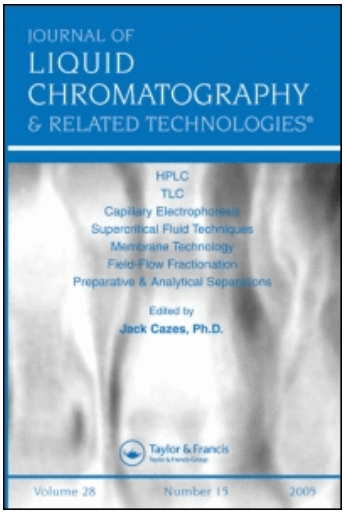

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P. Andrade[a]; F. Ferreres[b]; M. T. Amaral[c]
[a] Laboratório de Farmacognosia Faculdade de Farmácia da Universidade do Porto, Porto, Portugal [b] Departamento de Ciencia y Tecnología de Alimentos, Laboratório de Fitoquímica, Murcia, Spain [c] Laboratório de Farmacognosia Faculdade de Farmàcia da Universidade de Coimbra, Coimbra, Portugal

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ANALYSIS OF HONEY PHENOLIC ACIDS BY HPLC, ITS APPLICATION TO HONEY BOTANICAL CHARACTERIZATION

Paula Andrade,[1]* Federico Ferreres,[2] M. Teresa Amaral[3]

[1] Laboratório de Farmacognosia
Faculdade de Farmácia da Universidade do Porto
4050 Porto, Portugal

[2] Laboratório de Fitoquímica
Departamento de Ciencia y Tecnología de Alimentos
CEBAS (CSIC)
P.O. Box 4195
Murcia 30080, Spain

[3] Laboratório de Farmacognosia
Faculdade de Farmácia da Universidade de Coimbra
3000 Coimbra, Portugal

ABSTRACT

In this work we described an analytical technique that allowed the identification of 12 phenolic acids in honey samples, with emphasis on those phenolics acids which are markers of the botanical origin. After optimization of the HPLC conditions, this was applied to the phenolic acids analysis of 20 *Erica* sp. (heather) and 20 *Lavandula stoechas* (lavander) portuguese honeys.

A close correlation between the phenolic acids patterns and the botanical origin of honey has been found. *Erica* sp. honeys are characterized by ellagic, *p*-hydroxybenzoic, syringic and *o*-coumaric acids, and *Lavandula stoechas* honeys by gallic acid.

INTRODUCTION

The botanical origin of honey is one of its main quality parameters, and its price is very often related to this floral origin.[1] Recent studies have revealed that the analysis of phenolic compounds constitute a very promising technique to study the geographical and floral origin of honey.[2-7]

Reversed-phase HPLC is considered as the method of choice in phenolic compounds analysis,[8,9] however, HPLC of honey phenolic acids has rarely been reported. Thus, Amiot et al.[10] analysed flavonoids and phenolic acids in floral French honeys.

The aim of the present work is the analysis of honey phenolic acids by HPLC, and this analysis will be applied to the study of the phenolic acids present in some selected honey samples of different floral origin.

MATERIALS AND METHODS

Honey Samples

The *Erica* sp. (heather) and *Lavandula stoechas* (lavander) honey samples used in this study came from the Serra da Lousã and Trás-os-Montes regions (Portugal), respectively, and were directly provided by the beekeepers. The honey samples had not been industrially processed. To minimise any alterations, the samples were stored at -20°C.

Sample Preparation

Phenolic compounds for HPLC analysis were extracted from honey as reported previously.[11-13] The available honey samples (ca. 50 g) were thoroughly mixed with five parts of water (pH 2 with HCl) until completely fluid and filtered through cotton to remove solid particles. The filtrate was then passed through a column (25 x 2 cm) of Amberlite XAD-2 (Fluka Chemie; pore size 9 nm, particle size 0.3-1.2 mm). The phenolic compounds present in honey

remained in the column while sugars and other polar compounds were eluted with the aqueous solvent. The column was washed with acid water (water pH 2 with HCL, 100 mL) and subsequently with distilled water (ca. 300 mL). The whole phenolic fraction was eluted with methanol (ca. 300 mL). This fraction was concentrated under reduced pressure and purified by dissolving them in methanol and passing the solution through a Sephadex LH-20 column (15 x 1 cm). Phenolic fraction was clearly visualized under UV light (360 nm). The phenolic fraction was evaporated to dryness under reduced pressure (40°C), redissolved in methanol (0.5 mL) and analysed by HPLC.

HPLC Analysis of Honey Flavonoids

HPLC analysis was carried out in a Gilson system (Gilson Medical Electronics, Villiers le Bel France) equipped with a type 305 pump, a type 302 pump and a type 7125 Rheodyne Injector with a 20 μL loop. The chromatographic separation was achieved with a Merck Lichrospher 100 RP-18 (125x3 mm; 5 μm particle size) column, using water-formic acid (19:1) (solvent A) and methanol (solvent B) as solvents.

After trying different solvent gradients, the best resolution was obtained at a solvent flow rate of 0.4 mL/min, starting with 5% methanol and installing a gradient to obtain 15% B at 10 min, 30% B at 15 min., 35% B at 25min., 80% B at 40 min, and which then became isocratic until 45 min. Detection was achieved with a diode array detector, and chromatograms were recorded at 320 and 280nm.

The different phenolics compounds were identified by their UV spectra recorded with the diode array detector and by chromatographic comparisons (retention times) with authentic markers.

Phenolic acids quantification was achieved by the absorbance recorded in the chromatograms relative to external standards of phenolic acids with detection at 320nm for chlorogenic and caffeic acids and 280nm for the others.

RESULTS AND DISCUSSION

The technique presented here for the analysis of phenolic acids in honey is quite useful. The use of Amberlite XAD-2 allows the elimination of sugars and polar compounds from honey, yielding a phenolic acid fraction which also contains other phenolic compounds (flavonoids).[11] As we need a phenolic acid

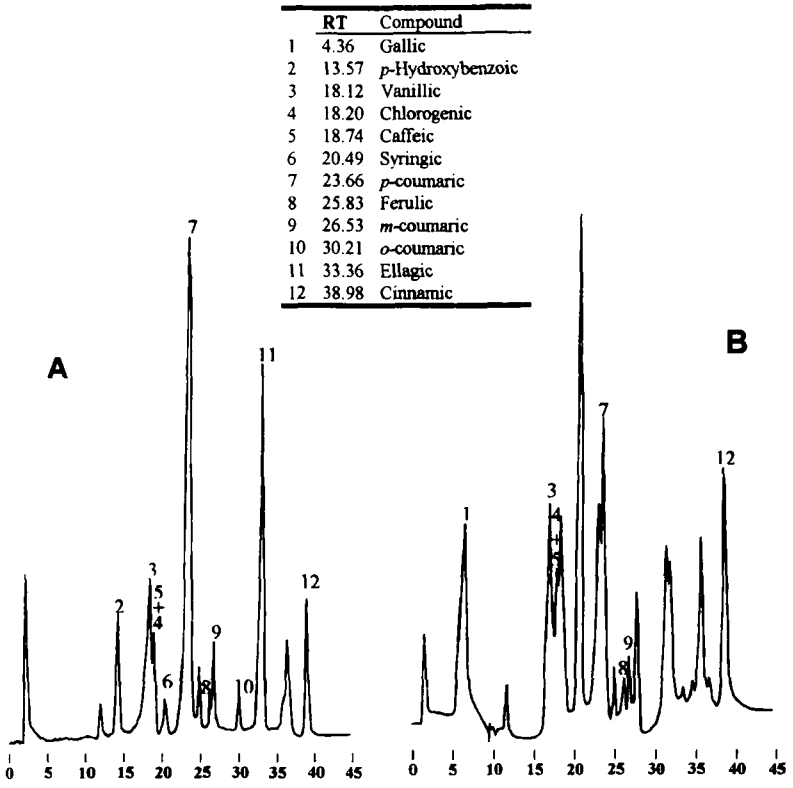

Figure 1. HPLC of honey phenolic acids. (A) Heather honey; (B) Lavender honey. Detection at 280 nm.

fraction which is sufficiently purified to permit phenolic acid analysis and quantification of the various compounds without problems of interference from other UV-absorbing substances (other phenolics), a purification through Sephadex LH-20 with methanol becomes necessary.

The phenolic acids present in the honeys samples were extracted and analysed by HPLC and the results are summarised in Tables 1 and 2. All the analysed samples contained a common phenolic acid profile including vanillic, chlorogenic, caffeic, *p*-coumaric, ferulic, *m*-coumaric, and cinnamic. On the other hand, some compounds were only detected in only one unifloral honey type, and could be considered as potential floral markers. Thus, gallic acid and an unidentified peak (RT 20.71 min) seems to be characteristic of *Lavandula*

Table 1
Phenolic Acid Content of *Lavandula Stoechas* Honeys*

Samples	Gallic	p-OH-benzoic	Vanillic	Chlorogenic	Caffeic	Siringic	p-Coumraric	Ferulic	m-Coumaric	o-Coumaric	Ellagic	Cinnamic
1	237.20	---	0.32	0.49	0.05	---	0.03	0.12	0.05	---	---	0.08
2	209.90	---	0.54	0.51	0.08	---	0.07	0.31	0.06	---	---	0.09
3	195.90	---	0.44	0.47	0.06	---	0.02	0.35	0.07	---	---	0.09
4	212.85	---	0.65	0.55	0.05	---	0.06	0.10	0.04	---	---	0.10
5	202.89	---	0.38	0.45	0.06	---	0.04	0.12	0.07	---	---	0.10
6	209.34	---	0.47	0.59	0.07	---	0.04	0.18	0.04	---	---	0.08
7	211.63	---	0.35	0.43	0.06	---	0.04	0.21	0.07	---	---	0.07
8	199.57	---	0.52	0.56	0.07	---	0.06	0.36	0.08	---	---	0.08
9	214.76	---	0.58	0.44	0.05	---	0.01	0.11	0.06	---	---	0.08
10	221.51	---	0.40	0.60	0.06	---	0.05	0.27	0.06	---	---	0.07
11	202.31	---	0.51	0.53	0.05	---	0.07	0.16	0.07	---	---	0.09
12	199.98	---	0.63	0.48	0.05	---	0.03	0.10	0.05	---	---	0.09
13	226.32	---	0.49	0.52	0.05	---	0.03	0.24	0.06	---	---	0.08
14	216.57	---	0.52	0.44	0.06	---	0.01	0.11	0.08	---	---	0.06
15	183.57	---	0.62	0.61	0.04	---	0.04	0.21	0.05	---	---	0.08
16	225.94	---	0.45	0.58	0.07	---	0.07	0.23	0.06	---	---	0.08
17	232.64	---	0.55	0.43	0.08	---	0.06	0.15	0.05	---	---	0.07
18	219.31	---	0.56	0.46	0.07	---	0.03	0.24	0.04	---	---	0.07
19	209.57	---	0.53	0.54	0.05	---	0.03	0.12	0.07	---	---	0.08
20	185.97	---	0.48	0.40	0.09	---	0.04	0.15	0.05	---	---	0.09
$\overline{X}$	210.89	---	0.50	0.50	0.06	---	0.04	0.19	0.06	---	---	0.08
sd	14.23	---	0.09	0.06	0.01	---	0.02	0.08	0.01	---	---	0.01
V_{min}	183.57	---	0.32	0.40	0.04	---	0.01	0.10	0.04	---	---	0.06
V_{mix}	237.20	---	0.65	0.061	0.09	---	0.07	0.36	0.08	---	---	0.10

*mg phenolic acid/100 g honey

$\overline{X}$ - mean; sd - Standard deviation; V_{min} - Minimum value; V_{max} - Maximum value.

Table 2
Phenolic Acid Content of *Erica sp.* Honeys*

Samples	Gallic	p-OH-benzoic	Vanillic	Chlorogenic	Caffeic	Siringic	p-Coumraric	Ferulic	m-Coumaric	o-Coumaric	Ellagic	Cinnamic
1	---	0.15	0.11	0.07	0.00	0.00	0.94	0.01	0.06	0.05	0.19	0.08
2	---	0.05	0.16	0.47	0.15	1.25	0.54	0.11	0.01	0.01	0.44	0.31
3	---	0.19	0.18	1.00	0.02	0.16	1.48	0.36	0.11	0.01	0.40	0.40
4	---	0.38	0.29	0.34	0.07	0.15	0.80	1.42	0.14	0.10	0.13	0.39
5	---	0.36	0.73	1.03	0.11	0.10	2.65	1.10	0.30	0.05	0.19	0.13
6	---	0.98	0.80	0.54	0.16	0.32	1.39	1.12	0.27	0.02	0.24	0.27
7	---	0.13	0.21	0.33	0.04	0.09	0.84	0.01	0.01	0.03	0.17	0.06
8	---	0.27	0.20	0.14	0.01	0.10	2.05	0.44	0.03	0.02	0.55	0.07
9	---	0.05	0.04	0.22	0.01	0.04	1.59	0.04	0.00	0.02	0.40	0.04
10	---	0.09	0.12	0.34	0.05	0.15	1.59	1.30	0.21	0.02	0.43	0.40
11	---	0.74	0.90	0.54	0.16	0.32	1.39	1.18	0.27	0.02	0.55	0.27
12	---	0.50	0.99	0.27	0.03	0.14	1.51	1.01	0.28	0.48	0.61	0.29
13	---	0.63	0.69	0.90	0.05	0.04	1.64	0.04	0.01	0.02	0.09	0.54
14	---	0.48	0.82	0.28	0.02	0.12	1.44	1.18	0.09	0.11	0.15	0.27
15	---	0.09	0.01	0.08	0.02	0.05	2.69	0.39	0.02	0.02	0.12	0.15
16	---	0.17	0.14	0.10	0.03	0.04	0.99	0.22	0.01	0.01	0.19	0.12
17	---	0.34	0.04	0.24	0.01	0.04	1.59	0.04	0.00	0.02	0.15	0.04
18	---	0.25	0.20	0.48	0.02	0.28	1.83	0.09	0.01	0.03	0.13	0.06
19	---	0.57	0.21	0.88	0.02	0.08	1.29	0.11	0.13	0.09	0.53	0.25
20	---	0.15	0.13	0.89	0.02	0.06	0.93	0.11	0.06	0.09	0.17	0.11
$\overline{X}$	---	0.33	0.35	0.46	0.05	0.12	1.49	0.54	0.11	0.06	0.29	0.21
sd	---	0.25	0.33	0.32	0.05	0.09	0.52	0.52	0.10	0.10	0.18	0.15
V_{min}	---	0.05	0.01	0.07	0.00	0.00	0.80	0.01	0.00	0.01	0.09	0.04
V_{max}	---	0.98	0.99	1.03	0.16	0.32	2.69	1.42	0.30	0.48	0.61	0.54

* mg phenolic acid/100 g honey.

$\overline{X}$ - Mean; sd - Standard deviation; V_{min} Mininum value; V_{max} Maximum value.

stoechas honey, and *Erica* sp. honey is characterised by the presence of *p*-hydroxybenzoic, syringic, *o*-coumaric and ellagic acids (Figure 1). The presence of ellagic acid (a dimeric derivative of gallic acid) in *Erica* sp. honeys agrees with previous reports in which this phenolic acid was suggested as a marker for the floral origin of *Erica* sp. honeys.[14,15]

It seems that the relative amount of one individual phenolic acid could be related to the floral origin of honey. Thus, *Erica* sp. honey (Table 2) contains a considerable amount (around 39%) of *p*-coumaric.

These results are very promising, but more detailed studies are necessary to confirm which phenolic compounds could be useful floral markers for a particular monofloral honey.

To conclude, this study suggests that the technique presented here for the analysis of phenolic acids in honey is quite useful, since allows the separation of the main honey phenolic acids with a single analysis, with emphasis on those phenolic acids which could be markers of the floral origin.

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