at 60 °C. As shown, there was no change in the CD values between 0 and 6 h, while there was a steady increase in the CD values from 6 to 48 h as the reddish-brown pigment progressively faded. The CD values sharply increased from 48 to 96 h after which a peak was reached within 120 h. These indicate that as the storage time increased, hydroperoxides, free radicals and oxidation chain reaction increased (Hamam and Shahidi 2004).

TBARS is a widely used test for assessing the presence of secondary oxidation products such as aldehydes and dialdehydes in oxidized oils (Akanbi and Barrow 2014). As shown (Fig. 3), similar CD trends were observed for TBARS except that there was a sharp increase after 24 h and a peak was reached within 120 h, indicating an accumulation of secondary oxidation products. This is not surprising, since studies have shown that the amounts and nature of hydroperoxides produced during oxidation may increase the rate at which secondary oxidation products are formed (Hamam and Shahidi 2004; Akanbi and Barrow 2014). In comparison,

Table 2 Lipid class composition of mutton bird oil during hydrolysis by PPL

Time of digestion (min)	Lipid classes (%) ¹					
	WE	TAG	ST	FFA	DAG	MAG
0	66.0 ± 1.8 ^d	32.9 ± 0.9 ^d	1.1 ± 0.1 ^b	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a
30	64.0 ± 0.3 ^c	22.8 ± 0.9 ^c	0.7 ± 0.2 ^a	7.1 ± 0.2 ^b	3.1 ± 0.5 ^b	2.3 ± 0.0 ^b
60	62.7 ± 1.3 ^b	13.0 ± 1.6 ^b	0.9 ± 0.1 ^a	15.8 ± 0.4 ^c	4.0 ± 0.3 ^c	3.6 ± 0.1 ^c
120	59.7 ± 1.1 ^a	8.4 ± 0.7 ^a	0.8 ± 0.0 ^a	22.7 ± 0.3 ^d	2.9 ± 0.7 ^b	5.5 ± 0.6 ^d

Values are mean ± standard deviation. Mean values in a column with different letters are significantly different as determined by Tukey-Kramer HSD, $P < 0.05$

WE wax esters, TAG triacylglycerol, ST sterol, FFA free fatty acids, DAG diacylglycerol, MAG monoacylglycerol

¹ Determined by capillary chromatography, TLC–FID

anchovy oil containing EPA (18 %) and DHA (12 %) subjected to the same oxidation conditions oxidized much more rapidly than mutton bird oil, with CD of 15.7 and TBARS of 8.2 $\mu\text{mol/g}$ after 12 h. The greater stability of mutton bird oil versus anchovy oil should be advantageous in food fortification with omega-3 rich oils, as oxidative stability is a limiting factor in omega-3 functional food development.

The lipid classes, carotenoids and the degree of unsaturation of fatty acids are believed to have direct influence on the oxidative stability of mutton bird oil. For instance, the presence of wax esters in the oil may have contributed to the slow rate of oxidation within the first few hours, since omega-3 rich wax esters have been reported to oxidize slowly (Gorreta et al. 2002). Also, its carotenoids which are mainly β -carotene and astaxanthin appeared to have acted as antioxidants, slowing the rate of CD and TBARS increase during the time when carotenoid pigments faded. It has been reported

that β -carotene can act as a chain breaking antioxidant, thereby suppressing CD formation (Paloza and Krinsky 1991). Lastly, the production of hydroperoxides from the unsaturated fatty acids such as oleic acid (C18:1n9), EPA (C20:5n3) and DHA (C22:6n3) may have been delayed until carotenoids were fully oxidized and this may explain why the TBARS value increased sharply at 48 h.

To further determine if the carotenoids in the oil acted as antioxidants, the rates of oxidation of omega-3 fatty acids, particularly EPA and DHA, in mutton bird oil at different storage times under the accelerated oxidation conditions were studied by FTIR.

FTIR analysis

FTIR is a useful tool for characterising oils and fats (Vongsivut et al. 2014) as well as studying their degradation

Fig. 3 Conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) values of mutton bird oil when stored at 60 °C in the dark for 120 h (5 days) under a stream of air at a flow rate of 10 L/g. Values are mean ± SD for triplicate replications

under different storage conditions (Guillén et al. 2004). In addition, FTIR has been used simultaneously with other classic methods of analysing the oxidative stability of oils, including CD and TBARS (Chaijan et al. 2006). The FTIR spectrum of mutton bird oil provides qualitative information about its functional groups. Figure 4a shows the spectrum of mutton bird oil before oxidation. The bands were assigned as previously reported (Guillén et al. 2004) as: 3011 cm^{-1} , *cis* double-bond stretching; 2922 and 2853 cm^{-1} , asymmetrical and symmetrical stretching vibration of methylene ($-\text{CH}_2$) group; 1739 cm^{-1} , stretching ester carbonyl functional group of triglycerides; 1645 cm^{-1} , distributed *cis* $\text{C}=\text{C}$ of unsaturated acyl groups; 1463 cm^{-1} , bending vibrations of the CH_2 and CH_3 aliphatic groups; 1377 cm^{-1} , bending vibrations of CH_2 groups; 1240 and 1165 cm^{-1} , stretching vibration of the $\text{C}-\text{O}$ group; 915 cm^{-1} , out-of-plane bending vibration; 721 cm^{-1} , overlapping of the methylene ($-\text{CH}_2$) rocking vibration. The FTIR assignment of mutton bird oil is typical of fish oil (Guillén et al. 2004).

Fig. 4 **a** Full Fourier transform infrared (FTIR) spectra of mutton bird oil before oxidation. **b** Changes produced in the region between 2990 and 3040 cm^{-1} of the FTIR spectra of mutton bird oil when stored at $60\text{ }^{\circ}\text{C}$ in the dark for 120 h (5 days) under a stream of air at a flow rate of 10 L/g

To determine the rate at which the omega-3 fatty acids in mutton bird oil were oxidized, the spectral region at 3011 cm^{-1} representing the *cis* double bonds of PUFAs (Vongsvivut et al. 2014) were analysed at different storage times. Figure 4b shows that between 0 and 48 h of storage, the *cis* double bonds content decreased steadily. However, there was a sharp decline in the *cis* double bonds content after 48 h Fig. 4b. These results are consistent with those obtained for both CD and TBARS, since long chain fatty acids are the primary target of oxidation. Therefore, the apparent oxidative stability of mutton bird oil within the first 2 days of accelerated oxidation at $60\text{ }^{\circ}\text{C}$ under forced-air flow, could have been a result of the presence of carotenoids in the oil.

Conclusion

The lipid profiles, in vitro digestion and oxidative stability of mutton bird oil were investigated. It was shown using Iatroscan-FID that wax esters and TAG were the major lipid classes of mutton bird oil. GC results showed that there were significant differences ($P < 0.05$) between the fatty acid composition of the TAG and wax esters. Although, both contained relatively high total omega-3 (*n*-3) levels, TAG omega-3 content was lower ($24.1 \pm 0.6\%$) than wax esters omega-3 content ($30.7 \pm 1.4\%$). ^{13}C NMR analysis of its TAG showed that EPA was approximately randomly distributed between *sn*-1,3 and *sn*-2, while DHA was preferentially located in the *sn*-2 position. During in vitro digestion of the oil, pancreatic lipase found TAG as a better substrate than wax esters, releasing FFA, DAG and MAG. As reflected in the CD, TBARS and FTIR results, a slower rate of mutton bird oil oxidation was observed within the first 48 h of accelerated oxidation at $60\text{ }^{\circ}\text{C}$ but a rapid increase in oxidation was recorded thereafter, which could have been due to the degradation of carotenoids within the first 48 h.

The results of this study demonstrated that the lipid profiles of mutton bird oil are of a high nutritional quality. The high polyunsaturated and monounsaturated fatty acids contents of the oil as well as the presence of carotenoids make it potentially beneficial to health as an omega-3 oil. The oil may be suitable for lowering serum cholesterol, reducing the risk of dementia and Alzheimer's disease, regulating memory, mood and emotion and treatment and prevention of arthritis among others, although mutton bird oil has not yet been thoroughly studied for its health benefits. Mutton bird oil is a relatively stable omega-3 oil and so may be useful for omega-3 fortification of foods. Increased consumption of mutton bird oil will also help improve the economic prospects of the Aboriginal communities in Tasmania where this oil is produced.

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