Supplementary Material

Glucosinolates as Markers of the Origin and Harvesting Period for Discrimination of Bee Pollen by UPLC-MS/MS

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Table S1. Colony of origin and harvesting period for each bee pollen sample.

Colony	Harvesting period		
	April-May	**June**	**July-August**
C-FH-1	FH-1	-	-
C-FH-2	FH-2	-	-
C-FH-3	-	FH-4	FH-6
C-FH-4	FH-3	-	-
C-FH-5	-	FH-5	FH-7
C-MO-1	MO-1	MO-5	MO-10
C-MO-2	MO-2	MO-6	-
C-MO-3	-	MO-7	-
C-MO-4	MO-3	MO-8	MO-11
C-MO-5	MO-4	MO-9	MO-12
C-PI-1	PI-1	PI-14	
C-PI-2	PI-2	PI-15	PI-32
C-PI-3	PI-3	PI-16	PI-33
C-PI-4	PI-4	PI-17	PI-34
C-PI-5	-	PI-18	PI-35
C-PI-6	-	PI-19	PI-36
C-PI-7	PI-5	PI-20	-
C-PI-8	PI-6	PI-21	PI-37
C-PI-9	PI-7	-	-
C-PI-10	PI-8	PI-22	PI-38
C-PI-11	-	PI-23	PI-39
C-PI-12	PI-9	-	-
C-PI-13	PI-10	PI-24	PI-40
C-PI-14	-	PI-25	-
C-PI-15	PI-11	PI-26	PI-41
C-PI-16	-	PI-27	PI-42
C-PI-17	-	PI-28	PI-43
C-PI-18	PI-12	PI-29	PI-44
C-PI-19	PI-13	PI-30	PI-45
C-PI-20	-	PI-31	-

Table S1. Continued.

Colony	Harvesting period		
	April-May	**June**	**July-August**
C-TN-1	-	TN-1	TN-6
C-TN-2	-	TN-2	-
C-TN-3	-	TN-3	TN-7
C-TN-4	-	TN-4	-
C-TN-5	-	TN-5	TN-8

Table S2. Instrument/devices, Sample treatment, UPLC elution program and MS/MS conditions.

Instruments/devices	Syringe filters (17 mm, Nylon 0.45 μm) were purchased from Nalgene (Rochester, NY, USA), and ultrapure water was obtained from Millipore Milli-RO plus and Milli-Q systems (Bedford, MA, USA). An Eppendorf Centrifuge 5810R (Hamburg, Germany), an R-210/215 rotary evaporator 109 (Buchi, Flawil, Switzerland), Bond Elut NH2 (3 mL with 500 mg of sorbent) SPE cartridges from Agilent Technologies (Palo Alto, CA, USA), a 10-port Visiprep vacuum manifold (Supelco, St. Louis, MO, USA), a Moulinette chopper device from Moulinex (Paris, France), and a Vibromatic mechanical shaker and a drying oven both from J.P. Selecta S.A. (Barcelona, Spain) were used for the extractions.
Sample treatment	The preparation of the samples was performed according to [23]. Briefly, 1 g of bee pollen sample and 10 mL of heated water (70 °C) were transferred to a centrifuge tube. The mixture was shaken for 10 min at maximum speed (960 oscillations per minute) in a Vibromatic and then centrifuged for 10 min at 4 °C and 10414g. The supernatant was collected and loaded onto a Bond Elut NH_2 SPE cartridge (previously conditioned with 5 mL of methanol and 5 mL of 1% acetic acid in water (v/v)) at about 1 mL/min by means of a suction system. The SPE cartridge was then washed with 5 mL of 5% acetic acid in methanol (v/v); the rinse was discarded, and, after 5 min of drying time, the analytes were eluted with 10 mL of freshly prepared 2% solution of ammonium hydroxide in methanol. The resulting solution was evaporated to dryness at 40 °C in a rotary evaporator; the dry residue was reconstituted with 1 mL of ultrapure water, filtered through a nylon 0.45-μm filter, and injected into the UHPLC-QTOF system.
UPLC elution program	It was employed the same program as optimized in [18]. The mobile phase was composed of 0.1% (v/v) formic acid in water (solvent A) and 0.1% (v/v) formic acid in acetonitrile (solvent B) applied at a flow rate of 0.2 mL/ min in the following gradient mode: (**i**) 0.0–2.0 min (A:B, 100:0, v/v); (**ii**) 2.0–2.1 min (A:B, 90:10, v/v); (**iii**) 2.1–6.0 min (A:B, 90:10, v/v); (**iv**) 6.0–6.1 min (A:B, 50:50, v/v); (**v**) 6.1–7.5 min (A:B, 35:65, v/v); (**vi**) 7.5–7.6 min (A:B, 10:90, v/v); (**vii**) 7.6–9.5 min (A:B, 10:90, v/v); (**viii**) 9.5–11.0 min (A:B, 35:65, v/v); (**ix**) 11.0–12.5 min (A:B, 100:0, v/v); (x) 12.5–15.0 min (A:B, 100:0, v/v).
MS/MS conditions	Capillary voltage, −2250 V; drying gas flow, 12 L/min; drying gas temperature, 220 °C; nebulizer pressure, 2 bar. GSLs were quantified by generating extracted ion chromatograms with the precursor ions; meanwhile the most relevant fragments for each precursor ion were also used to confirm the presence of each GSL. These MS/MS experiments were carried out by using an isolation width of 10 m/z and a collision energy of 40 eV.

Table S3. Mean and confident interval (95%) values in μg/kg (dry weight) obtained for the GSLs in commercial (CO) samples. The GLSs not detected in those samples (GIB, PRO and GER) were not included.

MUESTRA	SIN	EPI	GRA	GNA	ALY	4-OH	GBN	GTL	GBC	NAS	4-ME	NEO
CO-1	<LOD	557±10	249±11	1216±70	<LOD	4193±53	<LOQ	286±3	<LOQ	45±0	<LOD	<LOD
CO-2	<LOD	<LOQ	<LOD	231±5	<LOD	639±141	<LOQ	228±17	<LOD	<LOQ	<LOD	<LOD
CO-3	<LOD	<LOD	69±5	60±10	<LOD	820±58	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CO-4	<LOD	57±4	<LOD	84±7	<LOD	1667±81	<LOD	158±5	419±0	623±30	<LOQ	<LOD
CO-5	<LOD	<LOD	<LOD	<LOQ	<LOQ	1159±35	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD
CO-6	<LOD	<LOD	189±2	98±9	<LOD	899±63	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CO-7	<LOD	<LOD	<LOD	<LOQ	<LOD	1199±87	<LOD	23±1	<LOD	<LOD	<LOD	<LOD
CO-8	<LOD	<LOD	<LOD	<LOD	<LOD	702±47	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
CO-9	<LOD	<LOD	<LOD	49±7	<LOD	953±103	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD
CO-10	<LOD	<LOD	170±2	304±0	<LOD	1216±61	<LOD	24±0	<LOD	<LOQ	<LOQ	<LOD
CO-11	92±6	365±1	<LOD	245±5	<LOD	1291±85	<LOD	1593±41	73±3	23±1	<LOD	<LOD

Table S4. Frequency and concentration range of each GSL from MO apiary.

GSL	Frequency[A] (%)	Concentration Range* (μg/kg; Dry Weight)
GIB	0	<LOD
PRO	42	299-4377
SIN	17	1374-1851
EPI	25	<LOQ
GRA	33	47-1036
GNA	58	34-359
ALY	33	266-3078
4-OH	42	195-3067
GBN	83	52-6595
GTL	0	<LOD
GER	42	36-102
GBC	33	98-453
NAS	50	50-4724
4-ME	8	<LOQ
NEO	75	51-4367

[A](number of samples in which a GLSs residue was detected/total number of samples (n = 12)) × 100. *Concentrations over LOQ.

Table S5. Mean and confident interval (95%) values in µg/kg (dry weight) obtained for the GSLs in the samples from MO apiary. The GLSs not detected in those samples (GIB and GTL) were not included.

SAMPLE	PRO	SIN	EPI	GRA	GNA	ALY	4-OH	GBN	GER	GBC	NAS	4-ME	NEO
MO-1	1266±4	1851±45	<LOD	270±11	77±6	1261±1	772±9	1968±30	37±1	98±5	859±30	<LOD	150±1
MO-2	2832±57	1374±29	<LOD	341±1	359±6	3078±113	285±3	5266±23	102±0	<LOD	4724±51	<LOD	959±18
MO-3	4377±64	<LOD	<LOD	1036±8	161±0	937±30	3067±5	2937±167	84±1	167±7	2324±63	<LOD	4367±107
MO-4	299±11	<LOD	<LOQ	47±0	34±1	266±5	491±22	580±14	<LOQ	<LOD	132±6	<LOD	<LOD
MO-5	<LOQ	<LOD	<LOD	<LOD	50±1	<LOD	<LOD	555±33	<LOD	453±14	997±9	<LOD	752±8
MO-6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	91±2	<LOD	<LOD	<LOD	<LOD	271±8
MO-7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	181±6	<LOD	<LOD	<LOD	<LOD	107±3
MO-8	<LOD	<LOD	<LOQ	<LOD	334±10	<LOD	195±0	102±3	<LOD	111±9	50±2	<LOQ	401±4
MO-9	<LOD	<LOD	<LOD	<LOD	110±2	<LOD	<LOD	6595±49	<LOD	<LOD	<LOD	<LOD	<LOD
MO-10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	36±1	<LOD	<LOD	<LOD	51±0
MO-11	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	153±5
MO-12	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	52±3	<LOD	<LOD	<LOD	<LOD	<LOD

Table S6. Frequency and concentration range of each GSL from PI apiary.

GSL	Frequency[A] (%)	Concentration Range * (μg/kg; Dry Weight)
GIB	0	<LOD
PRO	36	137-6690
SIN	44	84-2721
EPI	11	41-55
GRA	29	58-1172
GNA	62	27-1354
ALY	29	143-7916
4-OH	36	105-1539
GBN	76	31-8470
GTL	13	22-76
GER	36	34-126
GBC	27	39-1467
NAS	44	21-9936
4-ME	33	109-1664
NEO	60	29-3928

[A](number of samples in which a GLSs residue was detected/total number of samples (n = 45)) × 100; * Concentrations over LOQ.

Table S7. Mean and confident interval (95%) values in µg/kg (dry weight) obtained for the GSLs in the samples from PI apiary. The GLSs not detected in those samples (GIB) was not included.

SAMPLE	PRO	SIN	EPI	GRA	GNA	ALY	4-OH	GBN	GTL	GER	GBC	NAS	4-ME	NEO
PI-1	469±6	392±4	<LOQ	60±0	72±1	692±2	105±0	1939±31	<LOD	<LOQ	<LOD	552±25	<LOD	<LOD
PI-2	162±6	626±21	<LOD	62±5	27±1	232±0	<LOD	344±1	<LOD	<LOD	39±1	239±11	<LOD	34±3
PI-3	615±14	602±7	<LOD	79±1	63±1	711±13	158±3	1782±5	<LOD	<LOQ	42±0	555±6	<LOD	54±2
PI-4	1827±44	2665±188	<LOD	463±24	86±5	865±28	<LOD	1962±172	<LOQ	<LOQ	74±20	1782±176	118±5	<LOD
PI-5	4335±118	2158±15	<LOD	598±1	816±16	7916±105	<LOD	6158±65	<LOD	116±2	<LOD	9505±25	<LOD	203±1
PI-6	519±14	471±7	<LOD	68±3	70±3	740±10	<LOD	1565±33	<LOD	<LOQ	<LOD	315±9	<LOD	<LOD
PI-7	834±12	642±25	<LOD	101±1	77±1	708±14	139±4	2064±42	<LOD	<LOQ	56±2	517±384	<LOD	49±1
PI-8	679±14	327±4	<LOD	122±1	128±3	497±369	154±2	1729±9	<LOD	65±5	<LOQ	490±7	<LOD	66±1
PI-9	336±7	748±19	<LOD	58±4	30±1	200±3	<LOD	234±171	<LOD	<LOD	<LOQ	265±2	<LOD	1342±19
PI-10	722±20	157±5	<LOD	473±3	254±4	2017±24	<LOQ	1805±13	<LOD	35±0	<LOD	430±8	<LOD	946±702
PI-11	6690±137	2721±57	<LOD	1172±8	322±10	3866±43	<LOD	8470±47	<LOD	126±18	102±1	9936±0	277±1	3928±36
PI-12	414±8	475±14	<LOQ	61±2	43±0	374±9	<LOD	820±36	<LOD	<LOQ	<LOD	195±5	<LOD	<LOD
PI-13	294±1	<LOQ	41±1	62±0	27±0	143±2	<LOD	257±7	<LOD	<LOQ	<LOD	61±0	<LOD	<LOD
PI-14	<LOD	<LOD	<LOD	<LOD	75±1	<LOD	<LOD	34±1	<LOD	<LOD	<LOQ	<LOD	130±1	<LOD
PI-15	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	110±1	<LOD	<LOD	<LOD	<LOD	<LOQ	158±0
PI-16	<LOD	84±4	<LOD	<LOD	130±2	<LOD	201±4	<LOD	<LOQ	<LOD	<LOD	90±0	117±2	<LOD
PI-17	<LOD	516±1	<LOD	<LOD	69±2	<LOD	171±22	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	53±3

PI-18	<LOD	<LOD	<LOQ	<LOD	469±7	<LOD	218±15	76±6	<LOD	<LOD	<LOD	<LOD	<LOQ	95±34
PI-19	<LOD	<LOD	<LOD	<LOD	92±2	<LOD	235±8	643±11	<LOD	<LOD	<LOQ	61±2	203±1	<LOD
PI-20	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	137±0
PI-21	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
PI-22	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	699±17	<LOQ	47±1	<LOD	<LOD	<LOD	<LOD
PI-23	<LOD	<LOD	<LOD	<LOD	295±5	<LOD	<LOD	60±0	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD
PI-24	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	54±0	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOQ
PI-25	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ
PI-26	<LOQ	<LOD	<LOD	<LOD	62±3	<LOD	415±1	31±1	<LOD	36±1	<LOD	49±3	165±4	133±1
PI-27	137±6	<LOD	<LOD	<LOD	558±5	<LOD	1121±21	2732±1	76±4	<LOD	<LOD	<LOD	663±20	<LOD
PI-28	<LOD	706±22	55±3	<LOD	1354±50	<LOD	<LOD	41±1	<LOQ	<LOD	207±7	<LOD	1664±7	<LOD
PI-29	<LOD	292±6	<LOD	<LOD	90±0	<LOD	<LOD	336±19	<LOD	<LOD	<LOD	<LOD	<LOD	77±3
PI-30	<LOD	590±6	<LOD	<LOD	400±12	<LOD	296±2	60±3	<LOD	<LOD	1467±14	448±8	109±7	187±6
PI-31	<LOD	<LOD	<LOD	<LOD	189±7	<LOD	<LOD	364±15	<LOD	<LOD	<LOD	<LOD	<LOD	54±5
PI-32	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	106±4	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD
PI-33	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	466±28	22±4	<LOD	<LOD	<LOD	109±1	<LOD
PI-34	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	622±8
PI-35	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	974±34
PI-36	<LOD	121±3	<LOD	<LOD	160±4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	45±0	<LOD	685±3

PI-37	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	<LOD	21±0	<LOD	<LOD
PI-38	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	29±0
PI-39	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	34±6	<LOD	<LOD	<LOD	<LOD
PI-40	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	29±1
PI-41	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	143±2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1272±44
PI-42	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	891±5	<LOQ	<LOD	<LOD	63±1	<LOD	<LOD	<LOD
PI-43	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1539±24	117±6	<LOD	<LOD	<LOD	<LOD	<LOD	120±3
PI-44	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1182±22	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	567±2
PI-45	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	70±1

Table S8. Frequency and concentration range of each GSL from TN apiary.

GSL	Frequency[A] (%)	Concentration Range* (μg/kg; Dry Weight)
GIB	0	<LOD
PRO	0	<LOD
SIN	13	<LOQ
EPI	25	<LOQ
GRA	0	<LOD
GNA	25	162-411
ALY	13	<LOQ
4-OH	0	<LOD
GBN	38	79-221
GTL	13	<LOQ
GER	13	152
GBC	25	155-547
NAS	25	76-91
4-ME	38	346
NEO	38	36-48

[A](number of samples in which a GLSs residue was detected/total number of samples (n = 8)) × 100. *Concentrations over LOQ.

Table S9. Mean and confident interval (95%) values in µg/kg (dry weight) obtained for the GSL in the samples from TN apiary. The GSLs not detected in those samples (GIB, PRO, GRA and 4-OH) were not included.

MUESTRA	SIN	EPI	GNA	ALY	GBN	GTL	GER	GBC	NAS	4-ME	NEO
TN-1	<LOD	<LOQ	411±1	<LOD	79±9	<LOD	<LOD	155±12	76±4	<LOQ	48±2
TN-2	<LOD	<LOD	162±21	<LOD	221±52	<LOQ	<LOD	547±197	91±7	<LOQ	<LOQ
TN-3	<LOD	<LOD	<LOD	<LOQ	79±3	<LOD	<LOD	<LOD	<LOD	<LOD	36±3
TN-4	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
TN-5	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	152±0	<LOD	<LOD	<LOD	<LOD
TN-6	<LOQ	<LOQ	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
TN-7	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
TN-8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	346±0	<LOD

Table S10. Frequency and concentration range of each GSL from FH apiary.

GSL	Frequency[A] (%)	Concentration Range* (μg/kg; Dry Weight)
GIB	0	<LOD
PRO	43	130-1361
SIN	14	478
EPI	43	<LOQ
GRA	29	184
GNA	43	30-131
ALY	43	157-1018
4-OH	29	166-3370
GBN	43	548-1200
GTL	0	<LOD
GER	14	<LOQ
GBC	14	194
NAS	86	99-1497
4-ME	0	<LOD
NEO	71	35-5562

[A](number of samples in which a GLSs residue was detected/total number of samples (n = 7)) × 100. *Concentrations over LOQ.

Table S11. Mean and confident interval (95%) values in µg/kg (dry weight) obtained for the GSLs in the samples from FH apiary. The GSLs not detected in those samples (GIB, 4-ME and GTL) were not included.

SAMPLE	PRO	SIN	EPI	GRA	GNA	ALY	4-OH	GBN	GER	GBC	NAS	NEO
FU-1	1361±47	478±14	<LOD	184±0	131±6	1018±29	3370±86	904±2	<LOD	<LOD	279±4	5562±84
FU-2	130±0	<LOD	<LOQ	<LOD	<LOQ	157±2	<LOD	548±11	<LOD	<LOD	99±2	<LOQ
FU-3	365±5	<LOD	<LOQ	<LOQ	30±1	283±2	166±18	1200±24	<LOQ	<LOD	218±2	<LOD
FU-4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	132±2
FU-5	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	194±1	1497±88	426±8
FU-6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	35±1
FU-7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD

Table S12. Weights of the first three canonical variables for the 83 samples.

Variable	Can1	Can2	Can3
PRO_1	-0.029047313	0.016096285	0.049168694
PRO_2	0.002967934	0.153952971	0.008411896
PRO_3	0.021118629	-0.171696190	-0.061440690
SIN_1	-0.053151810	0.022857796	0.029459475
SIN_2	0.015057546	-0.010756261	0.131756633
SIN_3	0.040197830	-0.005046324	-0.162544499
EPI_1	-0.582930260	0.675436703	-1.306376552
EPI_2	0.166944122	0.733024926	-0.927492934
EPI_3	0.403538184	-1.405024433	2.229260254
GRA_1	-0.225422837	-0.337479147	-0.120504978
GRA_2	-0.468469099	-0.467611206	-0.144537582
GRA_3	0.707437189	0.804334119	0.261912824
GNA_1	-0.029831969	0.373605104	-0.062751251
GNA_2	-0.092247455	0.436590348	-0.061735658
GNA_3	0.124155346	-0.809705096	0.125962444
ALY_1	0.094260396	0.023241338	-0.136860563
ALY_2	0.092984425	0.080691769	-0.275359760
ALY_3	-0.187400719	-0.103817812	0.413809837
4_OH_1	-0.014177858	0.002349057	0.006244149
4_OH_2	-0.036351288	0.000981165	0.008906508
4_OH_3	0.053275145	-0.002501512	-0.014815507
GBN_1	0.022578965	-0.072821570	0.096316975
GBN_2	0.004767189	-0.011002234	0.098473225
GBN_3	-0.027374258	0.082880002	-0.194274574
GTL_1	-0.372029112	-0.298840960	0.153577452

GTL_2	-0.102526182	-0.991561539	0.303585402
GTL_3	0.490818188	1.279180896	-0.455472084
GER_1	-0.197759955	0.296168033	0.573327514
GER_2	-0.097211863	0.780934963	0.451198252
GER_3	0.292157883	-1.083347830	-1.012017517
GBC_1	0.038230513	-0.066631118	0.029027182
GBC_2	0.146430492	-0.273044898	0.114322420
GBC_3	-0.185790368	0.336828620	-0.141800512
NAS_1	-0.013098735	-0.011966354	-0.025735045
NAS_2	0.013444044	0.014822097	0.029096033
NAS_3	0.001481710	-0.002270233	-0.004064490
4_ME_1	0.226573498	-0.496440964	-0.076891914
4_ME_2	0.025263631	-0.109729842	-0.224630635
4_ME_3	-0.255027123	0.608698160	0.304750980
NEO_1	-0.013765178	-0.085644742	0.053401247
NEO_2	-0.031917217	-0.122330458	0.068038468
NEO_3	0.043939100	0.207736089	-0.120487023

Table S13. Mean values of the canonical variables 1 and 2 for the 83 samples.

Group	Nº Observations	Variable	Mean
FH	**7**	**Can1**	-1.0427355
		Can2	-1.5899360
		Can 3	-2.3746905
MO	**12**	**Can1**	-1.2987433
		Can2	-2.3660896
		Can 3	0.8481109
PI	**45**	**Can1**	-0.6077352
		Can2	1.0672124
		Can 3	0.0129179
TN	**8**	**Can1**	-0.9423825
		Can2	-0.5117047
		Can 3	0.6974254
CO	**11**	**Can1**	5.2519283
		Can2	-0.4007541
		Can 3	0.0258906

Table S14. Number of observations and percentage classified in each group using canonical discriminant analysis.

Origin	FH	MO	PI	TN	CO	Total
FH	4	1	0	2	0	7
%	57.14	14.29	0.00	28.57	0.00	100.00
MO	0	11	0	1	0	12
%	0.00	91.67	0.00	8.33	0.00	100.00
PI	0	0	32	10	3	45
%	0.00	0,000	71.11	22.22	6.67	100.00
TN	0	0	0	8	0	8
%	0.00	0.00	0.00	100.00	0.00	100.00
CO	0	0	0	0	11	11
%	0.00	0.00	0.00	0.00	100.00	100.00
Total	4	12	32	21	14	83
	4.82	14.46	38.55	25.30	16.87	100.00

Table S15. Weights of the first three canonical variables for the 72 samples (origin).

Variable	Can1	Can2	Can3
PRO_1	-0.078745945	-0.116207143	0.121683634
PRO_2	0.127590164	-0.154520816	0.076472635
PRO_3	-0.049191393	0.275374820	-0.197026038
SIN_1	-0.111818356	-0.008649097	0.072056303
SIN_2	-0.108485741	-0.201103259	0.026351561
SIN_3	0.226762108	0.209684686	-0.101693079
EPI_1	1.789579869	-0.813021601	-1.912587191
EPI_2	3.270330127	0.733363327	0.019271430
EPI_3	-5.082371063	0.068897542	1.823497872
GRA_1	1.208119911	0.555488170	-0.094246958
GRA_2	0.169633144	1.133910857	0.510230481
GRA_3	-1.403109611	-1.699194905	-0.424533089
GNA_1	0.675320477	0.742149277	0.020598370
GNA_2	0.615895505	0.322197264	-0.128720350
GNA_3	-1.285343203	-1.063587445	0.113914054
ALY_1	0.029711270	0.028684922	-0.180132726
ALY_2	0.031536749	0.089802646	-0.267282265
ALY_3	-0.057128787	-0.119667751	0.447992926
4_OH_1	0.007623856	0.047557136	0.059431932
4_OH_2	-0.040703973	-0.021372745	0.029864601
4_OH_3	0.034010811	-0.026943455	-0.089527495
GBN_1	-0.171788584	-0.086374718	0.013865749
GBN_2	-0.074148065	-0.057677099	0.014472501
GBN_3	0.244638563	0.144318114	-0.027903843
GTL_1	3.435596513	4.811908653	-0.166599369
GTL_2	0.082058717	1.344653238	-0.168433114
GTL_3	-3.507478458	-6.085263345	0.298816621

GER_1	0.481744379	-1.197332050	0.902250394
GER_2	0.925328929	-1.006774676	0.675306355
GER_3	-1.417511691	2.180542205	-1.566404109
GBC_1	-0.102153412	0.003737694	0.022095882
GBC_2	-0.512276796	-0.295470632	0.030253232
GBC_3	0.609992802	0.292287398	-0.050589254
NAS_1	-0.004408643	0.008856185	-0.006644458
NAS_2	0.005692356	-0.012198799	0.012125342
NAS_3	-0.000927427	0.004224894	-0.007445508
4_ME_1	-0.453362143	0.248120204	-0.168325744
4_ME_2	0.444436857	0.999971883	-0.102166986
4_ME_3	0.008175248	-1.259628513	0.270328426
NEO_1	-0.129622269	-0.068110374	0.109881240
NEO_2	-0.135379020	-0.040639459	0.108501425
NEO_3	0.265728523	0.109593534	-0.217093249

Table S16. Mean values of the canonical variables 1 and 3 for the 72 samples (apiary of origin).

Group	Nº Observations	Variable	Mean
FH	**7**	**Can1**	-1.5801771
		Can2	0.9612291
		Can 3	-2.2140864
MO	**12**	**Can1**	-2.5838455
		Can2	0.7672015
		Can 3	1.0680189
PI	**45**	**Can1**	1.2116470
		Can2	0.0771155
		Can 3	0.1027449
TN	**8**	**Can1**	-1.5570911
		Can2	-2.4256523
		Can 3	-0.2426428

Table S17. Number of observations and percentage classified in each apiary using canonical discriminant analysis.

Origin	FH	MO	PI	TN	Total
FH	5	0	0	2	7
%	71.43	0.00	0.00	28.57	100.00
MO	0	10	0	2	12
%	0.00	83.33	0.00	16.67	100.00
PI	3	0	39	3	45
%	6.67	0,000	86.67	6.67	100.00
TN	0	0	0	8	8
%	0.00	0.00	0.00	100.00	100.00
Total	8	10	39	15	72
	11.11	13.89	54.17	20.83	100.00

Table S18. Weights of the first two canonical variables for the 72 samples (harvesting periods).

Variable	Can1	Can2
PRO_1	-0.080201118	0.042524563
PRO_2	0.055348265	0.146737661
PRO_3	0.022739485	-0.176307024
SIN_1	-0.050814301	0.110659318
SIN_2	-0.216326233	0.056173528
SIN_3	0.267766485	-0.165866447
EPI_1	4.137764804	1.231739870
EPI_2	-0.595786849	-0.501886895
EPI_3	-3.267159906	-0.704273036
GRA_1	-1.228041451	-0.284852906
GRA_2	0.696769188	0.233886798
GRA_3	0.538952012	-0.007411957
GNA_1	0.525335229	-0.059027491
GNA_2	0.841944528	0.250136764
GNA_3	-1.385212738	-0.185903536
ALY_1	0.063254618	-0.037751351
ALY_2	-0.033033065	0.047784825
ALY_3	-0.020271029	-0.015473271
_4_OH_1	0.105084115	-0.011156037
_4_OH_2	0.020359864	0.000641472
_4_OH_3	-0.127581926	0.008882004
GBN_1	-0.062075012	-0.040828744
GBN_2	0.047185500	0.038062074
GBN_3	0.014457572	0.002441351
GTL_1	-0.176279802	2.354322840
GTL_2	0.171249741	1.358018936
GTL_3	0.199997669	-3.508700398

GER_1	-2.456659926	-2.933516584
GER_2	-1.796419403	-2.797354996
GER_3	4.213306275	5.745429203
GBC_1	-0.140745700	-0.100315260
GBC_2	-0.587321053	-0.350532939
GBC_3	0.732428202	0.452024171
NAS_1	-0.016210049	0.001481072
NAS_2	0.025092686	-0.003238585
NAS_3	-0.010932472	0.002922961
_4_ME_1	-0.225535705	0.126673852
_4_ME_2	-0.192901959	-0.301996518
_4_ME_3	0.416311984	0.173859513
NEO_1	-0.022736015	-0.004774465
NEO_2	0.026057150	0.029671365
NEO_3	-0.001712700	-0.025406925

Table S19. Mean values of the canonical variables 1 and 2 for the 72 samples (harvesting periods).

Origin	Nº Observations	Variable	Mean
April-May	**20**	**Can1**	6.2380705
		Can2	0.0144656
June	**22**	**Can1**	-2.4444886
		Can2	-1.3811192
July-August	**30**	**Can1**	-2.5319706
		Can2	0.9901445

Table S20. Number of observations and percentage classified in each group (harvesting period) using canonical discriminant analysis.

Origin	April-May	June	July-August	Total
April-May	20	0	0	20
%	100.00	0.00	0.00	100.00
June	0	21	1	22
%	0.00	95.45	4.55	100.00
July-August	0	10	20	30
%	0.00	34.48	66,67	100.00
Total	20	31	21	72
	27.78	43.06	29,17	100.00

Figure S1. UPLC-MS/MS extracted ion chromatogram obtained in positive mode using the quantification ions (see Table 1) from PI-11 bee pollen sample in which eleven GSLs were detected: PRO (1); SIN (2); GRA (3); GNA (4); ALY (5); GBN (6); GER (17); GBC (8); NAS (9); 4-Me (10); NEO (11). The UPLC-MS/MS conditions are summarized in Subsection 2.4 and Table 1.

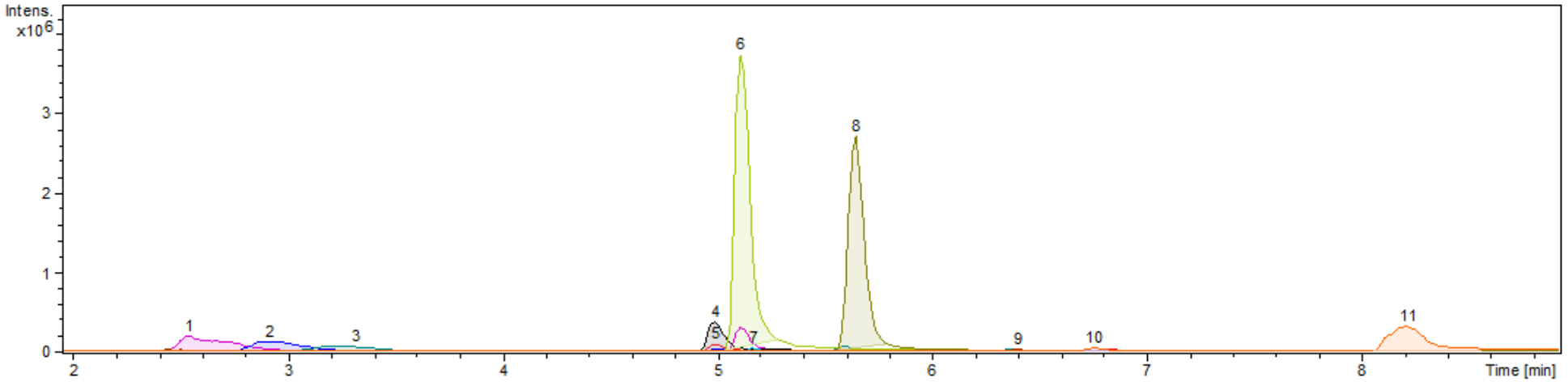

Figure S2. Representation of individual bee pollen samples (see Table 2) from the apiaries (FH, 1; MO, 2; PI, 3; TN, 4) and local markets (5) as function of the first two canonical variables.

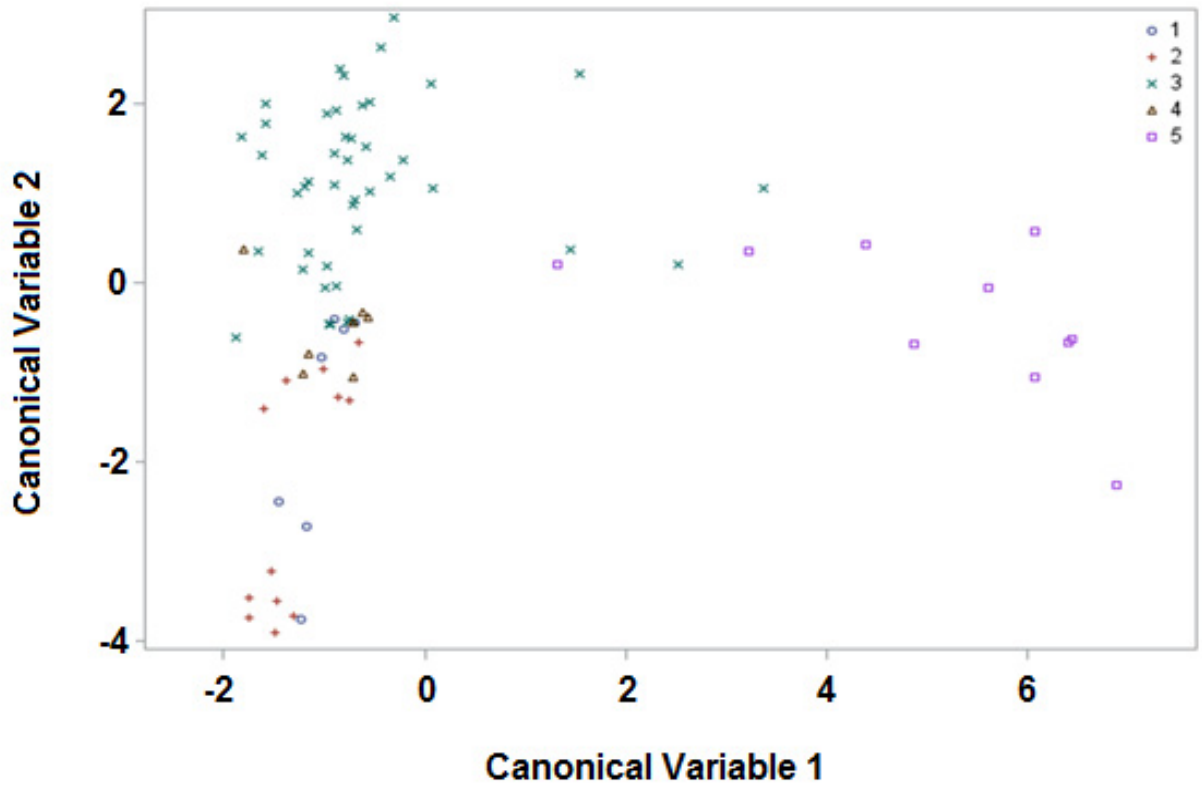

Figure S3. Representation of individual bee pollen samples (see Table 2) from the apiaries (FH, 1; MO, 2; PI, 3; TN, 4) as function of canonical variables 1 and 3.

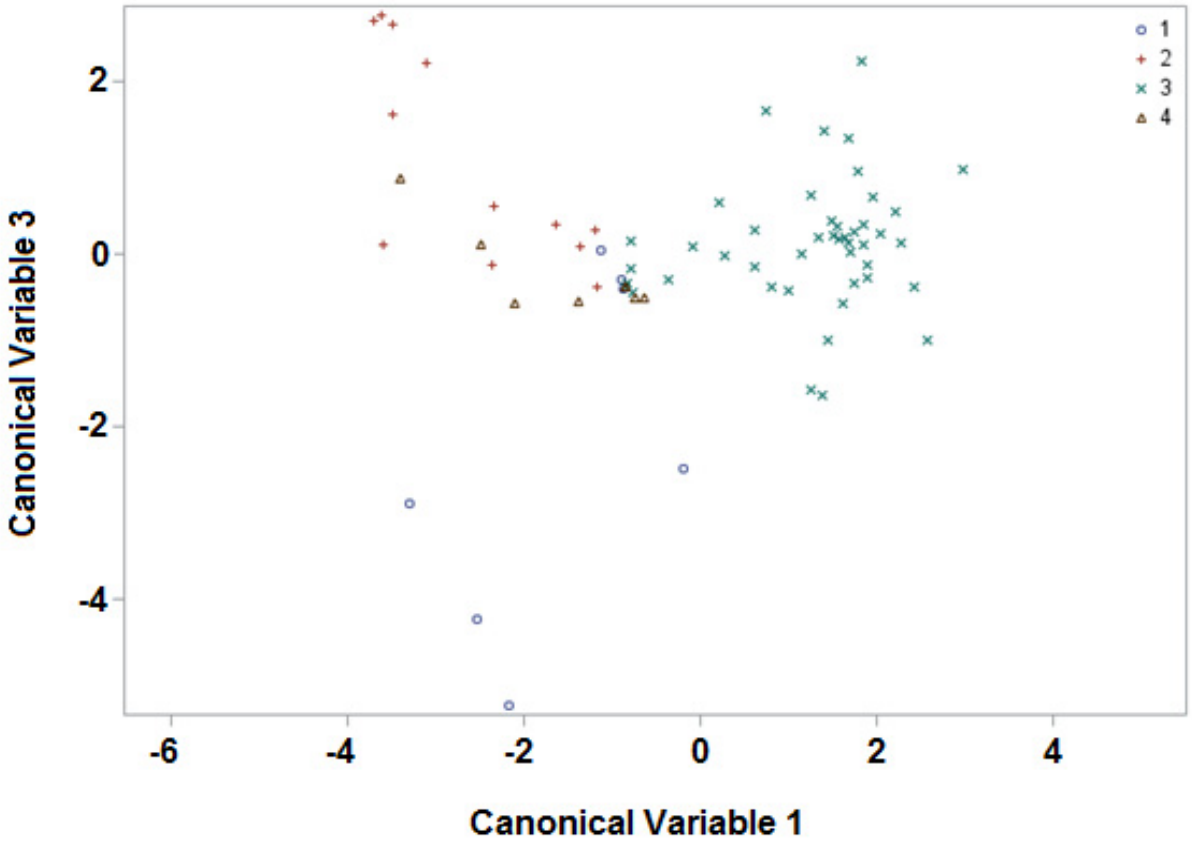

Figure S4. Representation of individual bee pollen samples (see Table 2) from the different harvesting periods (April-May, 1; June, 2; July-August, 3) as function of the first two canonical variables.