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Phenolic acids and abscisic acid in Australian *Eucalyptus* honeys and their potential for floral authentication

Lihu Yao [a,b], Yueming Jiang [a,*], Riantong Singanusong [c], Nivedita Datta [b], Katherine Raymont [d]

[a] *South China Institute of Botany, Chinese Academy of Sciences, Guangzhou ReYiJu 510650, People's Republic of China*
[b] *School of Land and Food Sciences, The University of Queensland, Gatton, Qld 4343, Australia*
[c] *Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environmental Sciences, Naresuan University, Muang, Phitsanulok 65000, Thailand*
[d] *School of Agriculture and Horticulture, The University of Queensland, Gatton, Qld 4343, Australia*

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Abstract

Seven phenolic acids related to the botanical origins of nine monofloral *Eucalyptus* honeys from Australia, along with two abscisic isomers, have been analyzed. The mean content of total phenolic acids ranges from 2.14 mg/100 g honey of black box (*Eucalyptus largiflorens*) honey to 10.3 mg/100 g honey of bloodwood (*Eucalyptus intermedia*) honey, confirming an early finding that species-specific differences of phytochemical compositions occur quantitatively among these *Eucalyptus* honeys. A common profile of phenolic acids, comprising gallic, chlorogenic, coumaric and caffeic acids, can be found in all the *Eucalyptus* honeys, which could be floral markers for Australian *Eucalyptus* honeys. Thus, the analysis of phenolic acids could also be used as an objective method for the authentication of botanical origin of *Eucalyptus* honeys. Moreover, all the honey samples analyzed in this study contain gallic acid as the main phenolic acid, except for stringybox (*Eucalyptus globoidia*) honey which has ellagic acid as the main phenolic acid. This result indicates that the species-specific differences can also be found in the honey profiles of phenolic acids. Furthermore, the analysis of abscisic acid in honey shows that the content of abscisic acid varies from 0.55 mg/100 g honey of black box honey to 4.68 mg/100 g honey of bloodwood honey, corresponding to the contents of phenolic acids measured in these honeys. These results have further revealed that the HPLC analysis of honey phytochemical constituents could be used individually and/or jointly for the authentication of the botanical origins of Australian *Eucalyptus* honeys.

Keywords: Honey; Phenolic acids; Abscisic acid; *Eucalyptus*; Botanical origin; Quality

1. Introduction

Eucalypts are native only to Australia, constituting about 95% of the trees and dominating the woodlands in this continent (Kelly, 1983). The phytochemical profiles of the *Eucalyptus* floral honeys produced in Australia could, therefore, be distinctive from the other types of honeys or honeys of other botanical origins. Phytochemical constituents are considered as very important parameters in the assessment of honey flavours and organoleptic quality and hence the quality (Cherchi, Spanedda, Tuberoso, & Cabras, 1994). Among these compounds, hydrocarbons (Bonaga, Giumanini, & Gliozzi, 1986), decomposed phenylalanines (Montag, 1987), aromatic aldehydes (Häusler & Montag, 1990), aromatic carboxylic acids and esters (Speer, Steeg, & Montag, 1988), and degraded carotenoids (Tan, Wilkins, Holland, & McGhie, 1989) in various floral honeys have been correlated with the flavour quality of honeys and/or their botanical origins. As such, the volatile compounds of unifloral honeys produced in New Zealand (Tan, Wilkins, Holland, & McGhie, 1990; Wilkins, Lu, & Tan, 1993) and Australia (D'Arcy, Rintoul, Rowland, & Blackman, 1997; Rowland, Blackman,

* Corresponding author. Tel.: +86-20-372-52525; fax: +86-20-372-52831.
E-mail address: ymjiang@scib.ac.cn (Y. Jiang).

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D'Arcy, & Rintoul, 1995) were found to be characteristic only of the corresponding honeys of specific botanical origins.

Non-volatile compounds, such as flavonoids (Ferreres, Andrade, & Tomás-Barberán, 1994; Ferreres, García-Viguera, Tomás-Lorente, & Tomás-Barberán, 1993; Ferreres, Giner, & Tomás-Barberán, 1994; Ferreres et al., 1994; Ferreres, Tomás-Barberán, Gil, & Tomás-Lorente, 1991), non-flavonoid phenolic compounds (Amiot, Aubert, Gonnet, & Tacchini, 1989; Andrade, Ferreres, & Amaral, 1997; Andrade, Ferreres, Gil, & Tomás-Barberán, 1997; Ferreres, Andrade, Gil, & Tomás-Barberán, 1996; Sabatier, Amiot, Tacchini, & Aubert, 1992), and abscisic acid (Ferreres, Andrade, & Tomás-Barberán, 1996; Tomás-Barberán, Martos, Ferreres, Radovic, & Anklam, 2001), have been used as indicators of botanical origin and hence the quality of honey. These compounds may be used to differentiate the *Eucalyptus* honeys originating from different *Eucalyptus* floral varieties (Amiot et al., 1989; Tomás-Barberán, Tomás-Lorente, Ferreres, & García-Viguera, 1989) and/or different geographical origins (Martos, Ferreres, & Tomás-Barberán, 2000; Martos et al., 2000; Tomás-Barberán, Ferreres, García-Viguera, & Tomás-Lorente, 1993). Among these honey components, phenolic acids have been found as a major group of phenolic compounds present in honeys (Andrade et al., 1997, 1997; Ferreres, Andrade, Gil, et al., 1996; Gil, Ferreres, Ortiz, Subra, & Tomás-Barberán, 1995; Martos, Cossentini, Ferreres, & Tomás-Barberán, 1997; Vit, Soler, & Tomás-Barberán, 1997). There are about 26 phenolic acids found in various honeys (Andrade et al., 1997), some of which could be related to the floral markers of honeys (Tomás-Barberán et al., 2001). Among these phenolic acids, ellagic acid has been proposed as the floral marker for French calluna (*Calluna vulgaris*) honey (Soler, Gil, García-Viguera, & Tomás-Barberán, 1995) and Portuguese heather (*Erica* spp.) honey (Andrade et al., 1997, 1997; Ferreres, Andrade, Gil, et al., 1996). However, no published data are available on phenolic acids and abscisic acid in the majority of Australian *Eucalyptus* honeys prior to this study. Thus, a great number of analyses on these phytochemical constituents could provide a database for aiding the authentication of botanical origins of Australian *Eucalyptus* honeys, particularly, when the pollen analysis is not as easily applicable as in other species, due to the difficulty of collection, and the propolis authentication is not as efficient as done in the northern hemisphere (Tomás-Barberán et al., 1993) due to the foraging properties of honeybees in the Australian practice (Winner, W.G., Capilano Honey Limited, Brisbane, Australia, 2000–2002, personal communications).

The present work aims the determination of phenolic acids and abscisic acid of monofloral *Eucalyptus* honeys from Australia. The objective determination of these compounds as floral markers and the occurrence of species-specific markers for Australian *Eucalyptus* honeys are also considered.

2. Materials and methods

2.1. Honey samples

The requested *Eucalyptus* honey samples for this study were sourced from the main honey production regions, namely, New South Wales (NSW) and Queensland (QLD), of Australia, by individual apiarists during the flowering seasons. The geographical locations, botanical origins and sourcing dates of these honeys are detailed in Table 1. All the samples were

Table 1
Australian unifloral *Eucalyptus* honeys used in this experiment

Sample code	Common name	Floral origin	Year	Origin
T/T LT1054[a]	Bloodwood	*E. intermedia*	1999	Kempsey, NSW
T/T LT286[a]	Bloodwood	*E. intermedia*	1999	Wauchope, NSW
B/wood A4131	Bloodwood	*E. intermedia*	1998	Maryborough, QLD
Yap Z8325	Yapunyah	*E. ochrophloia*	1995	Channel Country, QLD
Yap Z8174	Yapunyah	*E. ochrophloia*	1995	Channel Country, QLD
Yap A3911	Yapunyah	*E. ochrophloia*	1998	Quilpie, QLD
NL/IB A3988	Narrow-leaved ironbark	*E. crebra*	1998	Millmerran, QLD
BT/IB A3942	Blue top ironbark	*E. nubila*	1998	Western Creek, DLD
YB Q6734	Yellow box	*E. melliodora*	1997	Inverell, NSW
YB Y8879	Yellow box	*E. melliodora*	_[b]	_[b]
G/T A3895	Gum top	*E. moluccana*	1998	Barakula, QLD
RRG A5606	River red gum	*E. camaldulensis*	1999	Cunamulla, QLD
ST/Bark A4110	Stringybark	*E. globoidia*	1997	New England, NSW
Black/B A5629	Black box	*E. largiflorens*	1999	Goondiwindi, QLD

[a] While the code (T/T) was similar to that for tea tree honey, the floral source was confirmed by suppliers as bloodwood (*E. intermedia*) honey.
[b] Date and location could not be confirmed.

stored in a freezer at a temperature of −18 to −24 °C prior to analysis.

2.2. Australian practice of identification of honey floral source

In Australia, species-specific floral types of honey are obtained by the apiarist pursuing a particular floral species for honey production, through controlling the foraging of their honeybees by hive location (near to one species of plant) and season (flowering and honey flows of nectar) of production. In this study, the aroma, taste and colour characteristics, together with information about season, hive location and available floral sources, were utilised by supplying apiarists and honey packers to accurately identify the floral source of the honey samples. This procedure is the standard honey-sourcing method utilised by the Australian honey industry (D'Arcy et al., 1997; Rowland et al., 1995). Pollen analysis of *Eucalyptus* species is seen a challenge, due to the characteristic standing of the plants. Furthermore, beekeepers in Australia attempt to maintain high standards of hive construction. This reduces the need for bees to seal gaps, thus reducing the production of propolis (Winner, W.G., Capilano Honey Limited, Brisbane, Australia, 2000–2002, personal communications).

In addition, the honeybees in Australia show different foraging behaviour towards more honey production by collecting more nectar for honey production rather than the glues for propolis (Winner, W.G., Capilano Honey Limited, Brisbane, Australia, 2000–2002, personal communications). Thus, the source of propolis and its derivative compounds is limited in Australian honeys, resulting in less efficiency of floral authentication.

2.3. Chemical solvents and standards

Distilled water, analytical grade hydrochloric acid (HCl) and methanol were used for the column chromatography, whereas distilled water and analytical grade diethyl ether were used for re-extraction of phenolic and abscisic acids. HPLC grade methanol, distilled water and analytical grade formic acid, with double filtering, were used for HPLC analysis. The authentic chemical compounds used for the identification and quantification of phenolic and abscisic acids in this study were (chemical name bracketed): caffeic acid [3(3,4-dihydroxyphenyl)-prop-2-enoic acid], chlorogenic acid (3-caffeoyl quinic acid), *p*-coumaric acid [3(4-hydroxyphenyl)-prop-2-enoic acid], ellagic acid (4,4′, 5,5′,6,6′-hexahydroxydiphenic acid 2,6,2′,6′-dilactone), ferulic acid [3(3-methoxy-4-hydroxyphenyl)-prop-2-enoic acid], gallic acid (3,4,5-trihydroxybenzoic acid), abscisic acid [5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-methyl pentadienoic acid].

2.4. Sample extraction (column chromatography)

Extraction was carried out as described previously (Martos et al., 1997; Martos, Ferreres, Tomás-Barberán, 2000; Martos et al., 2000; Yao et al., 2003). Honey samples (100 g) were thoroughly mixed with five parts (500 ml) of distilled water, adjusted to pH 2 with concentrated HCl, until completely fluid. The fluid samples were then filtered through cotton wool to remove solid particles. The filtrate was mixed with 150 g of Amberlite XAD-2 (Supelco, Bellefonte, PA, USA, 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 adsorb honey phenolics with a recovery rate of more than 80% (Martos et al., 1997; Tomás-Barberán, Blazquez, García-Viguera, Ferreres, & Tomás-Lorent, 1992). The Amberlite particles were then packed in a glass column (42×3.2 cm) and 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 acids remain adsorbed on the column and could be eluted with methanol (Ferreres et al., 1991; Tomás-Barberán et al., 1992). Thus, the whole fraction of phenolic acids was then eluted with methanol (400 ml). This extract was concentrated to dryness under reduced pressure in a rotary evaporator at 40 °C. The residue was redissolved in distilled water (5 ml) and extracted with diethyl ether (5 ml × 3). The extracts were combined, and the diethyl ether was removed by flushing with nitrogen. The dried residue was redissolved in 1 ml of methanol and filtered through a 0.45 μm membrane filter for HPLC analysis.

2.5. HPLC analysis

The analyses of Australian unifloral honey extracts were conducted using a Shimadzu Class-VP HPLC system with a computer-controlled system containing 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 water-formic acid (19:1, v/v) (solvent A) and methanol (solvent B) at a constant solvent flow rate of 1 ml/min. The following gradient was used according to the method of Martos et al. (1997). Thirty percent methanol (B) flowed through the column isocratically with solvent A for 15 min, and was then 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 an SIL-10A XL Auto Injector and the phenolic acids were detected using a multichannel photodiode-array detector (SPD-M10A VP) to obtain the UV spectra of the various phenolics. In addition, the chromatograms were monitored at 290 and 340 nm, since most of the honey phenolic acids (also flavonoids) show their UV absorption maxima around these two wavelengths (Martos et al., 1997; Yao et al., 2003). The phenolic acids were identified and quantified according to the method reported previously (Andrade et al., 1997, 1997; Martos et al., 1997) by comparing their UV spectra and retention times against authentic compounds. When the authentic compounds for honey phenolics were unavailable, the stored UV spectra extracted by the same HPLC methods for honey analysis and their corresponding retention times were utilized for the identification. In this study, the phenolic acids, such as gallic, chlorogenic and coumaric acids, were quantified against their standards at 290 nm, and ellagic acid at 340 nm. The non-phenolics, abscisic acid, including both *trans*,*trans*- and *cis*,*trans*-isomers, were determined against the standard at 290 nm.

3. Results and discussion

3.1. Phenolic acids in Australian bloodwood (E. intermedia) and yapunyah (E. ochrophloia) honeys

The HPLC analysis of phenolic acids in samples of bloodwood and yapunyah honeys showed that gallic, chlorogenic, caffeic and coumaric acids were present in both floral types of honeys (Fig. 1). In bloodwood honey, the mean value of total phenolic acids reached 10.3 mg/100 g honey, which is the largest total amount of phenolic acids measured in the Australian *Eucalyptus* honeys (Table 2). Of these phenolic acids measured in bloodwood honey, gallic and chlorogenic acids represent 37.3% and 29.6% of the total content, respectively. Ellagic and coumaric acids are the secondary phenolic acids (13.1% and 10.3%, respectively) in this honey (Table 2).

Ellagic acid has been suggested as a floral marker for Portuguese heather honey, with the content ranging 0.10–0.60 mg/100 g honey (Ferreres, Andrade, Gil, et al., 1996), or 0.30–1.20 mg/100 g honey (Tomás-Barberán et al., 2001). In Australian bloodwood honey, ellagic acid ranges 1.02–1.48 mg/100 g honey, with an

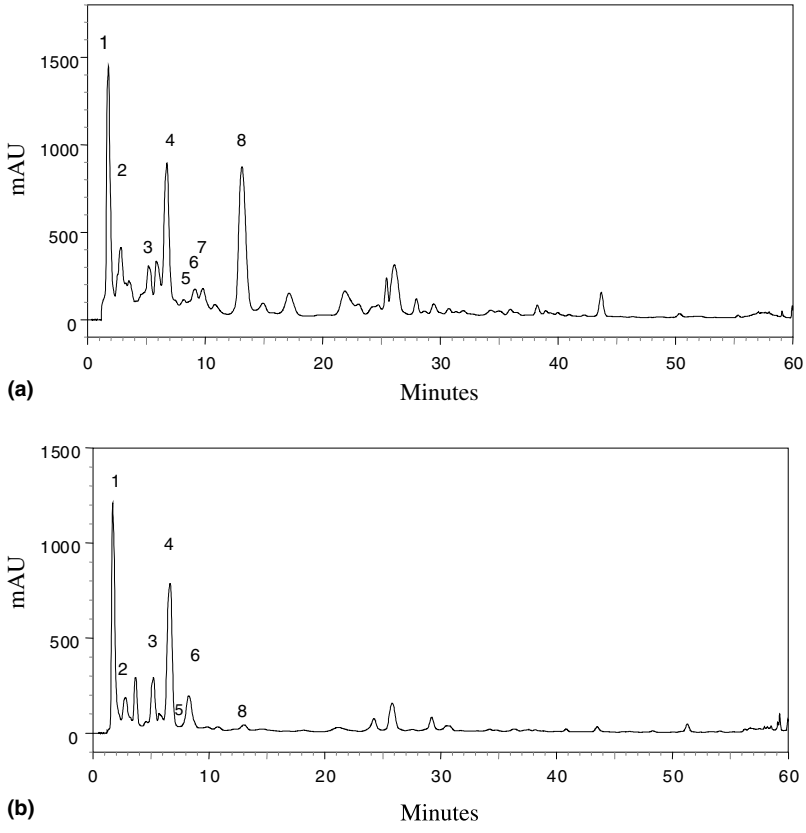

Fig. 1. HPLC chromatograms of phenolic acids and abscisic acid in Australian *Eucalyptus* honeys (290 nm). (a) Bloodwood (*E. intermedia*) honey; (b) yapunyah (*E. ochrophloia*) honey. Phenolic acids are identified as: (1) gallic acid, (2) chlorogenic acid, (3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, (6) *o*-coumaric, (7) ellagic acid, and (8) *trans*,*trans*-abscisic acid.

Table 2
Contents of phenolic acids in Australian unifloral *Eucalyptus* honeys

Sample[a]	Phenolic acids (mg/100 g honey)[b,c]							Total
	GA	CA	Caf	Cou	Fer	Ell	Ph1	
T/T LT1054	4.36 (42.2)	1.77 (17.1)	0.45 (4.3)	1.43 (13.8)	0.85 (8.3)	1.47 (14.3)		10.3
T/T LT286	1.44 (24.3)	2.53 (42.6)	0.31 (5.2)	0.24 (4.0)	0.40 (6.7)	1.02 (17.2)		5.94
B/wood A4131	6.62 (45.2)	4.26 (29.1)	0.71 (4.8)	1.88 (12.9)		1.16 (7.9)		14.6
Mean	4.14 (37.3)	2.85 (29.6)	0.49 (4.8)	1.18 (10.3)	0.63 (5.0)	1.22 (13.1)		
S.D.	2.59 (11.3)	1.28 (12.7)	0.20 (0.5)	0.85 (5.4)	0.32 (4.4)	0.23 (4.7)		
Yap Z8325	2.83 (39.0)	1.10 (15.2)	1.52 (20.9)	1.52 (20.9)	0.30 (4.1)			7.27
Yap Z8174	3.16 (49.2)	0.86 (13.4)	0.44 (6.9)	1.12 (17.5)	0.84 (13.0)			6.43
Yap A3911	1.06 (24.3)	1.16 (26.5)	0.25 (5.6)	1.07 (24.6)	0.39 (9.0)	0.44 (10.0)		4.37
Mean	2.35 (37.5)	1.04 (18.4)	0.74 (11.1)	1.24 (21.0)	0.51 (8.7)	0.22 (3.6)		
S.D.	1.13 (12.5)	0.16 (7.1)	0.69 (8.5)	0.25 (3.6)	0.29 (4.5)			
YB Q6734	6.58 (76.6)	0.47 (5.5)	0.40 (4.7)	0.59 (6.9)	0.27 (3.2)	0.28 (3.2)		8.60
YB Y8879	4.04 (68.9)	0.33 (5.6)	0.22 (3.7)	0.18 (3.1)	0.64 (10.8)	0.27 (4.7)	0.19 (3.2)	5.87
Mean	5.31 (72.7)	0.40 (5.6)	0.31 (4.2)	0.39 (5.0)	0.45 (7.0)	0.28 (4.0)	0.09 (1.3)	
S.D.	1.80 (5.4)	0.10 (0.1)	0.13 (0.7)	0.29 (2.7)	0.26 (5.4)			
NL/IB A3988	1.61 (33.2)	1.20 (24.7)	0.22 (4.5)	0.37 (7.5)	1.08 (22.3)	0.38 (7.8)		4.85
BT/IB A3942	1.20 (29.1)	0.80 (19.5)	0.18 (4.5)	0.98 (23.9)	0.19 (4.7)	0.76 (18.3)		4.12
G/T A3895	2.00 (35.5)	0.37 (6.7)	0.50 (8.9)	1.26 (22.3)	0.27 (4.8)	0.55 (9.7)	0.68 (12.1)	5.63
RRG A5606	0.69 (23.4)	0.13 (4.5)	0.39 (13.3)	0.60 (20.5)	0.15 (5.1)	0.54 (18.2)	0.44 (15.0)	2.94
ST/Bark A4110	0.34 (5.5)	0.36 (5.9)	0.71 (11.5)	0.59 (9.5)		2.66 (42.7)	1.55 (24.9)	6.22
Black/B A5629	0.43 (20.0)	0.47 (22.2)	0.17 (7.8)	0.38 (17.6)	0.15 (6.8)	0.33 (15.4)	0.22 (10.3)	2.14

[a] Common name and floral origin of honey samples are as in Table 1.
[b] GA: Gallic acid, CA: Chlorogenic acid, Caf: Caffeic acid, Cou: Coumaric acid, Fer: Ferulic acid, Ell: Ellagic acid, Ph1: Unknown phenolic acid.
[c] Values in parentheses are percent of each individual phenolic acid in total phenolic acids detected in the samples.

average of 1.22 mg/100 g honey (Table 2), which shows a higher concentration and is less variable than that in Portuguese heather honey. Thus, although ellagic acid is not the main phenolic acid present in bloodwood honey, the phenolic profile, comprising mainly gallic, chlorogenic, ellagic and coumaric acids, is characteristic and could be used as a marker for the floral origin of this Australian *Eucalyptus* honey.

In this study, the gradient elution of gallic acid has been found to be close to the solvent front during the HPLC separation. Thus, it is important to take special care for the accurate identification and quantification of this compound. Alternatively, an improvement in the mobile phase at an earlier stage of the separation may provide a better isolation of gallic acid from the solvent front. However, gallic acid was identified and quantified in this study by using both its retention time and full spectrum, relative to its standard. Thus, it has been included in the phenolic profiles of Australian *Eucalyptus* honeys. Furthermore, coumaric acid in this study is the sum of *p*-coumaric acid and *o*-coumaric acid. *o*-Coumaric acid was identified according to the library spectrum and chromatographic behaviour (Andrade et al., 1997, 1997). Another minor phenolic acid showed some spectroscopic properties of 4-hydroxybenoic acid, and was labelled in this study as an unknown phenolic acid (Ph1).

In yapunyah honey, the content of total phenolic acids is 6.02 mg/100 g honey (Table 2). The phenolic profile is dominated by gallic acid (Fig. 1), which represents 37.5% of the total phenolic acids, followed by coumaric, chlorogenic, and caffeic acids (Table 2). This phenolic profile is characteristic of yapunyah honey, and could be used as a marker for the floral origin of yapunyah honey.

The main difference between bloodwood and yapunyah honeys is that caffeic acid is a secondary compound in the latter (11.1%), while ellagic acid is a secondary compound for the former samples (13.1%). Additionally, coumaric acid represents 21.0% of total phenolic acids in yapunyah honey, which is larger than that found in bloodwood honey (10.3%) (Table 2). Furthermore, chlorogenic acid represents 29.6% of total phenolic acids in bloodwood honey, which is higher than that in yapunyah honey (18.4%).

3.2. Phenolic acids in Australian yellow box (E. melliodora) and stringybark (E. globoidia) honeys

The content of total phenolic acids is 7.24 mg/100 g honey in yellow box honey, with gallic acid having the highest mean value of a single phenolic acid among the *Eucalyptus* honeys analysed (Table 2). Gallic acid represents 72.7% of the total phenolic acids present in this honey. The other phenolic acids include chlorogenic, caffeic, coumaric, ferulic, ellagic and the unknown phenolic acid (Ph1) (Table 2), which are in small amounts. Thus, gallic acid could be used as a sole biochemical marker for the floral origin of Australian yellow box honey. Further studies on more yellow box honey samples sourced from different seasons and geographical

locations are suggested to confirm the findings in this study.

In stringybark honey, the main phenolic acid, ellagic acid, shows the highest level (2.66 mg/100 g honey), compared to the *Eucalyptus* honeys analysed so far, representing 42.7% of total phenolic acids of this honey (Fig. 2; Table 2). The unknown phenolic acid, Ph1, is shown to be the secondary acid, with caffeic and coumaric acids occurring in much smaller amounts (Table 2). Ellagic acid was proposed as the floral marker for French calluna (*Calluna vulgaris*) honey (Soler et al., 1995) and Portuguese heather (*Erica* spp.) honey (Andrade et al., 1997, 1997; Ferreres, Andrade, Gil, et al., 1996). Vit et al. (1997) reported that the presence of ellagic acid was a characteristic of *A. mellifera* honeys from Venezuela. Thus, further studies are necessary to confirm whether ellagic acid could also be used as a biochemical marker for the floral origin of Australian stringybark honey. However, the phenolic profile, comprising a large amount of ellagic acid, together with caffeic and coumaric acids, could be used as a floral marker for Australian stringybark honey because there has been no other reports showing a similar phenolic profile in any other honeys.

3.3. Phenolic acids in Australian gum top (*E. moluccana*) honey

Examination of the HPLC chromatogram of phenolic acids in gum top honey reveals that gallic and coumaric acids are major phenolic acids (Fig. 3; Table 2). Gallic acid represents 35.5%, while coumaric acid 22.3%, of total phenolic acids in this honey (Table 2). This phenolic profile, comprising mainly gallic and coumaric acids, may be considered as a floral marker for gum top honey as no other honey types show such a characteristic phenolic profile.

3.4. Phenolic acids in Australian narrow-leaved ironbark (*E. crebra*) and blue top ironbark (*E. nubila*) honeys

The main phenolic acids in narrow-leaved ironbark honey are gallic, chlorogenic and ferulic acids (Table 2). Gallic acid represents 33.2% of total phenolic acids in

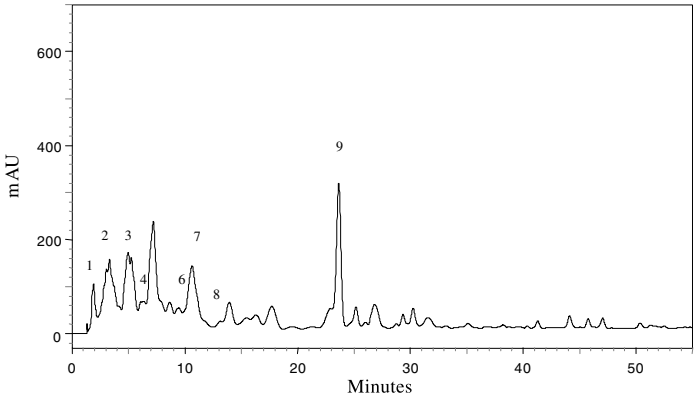

Fig. 2. HPLC chromatogram of phenolic and abscisic acids in Australian stringybark (*Eucalyptus globoidia*) honey (290 nm). Phenolic acids are identified as: (1) gallic acid, (2) chlorogenic acid, (3) caffeic acid, (4) *p*-coumaric acid, (6) *o*-coumaric acid, (7) ellagic acid, (8) *trans*,*trans*-abscisic acid and (9) *cis*,*trans*-abscisic acid.

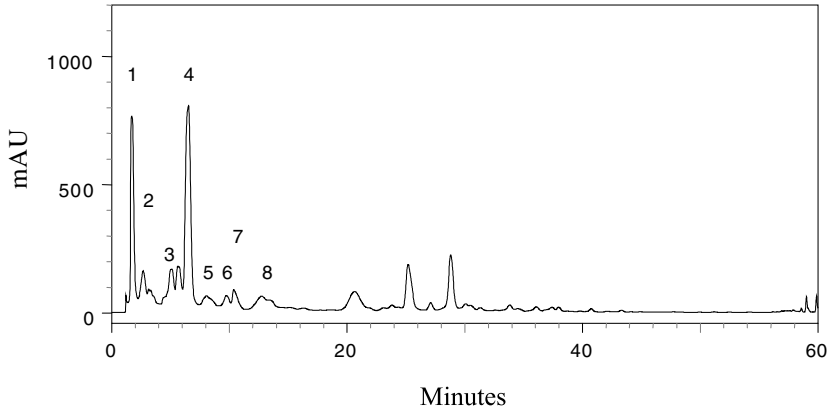

Fig. 3. HPLC chromatogram of phenolic acids and abscisic acid in Australian gum top (*E. moluccana*) honey (290 nm). Phenolic acids are identified as: (1) gallic acid, (2) chlorogenic acid, (3) caffeic acid, (4) *p*-coumaric acid, (5) ferulic acid, (6) *o*-coumaric acid, (7) ellagic acid, and (8) *trans*,*trans*-abscisic acid.

this honey, followed by chlorogenic and ferulic acids (24.7% and 22.3%, respectively) (Table 2). The percentage of ferulic acid found in narrow-leaved ironbark honey distinguishes it from other *Eucalyptus* honey examined in this study. These three compounds together represent 80.2% of total phenolic acids measured in this honey, suggesting that they could be related to the floral origin of this type of honey. In contrast, the phenolic profile for blue top ironbark honey mainly comprises gallic, coumaric, chlorogenic, and ellagic acids (Table 2). These four compounds represent 90.8% of total phenolic acids present in this honey, with gallic and coumaric acids being the main, and chlorogenic and ellagic acids being the secondary phenolic acids (Table 2). Thus, this phenolic profile is characteristic of blue top ironbark honey and could be used as a floral marker for it.

The main difference between the phenolic profiles of narrow-leaved ironbark honey and blue top ironbark honey is that, in the former, ferulic acid is the secondary phenolic acid, representing 22.3% of total phenolic acids while, in the latter, it represents only 4.7% of total phenolic acids. The secondary phenolic acids present in blue top ironbark honey are coumaric and ellagic acids. This result indicates that these two types of honeys have their own species-specific phenolic profiles and can thus be differentiated by them.

3.5. Phenolic acids in Australian river red gum (*E. camaldulensis*) and black box (*E. largiflorens*) honeys

The content of total phenolic acids present in Australian river red gum honey is the second lowest among the *Eucalyptus* honeys analysed (Table 2). Gallic, coumaric, and ellagic acids are the main compounds in this honey. Gallic acid represents 23.4%, whereas coumaric acid, ellagic acid and an unknown phenolic acid (Ph1) represent 20.5%, 18.2%, and 15.0% of total phenolic acids, respectively (Table 2). These four phenolic acids, together accounting for 77.1% of the total phenolic acids, could thus be used as the floral marker for river red gum honey.

In Australian black box honey, the content of total phenolic acids is the lowest among the *Eucalyptus* honey samples analysed (Table 2). Chlorogenic and gallic acids are the main phenolic acids, representing 22.2% and 20.0% of total phenolic acids, respectively. Coumaric and ellagic acids are the secondary phenolic acids (Table 2). These results show a relatively even contribution of the main phenolic acids in the phenolic profile, with chlorogenic acid contributing a greater percentage than gallic acid to the total phenolic acids. Thus, this phenolic profile could be used as a subsidiary floral marker for flavonoids and other objective indicators in authenticating the origin of Australian black box honey.

3.6. Abscisic acid in Australian Eucalyptus honeys

A large amount of abscisic acid has been found in some of the analysed *Eucalyptus* honeys. Abscisic acid is not a phenolic acid. However, it shows very similar chromatographic behaviour to the phenolic acids in honey, with very strong UV absorbances at 238 and 260 nm. Thus, it has been considered as one of the important phytochemical constituents for authentication of honey.

In this study, abscisic acid is one of the main phytochemical constitutes in Australian bloodwood honeys, in which the total content of two isomers of abscisic acid is 4.68 mg/100 g honey (Table 3). However, there are much smaller amounts of abscisic acid in the other *Eucalyptus* honeys, such as at a mean level of 1.86 mg/100 g honey in Australian yellow box honey (Table 3). Thus, abscisic acid could be used to differentiate honeys of one *Eucalyptus* species from another.

Abscisic acid has been found in many honeys, in various concentrations, including Australian blue gum (*E. leucoxylon*) honey and leatherwood (*Eucryphia lucida*) honey (Lipp, 1990; Sun, 1995). In Portuguese heather (*Erica* spp.) honey, abscisic acid ranged from 2.5–16.6 mg/100 g honey, and was thus proposed as a floral marker (Ferreres, Andrade, Tomás-Barberán, 1996). In this study, if the phenolic profile, comprising

Table 3
Content of abscisic acid in unifloral Australian *Eucalyptus* honeys

Common name	Botanical name	Abscisic acid (mg/100 g honey)[a]		Total
		Trans,trans-ABA	*Cis,trans*-ABA	
Bloodwood	*E. intermedia*	4.03	0.65	4.68
Yapunyah	*E. ochrophloia*	0.94	0.22	1.16
Yellow box	*E. melliodora*	1.04	0.82	1.86
Narrow-leaved ironbox	*E. crebra*	1.02	0.44	1.45
Blue top ironbark	*E. nubila*	0.78		0.78
Gum top	*E. moluccana*	0.49	0.28	0.77
River red gum	*E. camaldulensis*	0.68		0.68
Stringbark	*E. globoidia*	0.44	2.24	2.68
Black box	*E. largiflorens*	0.55		0.55

[a] ABA: Abscisic acid.

gallic, chlorogenic, caffeic and coumaric acids alone, could not be used as a marker for the floral origin of Australian bloodwood honey, a combination of abscisic acid with the profile of these four phenolic acids could be used to authenticate bloodwood honey (Fig. 1a). Thus, using a chemical approach, abscisic acid may be one of the biochemical markers, together with phenolic compounds, for the authentication of the floral origin of Australian *Eucalyptus* honeys.

4. Conclusion

Gallic acid has been found in all the samples of Australian *Eucalyptus* honeys. It is also the main phenolic acid for all the honey types, except for stringybox honey which is dominated by ellagic acid. This result may indicate that gallic acid could be used as a floral marker of Australian *Eucalyptus* honeys because there have been no published reports to date to indicate that this compound is present in other types of honey in such an amount. Meanwhile, the phenolic profiles for Australian *Eucalyptus* honeys also show characteristic patterns. The absence or presence of an individual phenolic acid in the profiles may be used to differentiate each species-specific floral type of honey among the *Eucalyptus* honeys.

The results of this current study suggest that distribution of phenolics in honeys is affected by the floral origins of honeys; thus honeys could be differentiated by their profiles of phenolic acids. For honeys of similar floral origins, if the profiles of phenolic acids cannot always assist in the differentiation, the contents of individual or total phenolics may be used. A non-phenolic phytochemical, abscisic acid, occurring in some honeys in large amounts, may also assist in the differentiation of floral origins of honeys. Thus, differentiation of these honey types may need a number of chemical parameters. As the phenolic acids and abscisic acid vary quantitatively and largely among and across the samples, due to the difficulty of collecting more species-specific *Eucalyptus* honeys for the analysis, further studies are necessary to analyze more samples of each of the selected honeys, in combination with the analysis of bee pollens and/or propolis where applicable, before a full conclusion can be drawn. However, the profiles of phenolic acids, together with abscisic acid, appear to be promising for the objective authentication of the floral origins of Australian honeys.

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