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ORIGINAL RESEARCH ARTICLE

Simple lipids and hydrocarbons of New Zealand propolis wax

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The composition of neutral lipids of New Zealand propolis wax was determined with the use of Thin Layer Chromatography, Solid Phase Extraction, Gas Chromatography, and Gas Chromatography-Mass Spectrometry. Neutral lipids in the sample were represented mostly by wax esters, long-chain hydrocarbons, and free fatty acids. Low levels of free fatty alcohols were also observed, accompanied by even lower levels of 1-O-alkylglycerols. Wax esters consisted mostly of saturated non-hydroxylated and mono-hydroxylated fatty acids and alcohols, with some monounsaturated non-hydroxylated esters also present. Non-hydroxylated fatty acids contained from 16 to 36 carbon atoms, whilst hydroxylated acids contained from 14 to 26 carbon atoms. While almost 11% of non-hydroxylated fatty acids were monounsaturated, only trace levels of monounsaturated hydroxylated fatty acids were observed. Fatty alcohol moieties of wax esters were predominantly saturated and contained mostly from 24 to 34 carbon atoms per molecule. No polyhydroxylated fatty components were detected in the sample. Overall, the composition of the sample resembled that of beeswax.

Lípidos e hidrocarburos simples en la cera de propóleos neozelandeses

La composición de los lípidos neutros de la cera de propóleos neozelandeses se determinó con el uso de cromatografía de capa fina, extracción en fase sólida, cromatografía de gases y cromatografía de gases-espectrometría de masas. Los lípidos neutros de la muestra estaban representados principalmente por ésteres de cera, hidrocarburos de cadena larga y ácidos grasos libres. También se observaron bajos niveles de alcoholes grasos libres, acompañados de niveles aún más bajos de 1-O-alquilgliceroles. Los ésteres de cera consistieron principalmente en 10 ácidos grasos y alcoholes saturados no hidroxilados y monohidroxilados, con algunos ésteres monoinsaturados no hidroxilados también presentes. Los ácidos grasos no hidroxilados contenían de 16 a 36 átomos de carbono, mientras que los ácidos hidroxilados contenían de 14 a 26 átomos de carbono. Mientras que casi el 11% de los ácidos grasos no hidroxilados fueron monoinsaturados, sólo se observaron niveles de ácidos grasos monoinsaturados hidroxilados. Los ésteres de cera en forma de alcohol graso fueron predominantemente saturados y contenían principalmente de 24 a 34 átomos de carbono por molécula. No se detectaron en la muestra ningún componente graso poli- 15 hidroxilado. En general, la composición de la muestra se asemejaba a la de la cera de abejas.

Keywords: propolis; lipid; wax; fatty acid; fatty alcohol; New Zealand

Introduction

Propolis is a heterogenous mixture, consisting of exudates collected by bees from certain plant species, which is admixed with beeswax secreted from the hypopharangeal glands of worker bees. The wax content of crude propolis is highly variable, and can range from 16 to 80% (Burdock, 1998). The amount of wax is postulated to vary according to the availability of suitable plant exudates (Burdock, 1998). The majority of crude propolis collected for use in propolis products is processed to remove the wax, usually by ethanol or ethanol/water extraction to give a propolis resin. Some wax is also removable through separation from the resin by flotation (Burdock, 1998). Depending upon the processing method used, the wax separated from the New Zealand propolis resin is discarded, or returned to beekeepers. There is the potential for this wax to be used in the same applications as beeswax if the composition is similar. The industrial uses for beeswax are diverse and include in the beehive to store honey, as a protective coating for cheese, as an excipient in dietary supplements (food additive E901), for the manufacture of candles, cosmetics including lip balms, hand creams, eye shadow and hair pomades, and as a polish ingredient for shoes (Bogdanov, 2009; Kuznesof, 2005). The high "policosanol" (generic name for a mixture of long chain fatty alcohols) content of beeswax is of interest for dietary supplements to manage cholesterol (Hargrove et al., 2004). A preparation based on long chain alcohols isolated from Cuban beeswax, Abexol or D-002, has been tested for a variety of health conditions (Pérez, Oyárzabal, Mas, Molina, & Jiménez, 2013 and references therein).

The lipid composition of European honey bee beeswax has been previously reported by Aichholz and Lorbeer (1999), and Maia and Nunes (2013) and found to consist of mostly hydrocarbons, wax esters and small quantities of free fatty acids. The composition of

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European honey bee propolis wax has also been reported by Seifert and Haslinger (1989, 1991) and found to contain similar compounds. Seifert and Haslinger extracted the wax from the crude propolis using a hydrocarbon solvent. The lipid composition of a number of Brazilian propolis wax samples collected across Brazil has been determined by Negri, Marcucci, Salatino, and Salatino (1998) and compared with that of comb wax by Custodio, Ferreira, Negri, and Salatino (2003). The wax composition from both sources was found to be similar, demonstrating that the majority of the wax compounds are sourced from the bee, and not from botanical sources. The wax was separated from the propolis resin by ethanol extraction followed by cooling and filtration. However, Negri, Marcucci, Salatino, and Salatino (2000) also reported the presence of pentacyclic triterpenoids α- and β-amyrin and β-amyrin acetate in some samples of propolis wax but not in corresponding comb samples, which are most likely originated from botanical sources. Surprisingly, the composition of New Zealand propolis wax has not been reported. In this work, we report the simple lipids and hydrocarbons composition of NZ-sourced propolis wax.

Materials and methods

Materials

Propolis wax was obtained from the hydrocarbon extraction of crude New Zealand propolis supplied by Manuka Health. A reference standard of a wax ester, oleyl tricosanoate, was synthesised from tricosanoic acid (T6543, Sigma) and oleyl alcohol (O8880, Sigma) using the method described by Al-Arafi and Salimon (2012), and purified using silica Solid phase extraction (SPE). The purity >99% was confirmed by GC under conditions specified in the relevant section below. Reference materials also used in Thin layer chromatography (TLC) and GC experiments were stearic acid (as a free fatty acid), and squalene (a hydrocarbon).

Thin layer chromatography

TLC analysis was performed on Silica Gel 60 aluminium foil backed plates (Merck, Germany) using chloroform with 1% ethanol as the solvent system. Spots were visualised by charring after dipping in 5% H_2SO_4 solution in ethanol.

Solid phase extraction fractionation

Propolis wax sample, 21.6 mg, was dissolved in 1 ml hexane and fractionated using 0.5 g SI-1 SPE cartridges (Phenomenex, USA) pre-washed with 4 ml of hexane. The fractions were collected as follows: hydrocarbons were eluted by 6 ml hexane, yield – 1.5 mg (7% of the sample); non-hydroxylated wax esters – by 6 ml hexane/diethyl ether (99:1, by v/v) followed by 6 ml hexane/diethyl ether (9:1, by v/v), yield – 11.5 mg (53% of the sample); monohydroxylated wax esters, free fatty acids (FFAs) and free alcohols – by 10 ml hexane/diethyl ether (8:2, by v/v), 10 ml hexane/diethyl ether (1:1, by v/v) and 10 ml diethyl ether, yield – 7.2 mg (33% of the sample). The total recovered yield was 20.2 mg (93% of the sample). These fractions were analysed by TLC and GC.

Methanolysis of the sample

Propolis wax sample, 15.3 mg, was dissolved in 0.5 ml 1% sodium methoxide in dry methanol (30 min, 80 °C), then 1 ml of 5% anhydrous HCl in methanol was added and kept for 30 min at 80 °C. The products of methanolysis were taken up in hexane and analysed by GC and GCMS. FFAs were methylated by redissolving the FFA fraction in 0.5 ml of 5% anhydrous HCl in methanol (10 min, 60 °C). Fatty acids methyl esters were taken up in hexane and analysed by GC and GCMS.

GC and GCMS analysis

GC analysis of hydrocarbons, FFAs, free alcohols and methanolysed propolis wax was performed on a Trace GC Ultra (Thermo Fisher Scientific, USA) gas chromatograph equipped with a flame ionization detector (FID) and ZB-5 ms (30 m × 0.25 mm i.d., 0.25 μm) capillary column (Phenomenex, USA). Helium was used as carrier gas, split ratio – 1:30. Injector and detector temperatures were both 350 °C. Oven temperature programme started at 150 °C (hold for 2 min), followed by a rise to 335 °C at a rate of 4 °C/min, and maintained at the final temperature for 45 min. Quantitative GC analysis of wax esters was performed on a Trace GC Ultra (Thermo Fisher Scientific, USA) gas chromatograph equipped with a flame ionization detector (FID) and TAP CB UltiMetal (25 m x 0.25 mm i.d., 0.1 μm) capillary column (Agilent, USA). Helium was used as carrier gas, split ratio – 1:30. Injector and detector temperatures were both kept at 370 °C. Oven temperature was raised from 150 to 370 °C at a rate of 3 °C/min, and maintained at the final temperature for 10 min. Wax esters were identified by using qualitative data produced by GCMS. Oleyl tricosanoate was used as an internal standard for quantification of wax esters. Tricosanoic acid methyl ester was a reference for quantification of fatty acids, hydrocarbons and fatty alcohols.

GCMS analysis was performed on GCMS-QP2010Ultra (Shimadzu, Japan) gas chromatograph mass spectrometer equipped with FID using RTX5 ms (30 m × 0.25 mm i.d., 0.25 μm) capillary column (Restek, USA). Helium was used as carrier gas in a splitless mode. Injector and detector temperatures were both 350 °C. Oven temperature programme was started at 150 °C (hold for 2 min), followed by a rise to 335 °C at a rate of 4 °C/min, and maintained at the final temperature for 45 min. Components were identified by mass spectra with the use of the NIST NBS75 K database. All samples, including the original propolis wax

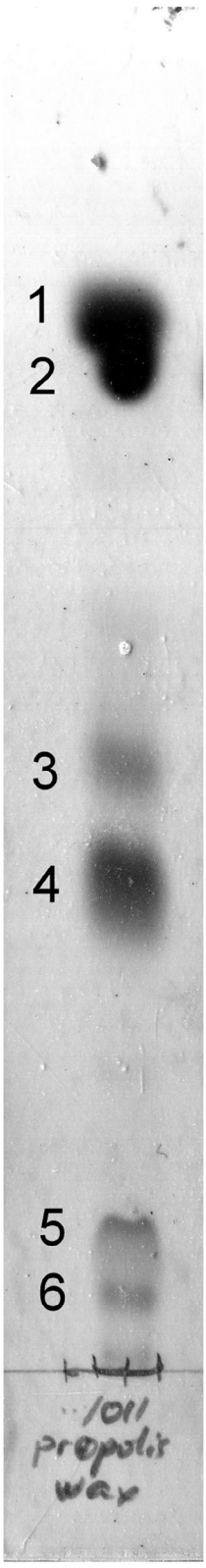

Figure 1. TLC of the propolis wax sample in $CHCl_3$ with 1% EtOH. Spots were visualised by charring after dipping in 5% H_2SO_4 solution in EtOH. 1, 2 Hydrocarbons and non-hydroxylated wax esters; 3. Hydroxylated wax esters of 14-hydroxy palmitic acid; 4. Hydroxylated wax esters of ω-2 hydroxy fatty acids; 5. Free very long-chain fatty acids (over 20 carbon atoms); 6. Free fatty acids (18–20 carbon atoms).

sample, were treated with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) that contained 1% trimethylchlorosilane (TMCS) prior to GC/GCMS analysis to ensure that hydroxylated components are able to be analyzed. Components were identified by comparison with reference materials and by GCMS, and quantified by their FID signals' area.

Results

Analysis by thin layer chromatography

One dimensional TLC with the use of reference materials revealed the presence of hydrocarbons and wax esters (spots 1 and 2), and free fatty acids with chromatographic mobility similar to that of stearic acid (spot 6), see Figure 1. The identities of the remaining spots were revealed by fractionation with preparative TLC followed by GCMS analysis of the resulting fractions. The analysis showed that the fraction corresponding to spot 3 was enriched with wax esters containing 14-hydroxy palmitic acid (i.e., ω3-hydroxyacid), the fraction corresponding to spot 4 represented wax esters that contained 15-hydroxy palmitic acid (i.e., ω2-hydroxyacid), the fraction corresponding to spot 5 was enriched with very long-chain (over 20 carbon atoms) non-hydroxylated fatty acids, and the fraction corresponding to spot 6 contained long-chain (18–20 carbon atoms) non-hydroxylated free fatty acids.

Analysis by gas chromatography and gas chromatography–mass spectrometry

GCMS analysis of the propolis wax sample confirmed the presence of hydrocarbons, free fatty acids, and wax esters. It also revealed the presence of free fatty alcohols, which were not detected by TLC due to their low levels in the wax (see Figure 2 and Table 1).

Wax esters were the major class of compounds (70.0%) present in the propolis wax. Four major series of wax esters were observed, namely palmitate esters, oleate esters, 14-hydroxypalmitate (ω3-hydroxy fatty acid-containing) esters and 15-hydroxypalmitate (ω2-hydroxy fatty acid-containing) esters. All wax esters, except the palmitate esters, were found in a homologous series containing an even-numbered alcohol with 24 to 34 carbon atoms. Eight palmitic acid-based esters with total number of carbon atoms varying from 38 to 52 were detected, indicative of esters containing fatty alcohol residues in the range from 22 to 36 carbon atoms. Mass spectra for all series of wax esters matched well with published spectra for honey comb-derived beeswax (Maia & Nunes, 2013). Beeswax is known to contain two types of wax diesters, namely diesters of diols, and acylated hydroxyacid esters (Aichholz & Lorbeer, 1999). Whilst the chromatographic properties of wax diesters under GC conditions used in our study did not allow their detection, the absence of diols (except

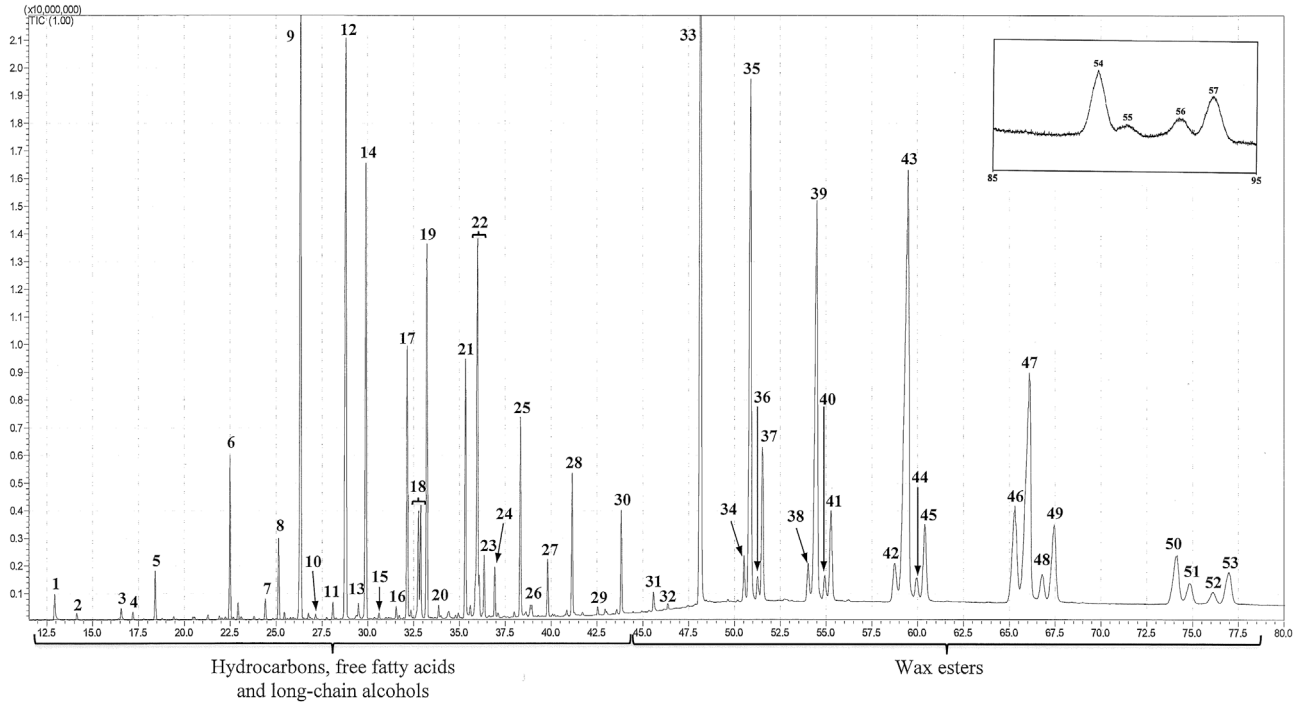

Figure 2. GCMS-FID trace of the silylated propolis wax sample on ZB5ms column (inset is not to scale). The identities and concentrations of numbered peaks are reported in Table 1.

for trace quantities of monoalkylglycerols) in the hydrolysed sample suggests that no diesters of diols were present in the sample. Additional experimental work is required to detect and quantify acylated hydroxyacyl esters, if present.

Hydrocarbons represented 13.6% of the propolis wax, with saturated odd-numbered hydrocarbons with 19 to 33 carbon atoms, and monounsaturated odd-numbered hydrocarbons with 27 to 35 carbon atoms being two major groups. This was similar to the average level for Brazilian propolis wax (Negri et al., 1998) at 15.8%. The hydrocarbon content of comb-derived wax (Maia & Nunes, 2013) was much higher, at around 40%. Even-numbered hydrocarbons with 26 to 30 carbon atoms were found at much lower levels. Heptacosane (27:0; 32.2% of total hydrocarbons, or 4.4% of the sample), followed by nonacosane (29:0; 19.1% of total hydrocarbons, or 2.6% of the sample), tritriacontene (33:1; 14.4% of total hydrocarbons, or 2.0% of the sample), and hentriacontane (31:0; 13.8% of total hydrocarbons, or 1.9% of the sample) were major hydrocarbons found in the sample. Overall, the hydrocarbon pattern composition was in line with that previously reported for honey-derived beeswax (Maia & Nunes, 2013), European propolis wax (Seifert & Haslinger, 1989), and averaged values for Brazilian propolis wax (Negri et al., 1998).

Free fatty acids were represented mainly by straight-chain even-numbered saturated acids with 26, 28 and 30 carbon atoms. Free fatty acids represented 11.5% of the propolis wax sample. Free fatty alcohols with 28, 30, and 32 carbon atoms were detected at low levels, with total free fatty alcohols accounting for 0.5% of the propolis wax sample. The free fatty acid composition differs substantially from the fatty acid composition of the wax esters, where the major fatty acid is C16:0 (approximately 50% of free fatty acids), followed by lower levels of C24:0, C18:1 and C16:0 ω2-OH.

Methanolysis products of propolis wax sample contained fatty acids (in the form of methyl esters), hydrocarbons and fatty alcohols (Table 2). Trace quantities of 1-*O*-glycerol ethers: 1-*O*-eicosylglycerol and 1-*O*-octadecylglycerol (less than 0.1% of the sample each) were also detected. A number of hydroxylated fatty acids were observed, including 14- and 15- (i.e., ω3- and ω2-, respectively) hydroxypalmitic acids identified earlier as components of monoesters. 11-, 13- and 16-hydroxy palmitic acids were also observed, albeit at trace levels (each less than 0.1%). Monohydroxy even-numbered fatty acids, of various chain lengths from 14 to 22 carbon atoms were observed in the hydrolysate, mostly in the form of the ω-2 isomer. As for the fatty alcohols, these were observed mostly as saturated components that contained from 18 to 36 carbon atoms. Low levels of monounsaturated alcohols were also detected, with chain length varying in the range of 32 to 34 carbon atoms. No dihydroxy fatty acids were detected in either the intact or hydrolysed propolis wax samples studied.

Table 1. Composition of the propolis wax sample as determined by GC-FID-MS (values in weight % presented as mean ± standard deviations, $n = 3$).

Components	Peak number (Figure 2)		
Hydrocarbons		*% of hydrocarbons*	*% of total wax*
21:0	2	0.5 ± 0.1%	0.1 ± 0.0%
23:0	5	2.3 ± 0.4%	0.4 ± 0.1%
25:0	6	5.9 ± 0.2%	0.9 ± 0.0%
26:0	7	0.7 ± 0.0%	0.1 ± 0.0%
27:0	9	31.5 ± 0.6%	5.0 ± 0.1%
28:0	11	0.7 ± 0.0%	0.1 ± 0.0%
29:1	13	0.4 ± 0.0%	0.1 ± 0.0%
29:0	14	18.7 ± 0.0%	3.0 ± 0.0%
30:0	16	1.3 ± 0.4%	0.2 ± 0.1%
31:1A	18	3.5 ± 0.1%	0.6 ± 0.0%
31:1B	18	3.4 ± 0.0%	0.5 ± 0.0%
31:0	19	13.2 ± 0.3%	2.1 ± 0.0%
33:1A	22	14.2 ± 0.1%	2.3 ± 0.0%
33:1B	22	0.9 ± 0.2%	0.1 ± 0.0%
33:0	23	2.0 ± 0.2%	0.3 ± 0.0%
35:1	26	0.8 ± 0.1%	0.1 ± 0.0%
Total hydrocarbons, g/100 g			*15.9 ± 0.7%*
Free fatty acids (FFA)		*% of FFA*	*% of total wax*
16:0	1	2.9 ± 1.7%	0.2 ± 0.2%
18:1	3	1.3 ± 0.2%	0.1 ± 0.0%
18:0	4	0.5 ± 0.2%	tr*
22:0	8	4.0 ± 0.2%	0.3 ± 0.0%
23:0	10	0.3 ± 0.0%	tr*
24:0	12	44.3 ± 2.6%	3.8 ± 0.2%
25:0	15	0.4 ± 0.0%	tr*
26:0	17	13.4 ± 0.1%	1.2 ± 0.0%
28:0	21	12.3 ± 0.5%	1.1 ± 0.0%
30:0	25	9.1 ± 0.9%	0.8 ± 0.1%
32:0	28	6.0 ± 1.6%	0.5 ± 0.1%
34:0	30	5.0 ± 1.5%	0.4 ± 0.1%
36:0	32	0.5 ± 0.4%	tr*
Total FFA, g/100 g			*8.7 ± 0.7%*
Free fatty alcohols		*% of alcohols*	*% of total wax*
28:0	20	11.8 ± 0.7%	0.1 ± 0.0%
30:0	24	43.1 ± 0.3%	0.3 ± 0.0%
32:0	27	39.1 ± 0.3%	0.3 ± 0.0%
34:0	29	6.0 ± 0.1%	tr*
Total fatty alcohols, g/100 g			*0.7 ± 0.0%*
Wax esters (acid–alcohol)		*% of wax esters*	*% of total wax*
38:0 (16:0–22:0)	31	0.2 ± 0.1%	0.1 ± 0.1%
40:0 (16:0–24:0)	33	15.3 ± 0.4%	11.5 ± 0.3%
42:1 (18:1–24:0)	34	0.6 ± 0.1%	0.4 ± 0.1%
42:0 (16:0–26:0)	35	10.4 ± 0.1%	7.8 ± 0.1%
40:0 (14-OH 16:0–24:0)	36	0.6 ± 0.2%	0.5 ± 0.1%
40:0 (15-OH 16:0–24:0)	37	2.0 ± 0.4%	1.5 ± 0.3%
44:0 (18:1–26:0)	38	0.6 ± 0.1%	0.4 ± 0.1%
44:0 (16:0–28:0)	39	10.8 ± 0.3%	8.1 ± 0.2%
42:0 (14-OH 16:0–26:0)	40	0.9 ± 0.2%	0.7 ± 0.1%
42:0 (15-OH 16:0–26:0)	41	1.6 ± 0.3%	1.2 ± 0.2%
46:1 (18:1–28:0)	42	1.1 ± 0.3%	0.8 ± 0.2%
46:0 (16:0–30:0)	43	20.9 ± 0.6%	15.6 ± 0.4%
44:0 (14-OH 16:0–28:0)	44	1.2 ± 0.3%	0.9 ± 0.2%
44:0 (15-OH 16:0–28:0)	45	2.0 ± 0.6%	1.5 ± 0.5%
48:1 (18:1–30:0)	46	3.5 ± 0.8%	2.6 ± 0.6%
48:0 (16:0–32:0)	47	14.7 ± 0.4%	11.0 ± 0.3%
46:0 (14-OH 16:0–30:0)	48	1.7 ± 0.1%	1.3 ± 0.1%
46:0 (15-OH 16:0–30:0)	49	3.3 ± 1.1%	2.5 ± 0.8%
50:1 (18:1–32:0)	50	2.6 ± 0.5%	1.9 ± 0.4%

(*Continued*)

Table 1. (*Continued*).

Components	Peak number (Figure 2)		
50:0 (16:0–34:0)	51	2.0 ± 0.0%	1.5 ± 0.0%
48:0 (14-OH 16:0–32:0)	52	0.9 ± 0.1%	0.6 ± 0.1%
48:0 (15-OH 16:0–32:0)	53	2.2 ± 0.9%	1.6 ± 0.7%
52:1 (18:1–34:0)	54	0.4 ± 0.0%	0.3 ± 0.0%
52:0 (16:0–36:0)	55	0.2 ± 0.0%	0.2 ± 0.0%
50:0 (14-OH 16:0–34:0)	56	0.2 ± 0.1%	0.1 ± 0.1%
50:0 (15-OH 16:0–34:0)	57	0.3 ± 0.1%	0.2 ± 0.1%
Hydroxylated		16.8 ± 4.3%	12.6 ± 3.2
Total wax esters, g/100 g			*74.8 ± 1.4%*

*Traces, <0.1% of total.

Table 2. Fatty acid and fatty alcohol components of the hydrolysed propolis wax sample, wt%.

Fatty acids		Hydroxylated fatty acids		Fatty alcohols	
16:0	42.2	14:0 ω2-OH	tr*	18:0	tr*
18:2	tr*	16:0 ω6-OH	tr*	22:0	tr*
18:1	7.9	16:0 ω4-OH	tr*	24:0	15.5
18:1	tr*	16:0 ω3-OH	2.9	26:0	11.7
18:0	1.9	16:0 ω2-OH	15.8	27:0	tr*
20:1	0.5	16:0 ω1-OH	tr*	28:0	13.8
20:0	tr*	18:0 ω4-OH	tr*	30:0	30.4
22:0	1.1	18:1 ω2-OH	tr*	32:1	1.5
24:0	11.0	18:0 ω3-OH	0.6	32:0	22.3
26:0	3.6	18:0 ω2-OH	1.3	34:1	0.7
28:0	3.5	20:1 ω3-OH	tr*	34:0	4.2
30:0	2.2	20:1 ω2-OH	tr*	36:0	tr*
32:0	1.8	20:0 ω3-OH	tr*	Total fatty alcohols	100.0
34:0	0.8	20:0 ω2-OH	0.8		
36:0	tr*	22:0 ω2-OH	0.4		
% of total fatty acids	76.5	24:0 ω2-OH	1.2		
		26:0 ω2-OH	0.5		
		% of total fatty acids	23.5		

*Traces, <0.1% of total.

Discussion

New Zealand propolis wax studied consists mostly of even carbon numbered wax esters at 70% by mass, long-chain odd carbon-numbered hydrocarbons at 14% by mass, and even carbon numbered free fatty acids at 11.5% by mass. Low levels of free fatty alcohols were also observed, accompanied by even lower levels of 1-*O*-alkylglycerols. Wax esters were represented mostly by saturated non-hydroxylated and hydroxylated fatty acids and alcohols, with some monounsaturated non-hydroxylated esters also present. Non-hydroxylated fatty acids contained from 16 to 36 carbons, whilst hydroxylated acids contained from 14 to 26 carbon atoms. While almost 11% of non-hydroxylated fatty acids were monounsaturated, only trace levels of monounsaturated hydroxylated fatty acids were observed. Fatty alcohol moieties of wax esters were predominantly saturated and contained mostly from 24 to 34 carbon atoms per molecule. No polyhydroxylated fatty components were detected in the sample. The composition of the sample strongly resembles that of honey comb-derived beeswax, suggesting that it may be used as a replacement for beeswax in food applications, such as food supplement manufacturing (soft gelatine capsules, tablet formulations), glazing and coatings, chewing gum, and as a carrier for food additives. Hydrolysis of the wax esters could give rise to a "Policosanol" (Hargrove, Greenspan, & Hartle, 2004) type product. Hydrolysis of the wax esters would also give rise to a free fatty acid fraction. Of potential applied interest in such a fraction are the hydroxylated fatty acids. A characteristic feature of these hydroxylated fatty acids is the position of the hydroxyl group, in the vicinity of the terminal methyl group (e.g., ω-2). Such or similar compounds were successfully used in the preparation of insect pheromones to attract stink bugs (Leal et al., 1998), which might have pest control application. A mixture containing royal jelly-derived hydroxylated and non-hydroxylated fatty acids was suggested as a potent deterrent of the parasitic *Varroa* mite (Drijfhout, Kochansky, Lin, & Calderone, 2005).

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Disclosure statement

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