ELSEVIER

Food Chemistry 81 (2003) 159–168

Food Chemistry

www.elsevier.com/locate/foodchem

Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand *Leptospermum* honeys

Lihu Yao[a], Nivedita Datta[a,*], Francisco A. Tomás-Barberán[b], Federico Ferreres[b], Isabel Martos[b], Riantong Singanusong[c]

[a]*Food Science and Technology, School of Land and Food Sciences, The University of Queensland, Gatton, Queensland 4343, Australia*
[b]*Laboratorio de Fitoquímica, Department of Food Science and Technology, CEBAS (CSIC), PO Box 4195, Murcia 30080, Spain*
[c]*Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environmental Sciences, Naresuan University, Muang, Phitsanulok 65000, Thailand*

Received 27 June 2002; accepted 29 July 2002

Abstract

Flavonoids, phenolic acids and abscisic acid of Australian and New Zealand *Leptospermum* honeys were analyzed by HPLC. Fifteen flavonoids were isolated in Australian jelly bush honey (*Leptospermum polygalifolium*), with an average content of 2.22 mg/100 g honey. Myricetin (3,5,7,3′,4′,5′-hexahydroxyflavone), luteolin (5,7,3′,4′-tetrahydroxyflavone) and tricetin (5,7,3′,4′,5′-pentahydroxyflavone) were the main flavonoids identified. The mean content of total phenolic acids in jelly bush honey was 5.14 mg/100 g honey, with gallic and coumaric acids as the potential phenolic acids. Abscisic acid was quantified as twice the amount (11.6 mg/100 g honey) of the phenolic acids in this honey. The flavonoid profile mainly consisted of quercetin (3,5,7,3′,4′-pentahydroxyflavone), isorhamnetin (3,5,7,4′-tetrahydroxyflavone 3′-methyl ethyl), chrysin (5,7-dihydroxyflavone), luteolin and an unknown flavanone in New Zealand manuka (*Leptospermum scoparium*) honey with an average content of total flavonoids of 3.06 mg/100 g honey. The content of total phenolic acids was up to 14.0 mg/100 g honey, with gallic acid as the main component. A substantial quantity (32.8 mg/100 g honey) of abscisic acid was present in manuka honey. These results showed that flavonoids and phenolic acids could be used for authenticating honey floral origins, and abscisic acid may aid in this authentication.
© 2002 Published by Elsevier Science Ltd.

Keywords: Honey; *Leptospermum*; Botanical origin; Flavonoids; Phenolic acids; Floral markers; Abscisic acid

1. Introduction

The quality of honey is judged by the botanical or floral origin and chemical composition (Cherchi, Spanedda, Tuberoso, & Cabras, 1994) and price of honey is based on its quality and, hence, its floral origin (Andrade, Ferreres, & Amaral, 1997). Traditionally, the floral source of a honey has been identified by the analysis of bee pollens present in the honey. However, Tan, Wilkins, Molan, Holland, and Reid (1989) suggested that chemical approaches might be more accurate and easily undertaken in the characterisation of the floral source of a honey. The use of phenolic compound analysis, including flavonoids, in the identification of honeys has been suggested (Amiot, Aubert, Gonnet, & Tacchini, 1989) and has since been used as a tool for studying the floral and geographical origins of honeys.

Earlier, researchers tried to use the analysis of amino acids (Bosi & Battaglini, 1978; Davies, 1976; Davies & Harris, 1982) to complement pollen analysis in the determination of the floral origins of honey. In recent years, volatile compounds (Bonaga, Giumanini, & Gliozzi, 1986; D’Arcy, Rintoul, Rowland, & Blackman, 1997), degradation products of phenylalanine (Speer & Montag, 1987), aromatic acids and their esters (Steeg & Montag, 1988a, 1988b, 1988c), aromatic and degraded carotenoid-like substances (Tan, Wilkins, Holland, & McGhie, 1989, 1990; Tan, Wilkins, Molan et al., 1989; Wilkins, Lu, & Molan, 1993) and aromatic aldehydes and heterocycles (Hausler & Montag, 1989, 1990, 1991)

* Corresponding author. Tel.: +61-754-601017; fax: +61-754-601171.
E-mail address: nd@fst.uq.edu.au (N. Datta).

0308-8146/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0308-8146(02)00388-6

have been analysed using gas chromatography connected with mass spectroscopy (GCMS) for the assessment of floral origins of honeys. The objective studies have been extended to better HPLC methods for the analysis of non-volatile phytochemical constituents of honeys, such as phenolic compounds (Amiot et al., 1989; Ferreres, Andrade, & Tomás-Barberán, 1994a; Ferreres, Garcia-Viguera, Tomás-Lorente, & Tomás-Barberán, 1993; Ferreres, Giner, & Tomás-Barberán, 1994; Sabatier, Amoit, Tacchini, & Aubert, 1992). These HPLC analyses have proven to be very useful in determining the authenticity of the floral origin of honey (Andrade, Ferreres, & Amaral, 1997; Andrade, Ferreres, Gil, & Tomás-Barberán, 1997b; Martos, Ferreres, & Tomás-Barberán 2000a; Martos, Ferreres, Yao, D'Arcy, Caffin, & Tomás-Barberán, 2000b).

Analysis of phenolic compounds has been regarded as a very promising technique for studying the floral and geographical origins of honeys (Amiot et al., 1989; Ferreres, Ortiz, Silva, Garcia-Viguera, Tomás-Barberán, & Tomás-Lorente, 1992; Sabatier et al., 1992; Tomás-Barberán, Ferreres, Garcia-Viguera, & Tomás-Lorente, 1993a; Tomás-Barberán, Garcia-Viguera, Vit-Olivier, Ferreres, & Tomás-Lorente, 1993). In these studies, the flavanone hesperetin has been used as a marker for citrus honey (Ferreres et al., 1993; Ferreres, Blazquez, Gil, & Tomás-Barberán 1994; Ferreres, Giner, & Tomás-Barberán, 1994); the flavone, kaempferol, for rosemary honey (Ferreres, Blazquez et al., 1994b; Ferreres, Juan, Perez-Arquillue, Herrera-Marteache, Garcia-Viguera, Tomás-Barberán, 1998), and quercetin for sunflower honey (Tomás-Barberán, Martos, Ferreres, Radovic, & Anklam, 2001). Moreover, some phenolic acids such as ellagic acid in heather honey have also been used as floral markers (Andrade, Ferreres, & Amaral, 1997; Ferreres, Andrade, Gil, & Tomás-Barberán, 1996; Ferreres, Andrade, & Tomás-Barberán, 1996), and the hydroxycinnamates (caffeic, *p*-coumaric and ferulic acids) in chestnut honey (Andrade, Ferreres, & Amaral, 1997). Pinocembrin, pinobanksin and chrysin are the characteristic flavonoids of propolis, and these flavonoid compounds have been found in most European honey samples (Tomás-Barberán et al., 2001).

In some honey samples, such as lavender and acacia honeys, no specific phenolic compounds have been found suitable as floral markers (Tomás-Barberán et al., 2001). Other possible phytochemicals markers may be found, such as abscisic acid for heather honey (Ferreres, Andrade, & Tomás-Barberán, 1996). Abscisic acid has also been detected in rapeseed, limetree and acacia honeys (Tomás-Barberán et al., 2001).

This study employs the HPLC analyses of flavonoids and phenolic acids, along with abscisic acid, in Australian and New Zealand *Leptospermum* honeys, and correlates these constituents with the honey floral origins as potential biochemical markers.

2. Materials and methods

2.1. General

All chemicals used were of HPLC grade. Pinocembrin, chrysin and quercetin were purchased from Extrasynthese, 69726 Genay Cedex, France; kaempferol, kaempferol 8-methyl ether, isorhamnetin, and luteolin were kindly provided by the Laboratory of Phytochemistry, Department of Food Science and Technology, CEBAS(CSIC), Murcia, Spain; gallic, coumaric and ellagic acids were purchased from Sigma Chemicals Co., St Louis, MO, USA; ferulic acid was purchased from Aldrich Chemicals Co., Milwaukee, WI, USA; abscisic acid was kindly provided by the Laboratory of Plant Physiology, Department of Botany, The University of Queensland, Brisbane, Australia. Amberlite XAD-2 was from Supelco, Bellefonte, PA, USA and the 0.45 μm membrane filter was supplied by Alltech Associates (Aust) Pty. Ltd., Baulkham Hills, NSW, Australia.

2.2. Honey samples

During the 1997–1998 and 1998–1999 flowering seasons, 12 jelly bush (*Leptospermum polygalifolium*) honey samples were collected and supplied by individual Australian apiarists from the North Coast, New South Wales (NSW). Two manuka (*Leptospermum scoparium*) honey samples were sourced from New Zealand. All the samples were stored in a freezer of −18 to −24 °C before analysis. Details of these *Leptospermum* honey samples used in this experiment are described in Table 1.

2.3. Extraction (column chromatography)

Extraction was performed according to methods described previously (Martos, Cossentini, Ferreres, & Tomás-Barberán 1997; Martos, Ferreres, & Tomás-Barberán, 2000; Martos, Ferreres, Yao et al., 2000). Namely, honey samples (100 g each) were thoroughly mixed with five parts (500 ml) of distilled water, adjusted to pH 2 with concentrated HCl, until completely fluid by stirring with a magnetic stirrer at room temperature. The fluid samples were then filtered through cotton wool to remove solid particles. The filtrate was mixed with 150 g Amberlite XAD-2 (pore size 9 nm, particle size 0.3–1.2 mm) and stirred in a magnetic stirrer for 10 min, which was considered enough to absorb honey phenolics with a recovery rate more than 80% (Martos et al., 1997; Tomás-Barberán, Blazquez, Garcia-Viguera, Ferreres, & Tomás-Lorente, 1992). The Amberlite particles were then packed in a glass column (42×3.2 cm) and the column was washed with acidified water (pH 2 with HCl, 250 ml) and subsequently rinsed with distilled water (300 ml) to remove all sugars and other polar constituents of honey. The phenolic

Table 1
Unifloral *Leptospermum* honey samples analysed in this study

Sample code	Common name	Floral origin	Year	Geographical origin
JB1052	Jelly bush	*L. polygalifolium*	1997	North Coast, NSW
JB1291	Jelly bush	*L. polygalifolium*	1997	North Coast, NSW
JB0041	Jelly bush	*L. polygalifolium*	1997	North Coast, NSW
JB0539	Jelly bush	*L. polygalifolium*	1997	North Coast, NSW
JB5367	Jelly bush	*L. polygalifolium*	1998	North Coast, NSW
JB0481	Jelly bush	*L. polygalifolium*	1997	North Coast, NSW
JB5364	Jelly bush	*L. polygalifolium*	1998	North Coast, NSW
JB0566	Jelly bush	*L. polygalifolium*	1997	North Coast, NSW
JB2174	Jelly bush	*L. polygalifolium*	1998	North Coast, NSW
JB1053	Jelly bush	*L. polygalifolium*	1997	North Coast, NSW
JB0140	Jelly bush	*L. polygalifolium*	1997	North Coast, NSW
JB0484	Jelly bush	*L. polygalifolium*	1997	North Coast, NSW
JB040	Jelly bush	*L. polygalifolium*	[a]	[a]
MIA01	Manuka[b]	*L. scoparium*	1998	New Zealand
MA01	Manuka[b]	*L. scoparium*	1998	New Zealand

[a] Date and location could not be confirmed.
[b] Supplied by Honey Research Unit, Department of Biological Sciences, University of Waikato, New Zealand.

compounds remained absorbed on the column (Ferreres, Tomás-Barberán, Gil, & Tomás-Lorente, 1991) and were eluted with methanol (400 ml). The methanolic extract was concentrated to dryness under reduced pressure in a rotary evaporator at 40 °C. The residue was resuspended in distilled water (5 ml) and extracted with diethyl ether (5 ml×3). The ether extracts were combined, and the diethyl ether was removed by flushing with nitrogen. The dried residue was then redissolved in 1 ml of methanol, filtered through a 0.45 μm membrane filter, and analysed by HPLC.

2.4. HPLC analysis

The HPLC analyses of flavonoids and other phenolic acids were performed by using a Shimadzu Class-VP HPLC system, a computer-controlled system with an upgraded Class-VP 5.03 software. Separations were carried out on a reversed phase column LiChroCART RP-18 (Merck, Darmstadt, Germany; 12.5 cm×0.4 cm, particle size 5 μm), using a mobile phase of 5% (v/v) aqueous formic acid (solvent A) and methanol (solvent B) at a constant solvent flow rate of 1 ml/min. The temperature of the column oven was set at 35 °C.

The gradient elution was established according to the method of Martos et al. (1997): 30% methanol (B) flowed through the column isocratically with solvent A for 15 min; this was increased to 40% methanol at 20 min, 45% methanol at 30 min, 60% methanol at 50 min, 80% methanol at 52 min, and 90% methanol at 60 min. Finally, isocratic elution with 90% methanol was done until 65 min.

The honey extracts were injected with a SIL-10A XL Auto Injector and the flavonoids were detected using a multichannel photodiode-array detector (SPD-M10A VP) to obtain the UV spectra of the various phenolic compounds. The chromatograms were monitored at 290 nm and 340 nm, since the majority of the honey flavonoids and phenolic acids show their UV absorption maxima around these two wavelengths (Martos et al., 1997).

2.5. Identification and quantification of flavonoids

The phenolic compounds (flavonoids) were identified and quantified as reported previously (Martos et al., 1997; Martos, Ferreres, & Tomás-Berberán, 2000; Martos, Ferreres, Yao et al., 2000), with reference to their standard compounds. For some of the minor honey flavonoids and phenolic acids, when their authentic compounds were unavailable, the stored UV spectra extracted from the same HPLC methods for honey analysis and their corresponding retention times, were utilised as library data for the comparison, and for identification.

The flavonoids were quantified by their absorbance in the HPLC chromatograms against external standards, and four standard flavonoids were used: pinocembrin at 290 nm (for flavanone), chrysin at 340 nm (for flavones with unsubstituted ring B), kaempferol at 340 nm (for flavone kaempferol and its methyl ether), quercetin at 340 nm (for the rest of flavones).

The phenolic acids such as gallic and coumaric acids, were quantified against their standards at 290 nm. Ellagic acid was quantified against its standards at 340 nm. The abscisic acid, both of *trans*,*trans*- and *cis*, *trans*- isomers, was determined against the standard at 290 nm.

3. Results and discussion

3.1. Flavonoid profiles of Leptospermum honeys

3.1.1. Flavonoids in Australian jelly bush (L. polygalifolium) honey

HPLC analysis of flavonoids in samples of Australian jelly bush honey showed that myricetin and luteolin are main flavonoids present in most of the samples analysed (Fig. 1a and Table 2). About 15 flavonoids have been found in this type of honey and most of them occur in small amounts. The content of total flavonoids was 2.22 mg/100 g honey, with myricetin being 0.37 mg/100 g honey (Table 2).

There were two minor compounds present in the jelly bush honey that showed similar flavonoid spectra and chromatographic behaviour but could not be identified, due to lack of availability of authentic compounds and literature data. These compounds had retention times of 24 and 30 min, respectively, with UV spectra (Fig. 2) similar to pinocembrin and pinobanksin (or the derivatives), where the major absorption peaks occur at ca 283 and ca 270 nm, respectively, suggesting flavanone compounds. These compounds were labelled F01 and F02 (Fig. 2).

Myricetin, luteolin and tricetin represented 27.2, 22.3 and 20.5% of the total flavonoids, respectively (Table 2) and pinobanksin, the unknown flavonoid (F01), and quercetin are the secondary flavonoids, representing 14.3, 12.6, and 11.5% of the total flavonoids, respectively (Table 2). Tricetin levels were variable amongst the samples studied; most of the samples did not contain this flavonoid. However, most of the samples show a common flavonoid profile, comprising myricetin, quercetin, luteolin, kaempferol and the unknown flavonoid (F01), suggesting that they could be used as floral markers for Australian jelly bush honeys.

The main similarity between Australian jelly bush honeys and *Eucalyptus* honeys (Martos, Ferreres, Yao et al., 2000) lies in the fact that they contain quercetin, luteolin and kaempferol in their flavonoid profiles. The main difference between these two types of honeys is that the flavonoid profiles in most of the jelly bush honeys are devoid of tricetin, instead, they have the unknown flavonoid (F01) as one of the main components in their flavonoid profile. Thus, the jelly bush

Table 2
Flavonoid content of Australian jelly bush (*Leptospermum polygalifolium*) and New Zealand manuka (*Leptospermum scoparium*) honeys

Sample	Content of flavonoids (mg/100 g honey)[a]															
	Myr	Tri	PB	F01	F02	Que	Lut	3MQ	Kae	8MK	PC	DMQ	IRM	Chr	Tch	Total
JB1052	0.58	0.68		0.41	0.08			0.19	0.13	0.13	0.10					2.30
JB1291	0.22			0.37		0.43	0.45	0.33	0.10	0.06	0.06	0.12	0.08			2.23
JB0041	0.08		0.14	0.01		0.07	0.08	0.03	0.01	0.09						0.51
JB0539	0.25	0.08				0.13	0.36		0.12				0.04		0.23	1.23
JB5367	0.12	0.07	0.06			0.06	0.06		0.01							0.37
JB0481	0.74		0.22	0.24		0.17	0.41				0.07					1.85
JB5364	0.06						0.03		0.04							0.14
JB0566		0.33	0.07	0.07	0.11	0.13	0.23	0.05	0.04	0.06		0.05	0.05			1.20
JB2174	0.22		0.23	0.36	0.12	0.09	0.21	0.06	0.08	0.10			0.11	0.09		1.67
JB1053	0.11		0.37	0.24	0.06	0.11	0.22	0.03	0.03							1.15
JB0140	0.10	0.16	0.04			0.14	0.26	0.02	0.02	0.02		0.03				0.78
JB0484	0.85					0.21	0.57									1.62
JB040	1.15		0.05	0.07	0.36	0.04		0.09	0.05	0.09	0.26	0.09	0.06			2.32
Mean	0.37	0.26	0.15	0.22	0.15	0.14	0.26	0.10	0.06	0.08	0.12	0.07	0.07	0.05	0.12	
S.D.	0.36	0.26	0.12	0.15	0.12	0.11	0.17	0.11	0.04	0.04	0.09	0.04	0.03			
%[b]	27.2	20.5	14.3	12.6	8.1	11.5	22.3	5.6	6.5	6.3	5.4	4.3	4.0	2.8	5.5	
MIA01				0.38	0.25	0.55	0.33	0.13	0.13	0.26	0.23	0.21	0.47	0.40		3.34
MA01	0.14			0.39		0.31	0.43	0.18	0.17	0.13	0.15	0.18	0.32	0.37		2.77
Mean	0.07			0.39	0.12	0.43	0.38	0.16	0.15	0.20	0.19	0.19	0.40	0.38		
S.D.				0.01		0.17	0.07	0.03	0.02	0.09	0.05	0.02	0.10	0.02		
%[b]	3.0			12.7	3.7	13.8	12.6	5.2	5.0	6.3	6.2	6.4	12.9	12.6		

[a] Myr—myricetin (3,5,7,3′,4′,5′-hexahydroxyflavone), Tri—tricetin (5,7,3′,4′,5′-pentahydroxyflavone), Que—quercetin (3,5,7,3′,4′-pentahydroxyflavone), Lut—luteolin (5,7,3′,4′-tetrahydroxyflavone), 3MQ—quercetin 3-methyl ether (5,7,3′,4′-tetrahydroxy-3-methoxyflavone), Kae—kaempferol (3,5,7,4′-tetrahydroxyflavone), 8MK—kaempferol 8-methyl ether (3,5,7,4′-tetrahydroxy-8-methoxyflavone), PC—pinocembrin (5,7-dihydroxyflavanone), DMQ—quercetin 3,3′-dimethyl ether (5,7,4′-trihydroxy-3,3′-dimethoxyflavone), IRM—isorhamnetin (3,5,7,4′-tetrahydroxy-3′-methoxyflavone), Chr—chrysin (5,7-dihydroxyflavone), PB—pinobanksin (3,5,7-trihydroxyflavanone), Tch—tectochrysin (5-hydroxy-7-methoxyflavone), F01—unknown flavonoid 01, F02—unknown flavonoid 02.

[b] Percentage of each individual flavonoid in the total flavonoids.

honeys can be readily differentiated from Australian *Eucalyptus* honeys by their flavonoid profiles.

3.1.2. Flavonoids in New Zealand manuka (L. scoparium) honey

In New Zealand manuka honeys, the flavonoid profile mainly consisted of quercetin (13.8%), isorhamnetin (12.9%), unknown flavonoid (F01) (12.7%), chrysin (12.6%) and luteolin (12.6%), together representing 64.6% of the total flavonoids (3.06 mg/100 g honey) in this honey (Fig. 1b and Table 2). Since only two manuka honey samples were analysed, it is necessary to analyse more samples to see whether this flavonoid profile could be used as a floral marker for this honey species.

The main similarity between Australian jelly bush honey and New Zealand manuka honey is that both honeys contain similar and relatively stable flavonoid profiles comprising quercetin, luteolin, quercetin 3-methyl ether, kaempferol and an unknown flavonoid (F01) (Table 2). This result indicated that this similar flavonoid profile could be used as a floral marker for *Leptospermum* honeys produced in the Oceanic areas.

The main difference between these honeys is that Australian jelly bush honey showed total flavonoids as 2.22 mg/100 g honey, i.e. lower than that found in New Zealand manuka honey (3.06 mg/100 g).

Weston, Mitchell, and Allen (1999) and Weston, Brocklebank, and Lu (2000) identified pinobanksin, pinocembrin, chrysin and galangin in New Zealand manuka honeys at a level of (totally) about 0.01 mg/100 g honey, which was much lower than the levels found for pinocembrin (0.19 mg/100 g honey) and chrysin (0.38 mg/100 g honey) in the manuka honey samples examined in this study (Table 2). The difference in the flavonoid contents of manuka honey measured between the present study and the studies by Weston et al. (1999, 2000) may be due to the differences of the extraction methods. In the method described by Weston et al. (1999, 2000), only 54–60 g of Amberlite XAD-2 was used for 150–236 g honey in the column chromatography, which could result in an incomplete extraction of honey flavonoids. Martos et al. (1997) advocated that at least 100 g Amberlite XAD-2 should be used for the extraction of flavonoids from 100 g honey. In the present study, 150 g of Amberlite XAD-2 was applied to the

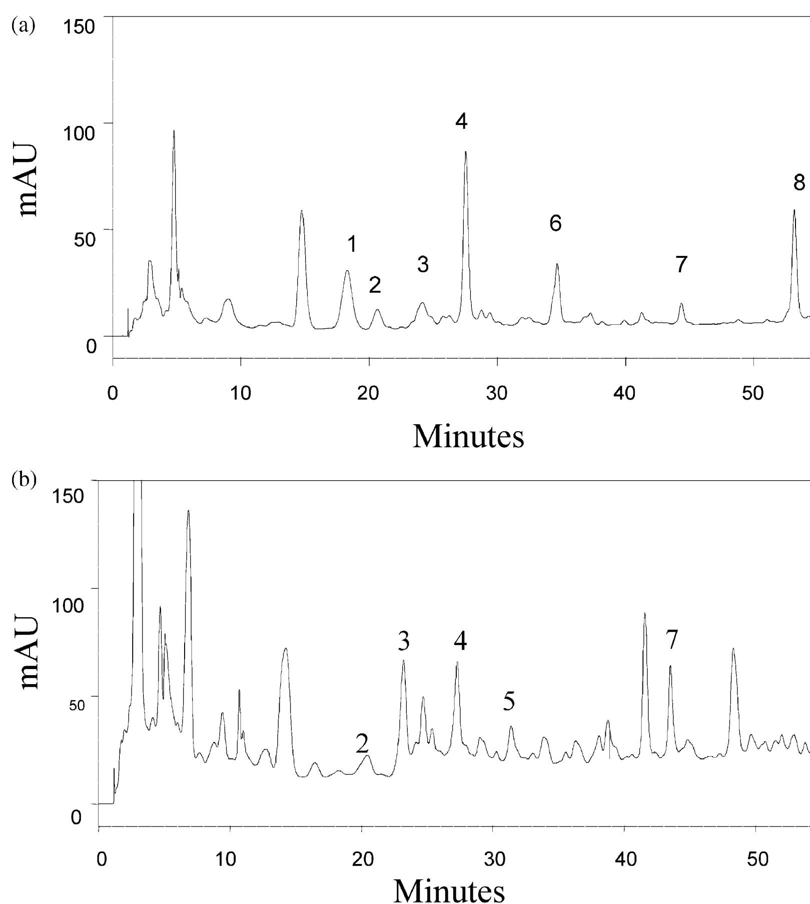

Fig. 1. HPLC chromatograms of flavonoids in *Leptospermum* honeys (340 nm): (a) Australian jelly bush (*L. polygalifolium*) honey; (b) New Zealand manuka (*L. scoparium*) honey. Flavonoids are: (1) myricetin, (2) tricetin, (3) quercetin, (4) luteolin, (5) kaempferol, (6) kaempferol 8-methyl ether, (7) pinocembrin, and (8) chrysin.

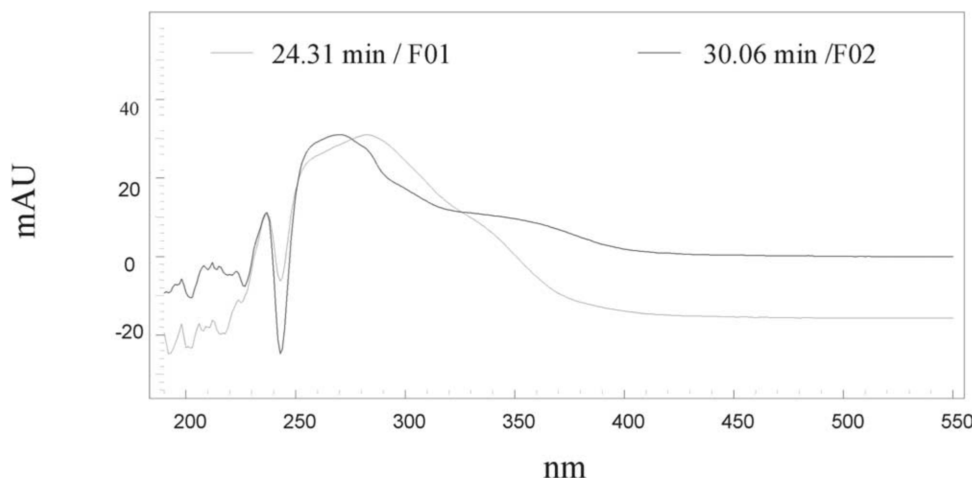

Fig. 2. Overlaid UV spectra of the unknown flavonoids in *Leptospermum* honeys.

column chromatography for 100 g of honey, which resulted in a similar analytical outcome to those analyses of other honeys by Martos et al. (1997), Martos, Ferreres, and Tomás-Barberán (2000) and Martos, Ferreres, and Yao et al. (2000).

Moreover, Weston et al. (2000) used the 280 nm wavelength for identification and quantification of flavonoids by an HPLC method, whereas the present methods, as well as other studies by Ferreres, Tomás-Barberán, Soler, Garcia-Viguera, Ortiz, and Tomás-Lorente (1994d); Ferreres, Andrade, Gil, and Tomás-Barberán (1996) and Martos et al. (1997), Martos, Ferreres, and Tomás-Barberán (2000) and Martos, Ferreres, and Yao et al. (2000) quantified the flavanones at 290 nm and the flavones at 340 nm, and the identification of flavonoids was based on both UV spectra and retention time in the HPLC with photodiode array detection. Therefore, further analysis of New Zealand manuka honey, using a larger number of samples with the same method under similar analytical conditions, is required for selection of an individual flavonoid or a flavonoid profile as floral markers for this type of honey.

3.2. *Phenolic acids in* Leptospermum *honeys*

3.2.1. *Phenolic acids in Australian jelly bush (*L. polygalifolium*) honey*

In the samples of Australian jelly bush (*L. polygalifolium*) honey, the content of total phenolic acids averaged 5.14 mg/100 g honey, with gallic acid (23.6%) and coumaric acid (22.2%) as the main components (1.23 and 1.36 mg/100 g honey, respectively) (Table 3 and Fig. 3). In addition, ellagic and chlorogenic acids were the secondary compounds (14.7 and 12.0%, respectively). The other phenolic acids present in this honey are relatively lower in proportion, ranging from 5.7% (ferulic acid) to 10.7% (an unknown phenolic acid, labelled as Ph1) of total phenolic acids. No similar phenolic profile was found among the honeys analysed except for Australian blue top ironbark honey (Yao et al., in preparation). However, blue top ironbark honey has a much higher percentage of gallic, coumaric, ellagic and chlorogenic acids. In addition, the contents of minor phenolic acids are much lower in blue top ironbark honey than in jelly bush honey.

Table 3
Phenolic acids in unifloral jelly bush and manuka honeys

Sample	Phenolic acids (mg/100 g honey)[a]								
	GA	CA	Ph01	Caf	Cou	Fer	Ell	Syr	Total
JB1052	1.65	0.36	2.14	0.77	2.50	0.59	1.18		9.20
JB1291	0.70	0.32	0.38	0.83	3.44	0.49	0.94	0.46	7.55
JB0041	0.38	0.64	0.06	0.17	0.33	0.10	0.46		2.14
JB0539	4.51	1.11	0.84	0.57	0.88	0.13	0.83		8.87
JB5367	0.58	0.05	0.26	0.36	0.13	0.27	0.32		1.97
JB0481	0.89	0.24	0.40	0.49	0.39	0.54	0.64	0.18	3.76
JB5364	0.08	0.07	0.06	0.03	0.09	0.04	0.08		0.44
JB0566	1.54		0.59	0.65	3.15	0.31	0.33		6.58
JB2174	2.78	0.58	0.96	0.62	4.74	0.53	1.37	0.33	11.9
JB1053	1.18	0.16	0.35	0.49	0.51	0.04	0.24	0.25	3.22
JB0140	0.09	0.16	0.08	0.05	0.05		0.17		0.59
JB0484	1.37	0.85	0.50	0.22	0.54	0.12	0.58		4.18
JB040	0.28	0.43	0.23	0.36	0.96	0.17	0.58	0.29	3.31
Mean	1.23	0.41	0.53	0.43	1.36	0.28	0.59	0.30	
S.D.	1.24	0.33	0.56	0.26	1.55	0.21	0.39	0.10	
%[b]	23.6	12.0	10.7	9.70	22.2	5.69	14.7	6.05	
MIA01	5.74	0.88	2.24	1.84	0.50	0.39	1.54		13.1
MA01	8.36	0.77	0.99	1.01	1.68	0.57	1.47		14.9
Mean	7.05	0.82	1.62	1.43	1.09	0.48	1.50		
S.D.	1.86	0.08	0.88	0.59	0.84	0.13	0.05		
%[b]	50.0	5.93	11.9	10.4	7.55	3.39	10.8		

[a] GA—gallic acid, CA—chlorogenic acid, Caf—caffeic acid, Cou—coumaric acid, Fer—ferulic acid, Ell—ellagic acid, Syr—syringic acid, Ph1—unknown phenolic acid.

[b] Percentage of each individual phenolic acid in the total phenolic acids.

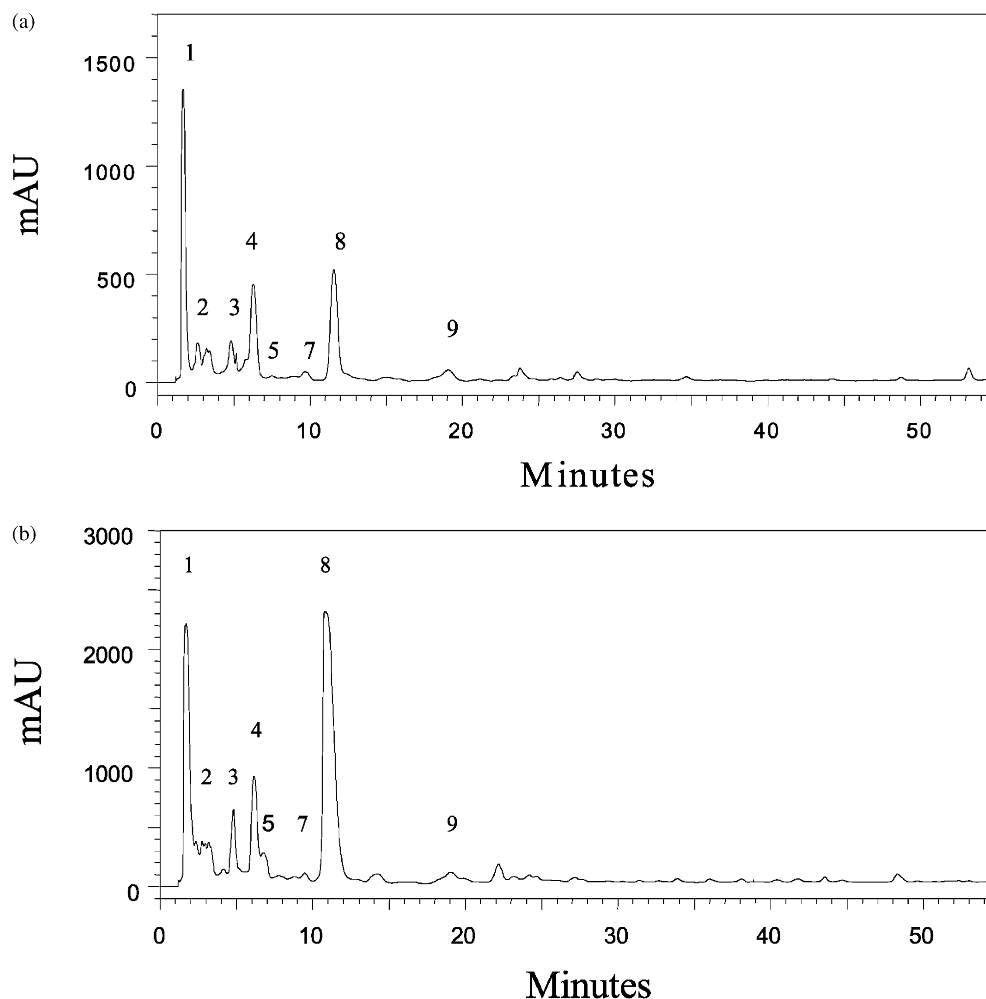

Fig. 3. HPLC chromatograms of phenolic acids and abscisic acid in uniformal *Leptospermum* honeys (290 nm): (a) Australian jelly bush (*L. polygalifolium*) honey; (b) New Zealand manuka (*L. scoparium*) honey. Phenolic acids are: (1) gallic acid, (2) chlorogenic acid, (3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, and (7) ellagic acid. Abscisic acids are: (8) *trans*,*trans*- and (9) *cis*,*trans*-abscisic acids.

3.2.2. *Phenolic acids in New Zealand manuka (*L. scoparium*) honey*

The content of total phenolic acids in New Zealand manuka (*L. scoparium*) honey was up to 14.0 mg/100 g honey, the highest amount of total phenolic acids measured in the honeys in this study so far (Yao, 2002). Of these phenolic acids, gallic acid was the highest phenolic acid detected, ranging 5.74–8.36 mg/100 g honey, with an average of 7.05 mg/100 g honey, representing 50.0% of total phenolic acids in manuka honey (Table 3). This level is the second highest among the floral types of honey studied, with only Australian yellow box honey having higher levels of gallic acid (Yao et al., in preparation). The unknown phenolic acid (Ph1), ellagic acid and caffeic acid were present in much smaller percentages in the phenolic profiles (11.9, 10.8, and 10.4%, respectively), and are considered as secondary phenolic acids in this honey. This phenolic profile, dominated by gallic acid, could be used as a floral marker for New Zealand manuka honey (Fig. 3 and Table 3).

The main differences between Australian and New Zealand *Leptospermum* honeys are the levels and percentage of total phenolic acids and of gallic acid (much higher in manuka honey) and coumaric acid (lower in manuka honey); these may be used to differentiate Australian jelly bush honey from New Zealand manuka honey.

Due to the differences in analytical methods discussed earlier, the levels of phenolic acids measured in New Zealand manuka honey by Weston et al. (1999, 2000) below than 0.01 mg/100 g honey while, in heather honey there was benzoic acid alone measured at a level of 0.39 mg/100 g honey (Weston et al., 2000). In this study, much larger amounts of phenolic acids were detected in Australian jelly bush honey (5.14 mg/100 g honey) and New Zealand manuka honey (14.0 mg/100 g honey). Again, the smaller quantities of phenolic acids detected in New Zealand manuka honey, in the previous studies, may be due to their incomplete extraction. Thus, further investigation on more samples of New Zealand manuka

honey is required to confirm the use of phenolic profiles as floral markers for this type of honey. Furthermore, because the elution of gallic acid from the column is close to the solvent front (in this HPLC analytical method) more specified studies are also required to confirm the gallic acid as one of the biochemical markers for authentication of the *Leptospermum* honeys.

3.3. Abscisic acid in Leptospermum honeys

Abscisic acid has been found in both Australian and New Zealand *Leptospermum* honey species in large amounts, with much higher concentrations than the total phenolic acids (Tables 3 and 4). In Australian jelly bush (*L. polygalifolium*) honey, the mean level of *trans*,*trans*-abscisic acid is 7.83 mg/100 g honey, while the level of its isomer, *cis*,*trans*-abscisic acid is 3.76 mg/100 g honey (Table 4). However, in New Zealand manuka (*Leptospermum scoparium*) honey, the mean level of *trans*,*trans*-abscisic acid is 31.0 mg/100 g honey, with the *cis*,*trans*-abscisic acid at a level of 1.87 mg/100 g honey (Table 4). Thus, the level of abscisic acid in New Zealand manuka honey is much higher than that in Australian jelly bush honey on that found in Portuguese heather (*Erica* spp.) honey (16.6 mg/100 g honey) (Ferreres, Andrade, & Tomás-Barberán, 1996).

In Fig. 3, the HPLC chromatograms of Australian and New Zealand *Leptospermum* honeys are dominated by gallic and abscisic acids. Thus, the high percentages and concentrations of these two phytochemical components could be used to differentiate *Leptospermum* honeys from the other types of honeys.

Previously, in New Zealand manuka honey, the level of *cis*,*trans*-abscisic acid was found only at a level of ca. 0.02 mg/100 g honey, while the *trans*,*trans*-abscisic acid was 0.07 mg/100 g honey (Lipp, 1990). These levels are much lower than those found in this study for two manuka honey samples (Table 4). In New Zealand willow (*Salix* spp.) honey, abscisic acid isomers were found to be as high as 14.8 mg/100 g honey, with *cis*,*trans*-abscisic acid being at a level of 10.6 mg/100 g honey and *trans*,*trans*-abscisic acid being at a level of 4.2 mg/100 g honey (Tan et al., 1990). In Australian leatherwood (*Eucryphia lucida*) honey, *trans*,*trans*-abscisic acid was present at a level of 0.65 mg/100 g honey, i.e. 6 times more than the level of its isomer *cis*,*trans*-abscisic acid (0.10 mg/100 g honey) (Lipp, 1990; Sun, 1995). In addition, both isomers of abscisic acid have been found in various European honeys (Tomás-Barberán et al., 2001) with total amounts as follows: Portuguese heather (*Erica* spp.) honey, 0.4–1.8 mg/100 g honey; rapeseed (*Brassica* spp.) honey, 0.03–0.25 mg/100 g honey; lime tree (*Tilia europaea*) honey, 0.05–0.5 mg/100 g honey, and acacia (*Robinia pseudoacacia*) honey, 0.10–0.25 mg/100 g honey. Again, the largest amount of these isomers were reported, ranging from 2.5 to 16.6 mg/100 g honey in Portuguese heather honey (Ferreres, Andrade, & Tomás-Barberán, 1996). These results suggest that the isomers of abscisic acid are present at variable levels in different types of honey. Therefore, the occurrence (and the levels) of abscisic acid could be used as a biochemical marker for aiding in the authentication of floral origin of honey (Tomás-Barberán et al., 2001).

Abscisic acid could be used alone as a floral marker for New Zealand manuka honey. This is because, in Australian jelly bush honey, the content of abscisic acid is less than the amount present in New Zealand manuka honey (ranging 31.4–34.3 with an average of 32.8 mg/100 g honey). This makes their differentiation possible. Moreover, abscisic acid has been found in quite large amounts in heather honey (2.5–16.6 mg/100 g honey) (Ferreres, Andrade, & Tomás-Barberán, 1996; Tomás-Barberán et al., 2001), but it is still much below that found in New Zealand manuka honey. Thus, abscisic acid may be suitable for authenticating the floral origin of the honey species alone. Further studies on more New Zealand manuka honey samples are necessary to confirm its use as a floral marker.

Table 4
Abscisic acid in unifloral jelly bush and manuka honeys

Sample	Abscisic acid (mg/100 g honey)[a]		
	trans,*trans*-ABA	*cis*,*trans*-ABA	Total
Jelly bush honey	7.83	3.76	11.6
Manuka honey	31.0	1.87	32.8

[a] ABA—abscisic acid.

4. Conclusion

The *Leptospermum* honeys, including Australian jelly bush and New Zealand manuka honeys, show a common flavonoid profile, mainly comprising quercetin, luteolin, quercetin 3-methyl ether, luteolin and an unknown flavonoid F01. This flavonoid profile is characteristic of these floral types of honeys and thus could be used as a biochemical marker for the botanical authentication of these honeys. Chrysin in New Zealand manuka honey, and myricetin, in jelly bush honey, could be used to distinguish these *Leptospermum* floral types from each other, suggesting a species-specific differentiation occurring between these two *Leptospermum* honeys. Gallic acid and abscisic acid are dominant compounds in New Zealand manuka honey, which could further assist in the authentication of New Zealand manuka honey. These results show that non-volatile HPLC analysis can be an objective tool for honey floral authentication.

Acknowledgements

We thank Mr. William G. Winner of Capilano Honey Ltd, Australia and Dr. Peter Molan, University of Waikato, New Zealand for the supply of honey samples and Ms. Katherine Raymont, Ms. Brenda Mossel, and Mr. Gavin Rintoul of the University of Queensland for their technical supports and sample sourcing. Appreciation is also expressed to the Rural Industries Research and Development Corporation (RIRDC, Australia) for financial support for this project, and Department of Education, Science and Training (DEST, formerly DETYA, Australia) for providing an IPRS (formerly OPRS) fund support. Our gratitude is extended to Dr. Bruce D'Arcy for discussion during the preliminary preparation of this work.

References

Amoit, M. J., Aubert, S., Gonnet, M., & Tacchini, M. (1989). The phenolic compounds in honeys: preliminary study upon identification and family quantification. *Apidologie*, *20*(2), 115–125.

Andrade, P., Ferreres, F., & Amaral, M. T. (1997). Analysis of honey phenolic acids by HPLC, its application to honey botanical characterization. *Journal of Liquid Chromatography & Related Technologies*, *20*(14), 2281–2288.

Andrade, P., Ferreres, F., Gil, M. I., & Tomás-Barberán, F. A. (1997). Determination of phenolic compounds in honeys with different floral origin by capillary zone electrophoresis. *Food Chemistry*, *60*(1), 79–84.

Bonaga, G., Giumanini, A. G., & Gliozzi, G. (1986). Chemical composition of chestnut honey: analysis of the hydrocarbon fraction. *Journal of Agricultural and Food Chemistry*, *34*(2), 319–326.

Bosi, G., & Battaglini, M. (1978). Gas chromatographic analysis of free and protein amino acids in some unifloral honeys. *Journal of Apiculture Research*, *17*(3), 152–166.

Cherchi, A., Spanedda, L., Tuberoso, C., & Cabras, P. (1994). Solid-phase extraction and high-performance liquid chromatographic determination of organic acids in honey. *Journal of Chromatography A*, *669*, 59–64.

D'Arcy, B. R., Rintoul, G. B., Rowland, C. Y., & Blackman, A. J. (1997). Composition of Australian honey extractives. 1. Norisoprenoids, monoterpenes, and other natural volatiles from blue gum (*Eucalyptus leucoxylon*) and yellow box (*Eucalyptus melliodora*) honeys. *Journal of Agricultural and Food Chemistry*, *45*(5), 1834–1843.

Davies, A. M. C. (1976). The application of amino acid analysis to the determination of the geographical origin of honey. *Journal of Food Technology*, *11*(5), 515–523.

Davies, A. M. C., & Harris, R. G. (1982). Free amino acid analysis of honeys from England and Wales: application to the determination of the geographical origin of honeys. *Journal of Apiculture Research*, *21*(3), 168–173.

Ferreres, F., Andrade, P., Gil, M. I., & Tomás-Barberán, F. A. (1996). Floral nectar phenolics as biochemical markers for the botanical origin of heather honey. *Zeitschrift fuer Lebensmittel Untersuchung und Forschung*, *202*(1), 40–44.

Ferreres, F., Andrade, P., & Tomás-Barberán, F. A. (1994). Flavonoids from Portuguese heather honey. *Zeitschrift fuer Lebensmittel Untersuchung und Forschung*, *199*, 32–37.

Ferreres, F., Andrade, P., & Tomás-Barberán, F. A. (1996). Natural occurrence of abscisic acid in heather honey and floral nectar. *Journal of Agricultural and Food Chemistry*, *44*(8), 2053–2056.

Ferreres, F., Blazquez, M. A., Gil, M. I., & Tomás-Barberán, F. A. (1994). Separation of honey flavonoids by micellar electrokinetic capillary chromatography. *Journal of Chromatography A*, *669*(1/2), 268–274.

Ferreres, F., Garcia-Viguera, C., Tomás-Lorente, F., & Tomás-Barberán, F. A. (1993). Hesperetin: a marker of the floral origin of citrus honey. *Journal of the Science of Food and Agriculture*, *61*, 121–123.

Ferreres, F., Giner, J. M., & Tomás-Barberán, F. A. (1994). A comparative study of hesperetin and methyl anthranilate as markers of the floral origin of citrus honey. *Journal of the Science of Food and Agriculture*, *65*, 371–372.

Ferreres, F., Juan, T., Perez-Arquillue, C., Herrera-Marteache, A., Garcia-Viguera, C., & Tomás-Barberán, F. A. (1998). Evaluation of pollen as a source of kaempferol in rosemary honey. *Journal of the Science of Food and Agriculture*, *77*, 506–510.

Ferreres, F., Ortiz, A., Silva, C., Garcia-Viguera, C., Tomás-Barberán, F. A., & Tomás-Lorente, F. (1992). Flavonoids of "La Alcarria" honey—a study of their botanical origin. *Zeitschrift-fuer Lebensmittel Untersuchung und Forschung*, *194*, 139–143.

Ferreres, F., Tomás-Barberán, F. A., Gil, M. I., & Tomás-Lorente, F. (1991). An HPLC technique for flavonoid analysis in honey. *Journal of the Science of Food and Agriculture*, *56*, 49–56.

Ferreres, F., Tomás-Barberán, F. A., Soler, C., Garcia-Viguera, C., Ortiz, A., & Tomás-Lorente, F. (1994). A simple extractive technique for honey flavonoid HPLC analysis. *Apidologie*, *25*, 21–30.

Hausler, M., & Montag, A. (1989). Isolation, identification and quantitative determination of the norisoprenoid (S)-(+)-dehydrovomifoliol in honey. *Zeitschrift fuer Lebensmittel Untersuchung und Forschung*, *189*, 113–115.

Hausler, M., Montag, A.(1990).Minor constituents of honey with aroma-relevance. III. Nectar flow-specific distribution of aromatic aldehydes, & presence of nitrogen, sulphur heterocyclic compounds (1990). *Deutsche Lebensmittel Rundschau*, *86*(6), 171–174.

Hausler, M., & Montag, A. (1991). Minor constituents of honey with aroma relevance. IV. Presence and nectar flow-specific distribution of the aroma compound precursor (S)-dehydrovomifoliol. *Deutsche Lebensmittel Rundschau*, *87*(2), 35–36.

Lipp, J. (1990). Detection and origin of abscisic acid and proline in honey. *Apidologie*, *21*(3), 249–260.

Martos, I., Cossentini, M., Ferreres, F., & Tomás-Barberán, F. A. (1997). Flavonoid composition of Tunisian honeys and propolis. *Journal of Agricultural and Food Chemistry*, *45*(8), 2824–2829.

Martos, I., Ferreres, F., & Tomás-Barberán, F. A. (2000). Identification of flavonoid markers for the botanical origin of Eucalyptus honey. *Journal of Agricultural and Food Chemistry*, *48*, 1498–1502.

Martos, I., Ferreres, F., Yao, L. H., D'Arcy, B. R., Caffin, N., & Tomás-Barberán, F. A. (2000). Flavonoids in monospecific Eucalyptus honeys from Australia. *Journal of Agricultural and Food Chemistry*, *48*, 4744–4748.

Sabatier, S., Amoit, M. J., Tacchini, M., & Aubert, S. (1992). Identification of flavonoids in sunflower honey. *Journal of Food Science*, *57*(3), 733–734.

Speer, K., & Montag, A. (1987). Phenylalanine decomposition products as flavour compounds in honey. *Deutsche Lebensmittel Rundschau*, *83*(4), 103–107.

Steeg, E., & Montag, A. (1988a). Minor constituents of honey, related to aroma. 1. Aromatic carboxylic acids and esters. *Deutsche Lebensmittel Rundschau*, *84*(4), 103–108.

Steeg, E., & Montag, A. (1988b). Minor constituents of honey, related to aroma. 2. Decomposition products of aromatic carboxylic acids, and glycoside-bound aromatic compounds that contribute to aroma. *Deutsche Lebensmittel Rundschau*, *84*(5), 147–150.

Steeg, E., & Montag, A. (1988c). Quantitative determination of aromatic carboxylic acids in honey. *Zeitschrift fuer Lebensmittel Untersuchung und Forschung*, *187*, 115–120.

Sun, Y. (1995). *A chemical investigation of some New Zealand native honeys*. MPhil thesis, University of Waikato, Hamilton, New Zealand.

Tan, S. T., Wilkins, A. L., Holland, P. T., & McGhie, T. K. (1989). Extractives from New Zealand unifloral honeys. 2. Degraded carotenoids and other substances from heather honey. *Journal of Agricultural and Food Chemistry*, *37*(5), 1217–1221.

Tan, S. T., Wilkins, A. L., Holland, P. T., & McGhie, T. K. (1990). Extractives from New Zealand honeys: 3. Unifloral thyme and willow honey constituents. *Journal of Agricultural and Food Chemistry*, *38*(9), 1833–1838.

Tan, S. T., Wilkins, A. L., Molan, P. T., Holland, P. T., & Reid, M. A. (1989). A chemical approach to the determination of floral sources of New Zealand honeys. *Journal of Apiculture Research*, *28*, 212–222.

Tomás-Barberán, F. A., Blazquez, M. A., Garcia-Viguera, C., Ferreres, F., & Tomás-Lorent, F. (1992). A comparative study of different amberlite XAD resins in flavonoid analysis. *Phytochemistry Analysis*, *3*, 178–181.

Tomás-Barberán, F. A., Ferreres, F., Garcia-Viguera, C., & Tomás-Lorente, F. (1993). Flavonoids in honey of different geographical origin. *Zeitschrift fuer Lebensmittel Untersuchung und Forschung*, *196*, 38–44.

Tomás-Barberán, F. A., Garcia-Viguera, C., Vit-Olivier, P., Ferreres, F., & Tomás-Lorente, F. (1993). Photochemical evidence for the botanical origin of tropical propolis from Venezuela. *Photochemistry*, *34*(1), 191–196.

Tomás-Barberán, F. A., Martos, I., Ferreres, F., Radovic, B. S., & Anklam, E. (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture*, *81*(5), 485–496.

Weston, R. J., Mitchell, K. R., & Allen, K. L. (1999). Antibacterial phenolic components of New Zealand manuka honey. *Food Chemistry*, *64*(3), 295–300.

Weston, R. J., Brocklebank, L. K., & Lu, Y. R. (2000). Identification and quantitative levels of antibacterial components of some New Zealand honeys. *Food Chemistry*, *70*, 427–435.

Wilkins, A. L., Lu, Y. R., & Molan, P. C. (1993). Extractable organic substances from New Zealand unifloral manuka (Leptospermum scoparium) honeys. *Journal of Apicultural Research*, *32*(1), 3–9.

Yao, L. H. (2002). *Flavonoids and other polyphenols in Australian tea and honey* (unpublished data).

Yao, L. H., Datta, N., Tomás-Barberán, F. A., Wansri, R., Ferreres, F., & Martos, I. An HPLC analysis of flavonoids in Australian *Eucalyptus* honey (in preparation).