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Determination of phenolic compounds in honeys with different floral origin by capillary zone electrophoresis

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Twenty-six phenolic compounds from honey samples with different floral origin were analysed by capillary zone electrophoresis. All the phenolics were separated on a fused-silica column (50 cm×50 μm) using 100 mM sodium borate buffer (pH 9.5)–20% methanol. This technique was applied to the separation of phenolic compounds from heather, lavender, acacia, rape, sunflower, rosemary, citrus, rhododendron, thyme, chestnut-tree and calluna honey samples, to establish correlations between the phenolics profiles and the botanical origin of the honey. Some individual honey samples showed potential floral markers. Thus, thyme honey was characterised by the presence of rosmarinic acid, heather honey by ellagic acid, citrus honey by hesperetin and lavender honey by naringenin. © 1997 Elsevier Science Ltd

INTRODUCTION

The botanical origin of honey is one of its main quality parameters, and its price is very often related to this floral origin (Tomás-Barberán *et al.*, 1994). Recent studies have revealed that the analysis of phenolic compounds constitute a very promising technique for studying the geographical and floral origin of honey (Amiot *et al.*, 1989; Sabatier *et al.*, 1992; Ferreres *et al.*, 1992; Tomás-Barberán *et al.*, 1993*a*,*b*).

The separation of honey flavonoids by micellar electrokinetic capillary chromatography has recently been achieved to assess the possible markers for its botanical origin (Delgado, 1993; Ferreres *et al.*, 1994*b*; Andrade, 1995).

In the present work, the phenolic compounds of 11 different floral origin honeys were analysed by capillary zone electrophoresis (CZE) to evaluate differences in their phenolics patterns that could be related to their botanical origin.

MATERIALS AND METHODS

Sample preparation

Phenolic compounds for CZE analysis were extracted from honey as reported previously (Ferreres *et al.*, 1994*a*,*d*). The available honey samples (approx. 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 cm×2 cm) of Amberlite XAD-2 (pore size 9 nm, particle size 0.3–1.2 mm; Fluka Chemie). 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

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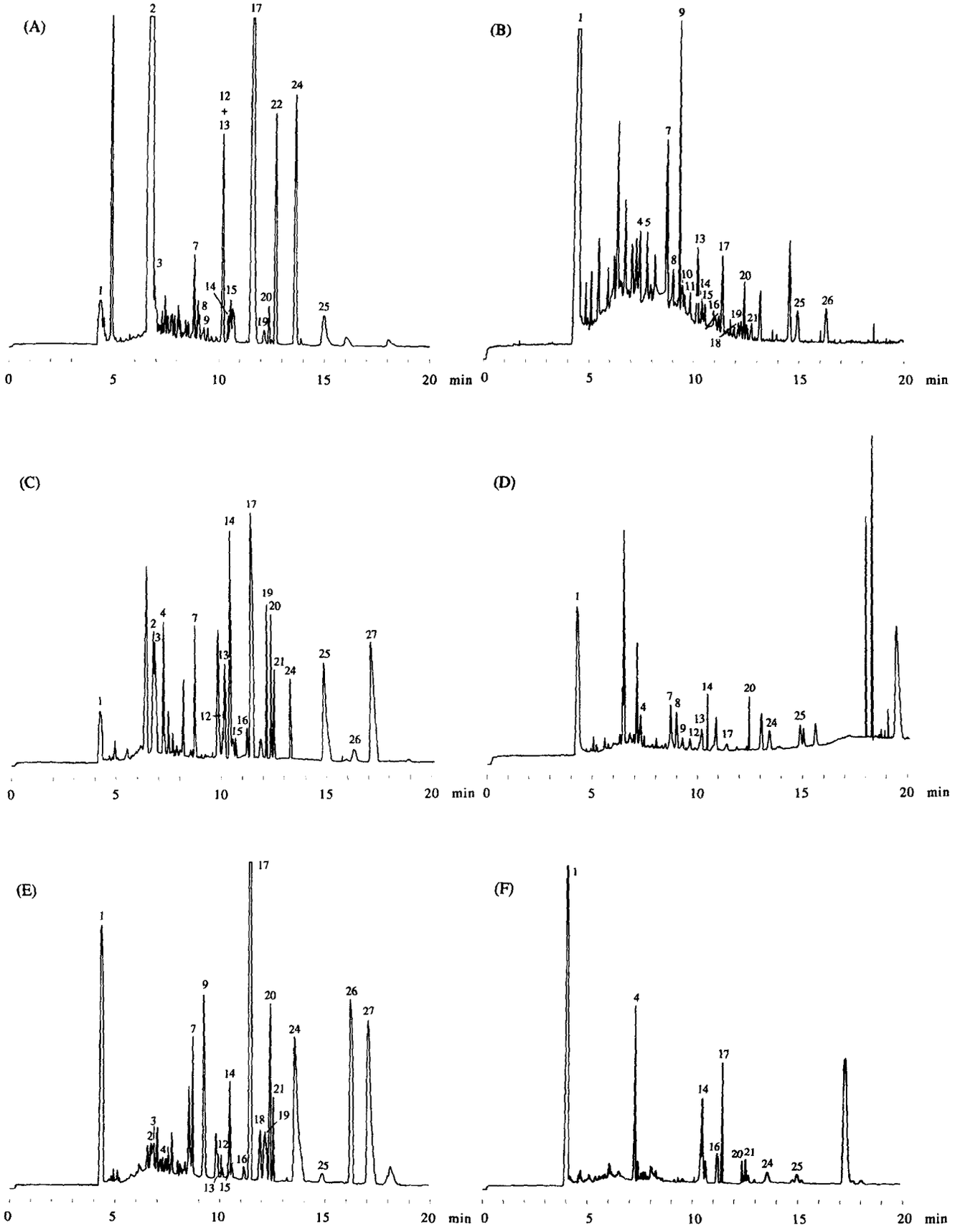

Fig. 1. CZE of phenolic compounds in honey samples: A, *Erica* sp. honey; B, *Lavandula stoechas* L. honey; C, *Brassica napus* L. honey; D, *Robinia pseudo-Acacia* L. honey; E, *Helianthuus annuus* L. honey; F, *Rosmarinus officinalis* L. honey; G, *Citrus* sp. honey; H, *Rhododendron ponticum* L. honey; I, *Thymus capitatus* Hoffgg. and LK. honey; J, *Castanea sativa* Miller honey; K, *Calluna vulgaris* (L.) Hull honey. For identification of phenolic compounds Table 1. Detection at 280 nm.

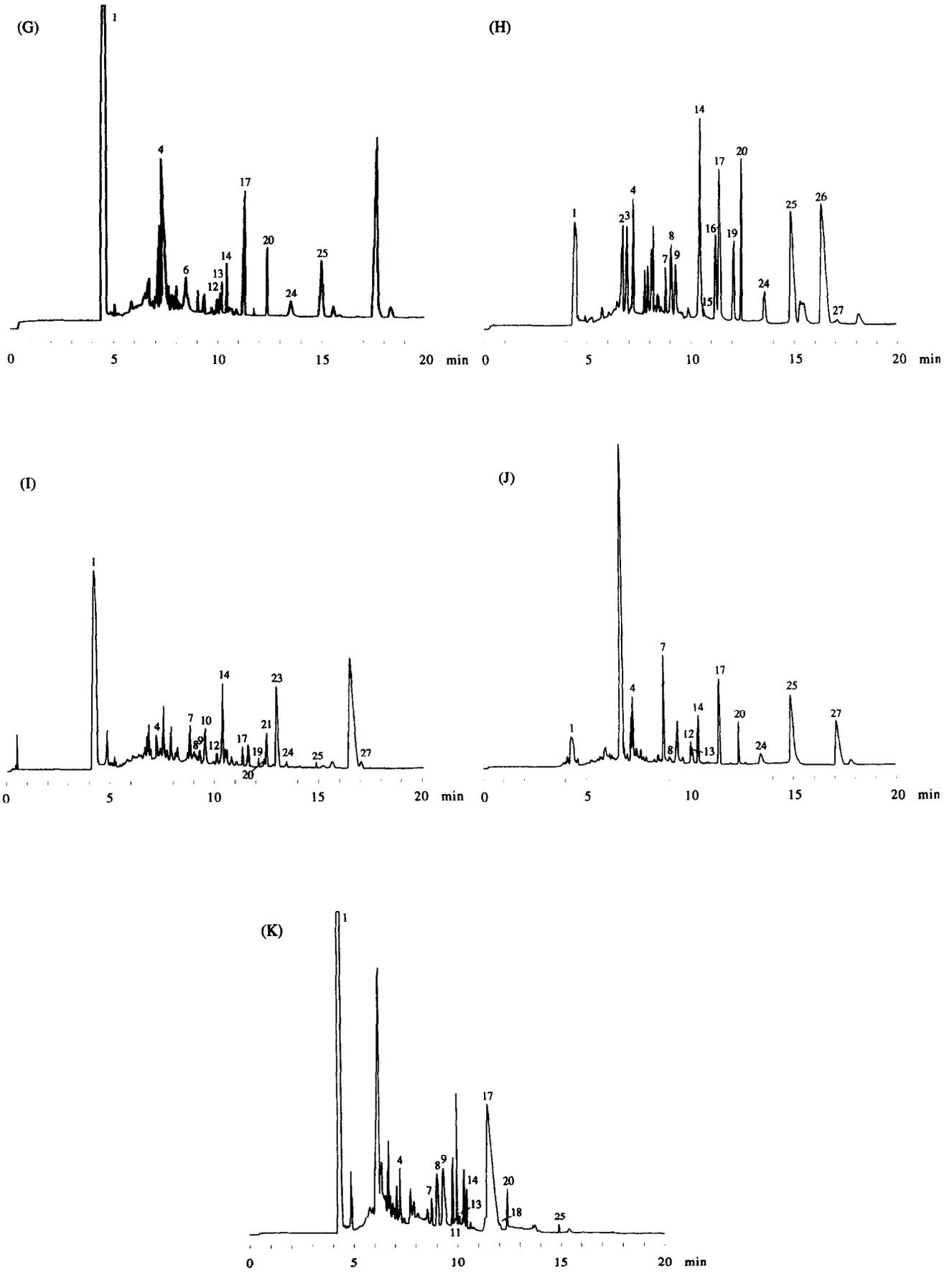

Fig. 1. *continued.*

Table 1. CZE migration times for authentic phenolic compounds identified in honey

	Migration time (min)	Compound
1.	4.36	Hydroxymethylfurfural
2.	6.81	Phenylethylcaffeate
3.	6.88	Dimethylallylcaffeate
4.	7.25	Pinobanksin
5.	7.70	Naringenin
6.	8.50	Hesperetin
7.	8.77	Cinnamic acid
8.	9.03	Chlorogenic acid
9.	9.25	*m*-Coumaric acid
10.	9.50	Quercetin
11.	9.92	Luteolin
12.	10.12	Syringic acid
13.	10.14	Ferulic acid
14.	10.45	Pinocembrin
15.	10.52	*o*-Coumaric acid
16.	11.23	Kaempferol
17.	11.41	*p*-Coumaric acid
18.	11.92	Apigenin
19.	12.10	Vanillic acid
20.	12.40	Chrysin
21.	12.50	Galangin
22.	12.64	Ellagic acid
23.	13.00	Rosmarinic acid
24.	13.50	*p*-Hydroxybenzoic acid
25.	14.88	Caffeic acid
26.	16.30	Gallic acid
27.	17.10	2,4-Dihydroxybenzoic acid

HCl, 100 ml) and subsequently with distilled water (approx. 300 ml). The whole phenolic fraction was eluted with methanol (approx. 300 ml). This fraction was concentrated under reduced pressure and purified by dissolving in methanol and passing the solution through a Sephadex LH-20 column (15 cm×1 cm). The 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 CZE.

Capillary zone electrophoresis (CZE)

CZE separations were carried out using a Beckman P/ACE System 2200 apparatus equipped with a fused-silica column (50 cm to detect 50 μm i.d.) at 30°C using 100 mM sodium borate as a buffer, pH 9.5, plus 20% methanol, at 20 kV (average current of 38 μA). Detection was achieved with a diode array detector which allowed the recording of the UV spectra relating to the different phenolic compounds. Electrophoretograms were recorded at 280 nm.

In order to improve the reproducibility of the migration times, the capillaries were conditioned daily as reported previously (Ferreres *et al.*, 1994*b*).

The different phenolic compounds were identified by their UV spectra recorded with the diode array detector and by electrophoretic comparisons (migration times) with authentic markers.

RESULTS AND DISCUSSION

To optimise the CZE conditions for the analysis of phenolic compounds from honey, an artificial mixture was prepared containing the twenty-six phenolic compounds previously reported from honey (Tomás-Barberán *et al.*, 1993*a*,*b*, 1994; Amiot *et al.*, 1989; Sabatier *et al.*, 1992; Ferreres *et al.*, 1992; Andrade, 1995).

Under the conditions of CZE described, the migration order observed is that indicated in Table 1.

After optimisation of the CZE conditions for the separation of the standard mixture, this was applied to the analysis of phenolic compounds in 11 honey samples of different floral and geographical origin (Fig. 1(A–K)). Honey samples from heather (*Erica* sp.), lavender (*Lavandula stoechas* L.), acacia (*Brassica napus* L.), rape (*Robinia pseudo-Acacia* L.), sunflower (*Helianthuus annuus* L.), rosemary (*Rosmarinus officinalis* L.), citrus (*Citrus* sp.), rhododendron (*Rhododendron ponticum* L.), thyme (*Thymus capitatus* Hoffgg. and LK.), chestnut-tree (*Castanea sativa* Miller) and calluna (*Calluna vulgaris* (L.) Hull) were analysed.

The differences in the phenolic compound profiles shown in the electrophoretograms are clear. Heather honeys have the higher contents of phenolic acid derivatives (Table 2) and smaller amounts of flavonoids (Fig. 1(A)). In contrast, citrus (Fig. 1(G)) and rosemary (Fig. 1(F)) honeys are characterized by small amounts of phenolic acid derivatives (Table 2).

In some cases, one individual phenolic compound was detected in only one unifloral honey type, and could be considered as a potential marker for the floral origin of honey. Thus, rosmarinic acid seems to be characteristic of thyme honey and naringenin of lavender honey. The presence of hesperetin seems to be characteristic of citrus honey, which agrees with previous reports on French and Spanish honeys (Ferreres *et al.*, 1993, 1994*c*; Soler *et al.*, 1995).The same applies to ellagic acid (a dimer of gallic acid) since it has recently been reported to be a marker of the botanical origin in heather honey (Ferreres *et al.*, 1996), and was found in our heather honey sample.

On the other hand, it seems that the relative amount of one individual phenolic acid derivative could be related to the floral origin of the honey. Thus, heather honey (Fig. 1(A)) has a considerable amount (around 35%) of phenylethylcaffeate, while the sample of lavender honey (Fig. 1(B)) contained *m*-coumaric acid as the principal phenolic compound.

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.

Table 2. Qualitative composition of phenolic acid derivatives from the various honey samples analysed

	Phenylethyl caffeate	Dimethyl allyl caffeate	Cinnamic	Chlorogenic	*m*-Coumaric	Syringic	Ferulic	*o*-Coumaric	*p*-Coumaric	Vanillic	Ellagic	Rosmarinic	*p*-Hydroxy-benzoic	Caffeic	Gallic	2,4-Dihydroxy-benzoic
Erica sp., Portugal	+	+	+	+	+	+	+	+	+	+	+	–	+	+	–	–
Lavandula stoechas L., Portugal	–	–	+	+	+	–	+	–	+	+	–	–	–	+	+	–
Helianthuus annuus L., France	+	+	+	–	+	+	+	+	–	+	–	–	+	+	+	+
Rosmarinus officinalis L., France	–	–	–	–	–	–	–	–	+	–	–	–	+	+	–	–
Thymus capitatus Hoffgg. e LK., Greece	–	–	+	+	+	+	–	–	+	+	–	+	+	+	–	+
Castanea sativa Miller, France	–	–	+	+	–	+	+	–	+	–	–	–	+	+	–	+
Citrus sp., Spain	–	–	–	–	·	+		–	+	–	–	–	+	+		–
Brassica napus L., France	+	+	+	–	·	+	+	+	+	+	–	–	+	+	+	+
Robinia pseudo-Acacia L., China	–	–	+	+	+	–	+	–	+	–	–	–	+	+	–	–
Rhododendron ponticum L., France	+	+	+	+	+	–	·	+	+	+	–	–	+	+	+	+
Calluna vulgaris (L.) Hull, Spain	+	+	+	+	–	+	–	–	+	+	–	–	+	+	–	+

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