ELSEVIER

Food Chemistry 79 (2002) 453–459

Food Chemistry

www.elsevier.com/locate/foodchem

Floral quality and discrimination of *Lavandula stoechas*, *Lavandula angustifolia*, and *Lavandula angustifolia*×*latifolia* honeys

Christine Guyot-Declerck, Sarah Renson, Amina Bouseta, Sonia Collin*

Université catholique de Louvain, Unité de Brasserie et des Industries Alimentaires, Croix du Sud 2/Bte 7, B-1348 Louvain-la-Neuve, Belgium

Received 11 January 2002; received in revised form 12 April 2002; accepted 25 April 2002

Abstract

Portuguese lavender honeys are generated from the nectar of *Lavandula stoechas*, whereas French lavender honeys are exclusively derived from *Lavandula angustifolia*, *Lavandula latifolia*, or hybrids of these two species. In the framework of the floral origin authentication of such honeys, volatile compounds from *L. stoechas*, *L. angustifolia*, and *L. angustifolia*×*latifolia* unifloral honeys were investigated. The aromatic profiles of French and Portuguese lavender honey samples showed major qualitative and quantitative differences, but no volatile compound is characteristic of *L. stoechas* honeys only. As expected, *n*-hexanal, *n*-heptanal, phenylacetaldehyde, and *n*-hexanol, previously proposed to authenticate French lavender honeys, were found at concentrations far above the published discrimination thresholds. Coumarin, previously proposed to characterize French lavender honeys, emerges here rather as an indicator of the freshness of lavender honey, being mainly released from glycosides during storage. Lastly, *L. angustifolia* honeys were distinguishable from hybrid-derived samples by their lower phenylacetaldehyde and higher heptanoic acid content.

Keywords: Honey; Lavender; Flavour; Floral origin marker; Quality marker; Coumarin

1. Introduction

During the summer, Mediterranean landscapes are resplendent with the blue hues of lavender fields. These plants of the genus *Lavandula* are cultivated or develop in a wild state. With their colours and odours peculiar to each species, the flowers of "fine lavender" (*Lavandula angustifolia*), "spike lavender" (*L. latifolia*), "lavandin" (*Lavandula angustifolia*×*latifolia*) and "stechas lavender" (*Lavandula stoechas*), constitute a prime nectar source for honey bees. Organoleptically, this specificity will be more or less pronounced in the honeys.

In France, lavender honey is protected by a "red label" (Gonnet, 1989). It derives exclusively from the nectar of *L. angustifolia,* sometimes *L. latifolia*, or hybrids of these two species, to the exclusion of *L. stoechas* (Journal Officiel de la République Française, 1976). Its production area is essentially limited to the Southwest of France, *L. stoechas* honey being produced in Portugal and Spain.

It is hard to distinguish "fine lavender" from "lavandin" honeys. Melissopalynological characterization of monofloral lavender honeys requires a percentage of pollen ranging from 10 to 20% (Louveaux, Maurizio, & Vorwohl, 1978). These values are usually reached in *L. angustifolia* samples, but never in *L. angustifolia*×*latifolia* honeys, because "lavandin" is sterile, and the number of pollen grains is especially low. For this reason, a threshold of 50 grains per 10 g honey was proposed as a necessary (but not sufficient) condition for allowing the monofloral appellation (Loublier, Piana, Pham Delègue, & Borneck, 1994). Sensory analysis can be used as a complement, but requires experts and remains subjective.

In recent years, alternatives to sensory assessments and pollen analyses (tedious and very dependent on expert ability and judgment) have been developed in order to characterize honeys more widely and accurately. *L. stoechas* honeys can be differentiated from honeys from 10 other floral origins—heather (*Erica* sp. and *Calluna vulgaris*), acacia, rape, sunflower, rosemary, citrus, rhododendron, thyme and chestnut tree—on the basis of phenolic compounds, some of them specific like naringenin, others predominant like *m*-coumaric acid (Andrade, Ferreres, Gil, & Tomas-Barberan, 1997). High

* Corresponding author. Tel.: +32-10-47-29-13; fax: +32-10-47-21-78.

E-mail address: collin@inbr.ucl.ac.be (S. Collin).

0308-8146/02/$ - see front matter
PII: S0308-8146(02)00216-9

contents in some aroma compounds, such as *n*-hexanal, *n*-heptanal, *n*-heptanol, phenylacetaldehyde and coumarin, are especially adequate for authenticating French lavender honeys among ten other floral origins (Bouseta, Collin, & Dufour, 1992; Bouseta, Scheirman, & Collin, 1996).

To our knowledge, no research has focused on establishing distinctive floral markers of different species within the genus *Lavandula*. Therefore, we have investigated the volatile compounds of honeys derived from three different species, *L. stoechas*, *L. angustifolia*, and *L. angustifolia*×*latifolia*, using an optimised Likens-Nickerson method yielding organoleptically highly representative extracts (Bouseta & Collin, 1995). This technique recently proved adequate for distinguishing *C. vulgaris* from *Erica arborea* heather honeys (Guyot, Scheirman, & Collin, 1999). In the present work, the same methodology was applied to define markers for each type of lavender honey. Amounts of coumarin were then compared in fresh and aged samples to assess both the quality of the honeys and the reliability of this previously proposed marker.

2. Materials and methods

2.1. Honey samples

All the honeys were directly provided by the beekeepers and had not been industrially processed. They were analysed just after harvest, unless otherwise stated. The samples were stored at 4 °C until analysis.

2.1.1. Determination of the floral markers

Five *L. stoechas* from Portugal, six *L. angustifolia*× *latifolia* (lavandin) and four *L. angustifolia* (fine lavender) unifloral honeys from the Southwest of France were used just after harvest in this study. Difficulties to find "pure-species" samples explain why no more fresh lavender honeys were analysed. Screening for floral purity was based on pollen analyses (Loublier et al., 1994; Louveaux et al., 1978; Pérez-Arquillué, Conchello, Ariño, Juan, & Herrera, 1995; Serra Bonvehi & Coll, 1993). Our data were also compared with ten French lavender honeys (without further genus characterization) previously analysed by Bouseta et al. (1996) after a 1-year storage at 4 °C.

The aromatic profiles of *Lavandula* species honeys were here discriminated from those of 12 other unifloral origins (*C. vulgaris* from France, Belgium, United Kingdom, Norway and Germany; chestnut from France and Italy; *E. arborea* from France, Greece and Italy; *Eucalyptus* from Australia, Italy and Spain; fir from France; lavender from France; lime tree from France; orange blossom from France, Mexico and Spain; rape from Belgium and France; robinia from Canada, France, Hungary, Russia and Spain; rosemary from France and Spain; sunflower from Belgium and France; white clover from Canada and New-Zealand; 10 of each) (Bouseta, 1994). Screening for floral purity was based on pollen analyses (Louveaux et al., 1978), sensory tests (Gonnet & Vache, 1984), conductivity, pH, titratable acidity (Journal Officiel de la République Française, 1977), and sugar composition (Pourtallier & Rognone, 1977).

2.1.2. Kinetics of the coumarin release and determination of the coumarin potential

Two lavandin (*L. angustifolia*×*latifolia*) honeys were used for this study. Each of them was divided into two halves stored at 4 and 40 °C for 7 months.

2.2. Reagents

n-Hexanal (98%), *n*-heptanal (95%), *n*-octanal (99%), *n*-nonanal (95%), benzaldehyde (90%), phenylacetaldehyde (90%), 5-methylfurfural (99%), furfurylalcohol (99%), *n*-heptanol (98%), heptanoic acid (99%) and coumarin were obtained from Aldrich (Bornem, Belgium), 3-methyl-2-buten-1-ol, 2-phenylethanol (99.5%), benzyl alcohol (99%), hexanoic acid (99%), *n*-octane (99%), *n*-nonane (99%) and 1-chloroheptane from Acros Chimica (Geel, Belgium), *n*-hexanol (97%) and sulfuric acid from UCB (Bruxelles, Belgium), and 2-furaldehyde from Acros (Geel, Belgium).

The solvents (dichloromethane and methanol) were of pure analytical grade (purity > 99.8%) and were purchased from Romil (Gent, Belgium). Dichloromethane was redistilled twice prior to use. The water used was ultra-pure water (Milli-Q water purification system, Millipore, Bedford, MA, USA). Sep-Pak classic short body C_{18} cartridges were obtained from Waters (Bruxelles, Belgium).

2.3. Honey flavour extraction

Aroma compounds isolation was performed by a dichloromethane dissolution, followed by a Likens–Nickerson steam distillation/solvent extraction according to the procedure described by Bouseta and Collin (1995). For each sample, two replicates were obtained. The reproducibility of the extraction method was previously determined by Bouseta and Collin (1995) from five consecutive analyses of a standard mixture. Depending on volatile compound, coefficients of variation were found below 12% and recovery factors above 70%.

2.4. Determination of the coumarin potential

The protocol used is adapted from Abott (1991). Honey (25 g) was dissolved in 100 ml of ultra-pure water. The solution was then filtered through a Sep-Pak C_{18} cartridge, previously activated by 50 ml of methanol

and 50 ml of ultrapure water. The cartridge was rinsed with 50 ml of ultra-pure water in order to eliminate sugars and other water-soluble substances. The glycosides were then eluted using 50 ml of methanol. The methanol extract was evaporated to dryness in a rotavapor (Heidolph, Germany). Fifty milliliters of H_2SO_4 6N were added to the residue and the solution was placed in a water-bath for 16 h at 37 °C ± 1 °C and then for 5 h at 60 ± 1 °C. The free coumarin isolation was performed by three successive extractions with 50 ml of dichloromethane. The dichloromethane extract was concentrated to 1 ml in a Snyder Kuderna apparatus at 45 ± 1 °C with 50 μL of a 1000 μg g^{-1} solution of 1-chloroheptane in dichloromethane, added as external standard. The extract was further analysed by GC. For each sample, two replicates were obtained.

2.5. Gas chromatography–FID (GC–FID) analytical conditions

A Hewlett Packard Model 5890 gas chromatograph was used, equipped with a Hewlett Packard Model 7673 automatic sampler, a cold on-column injector, a flame ionization detector, and a Shimadzu CR4A integrator. Analysis of honey volatile compounds was carried out on a 50 m × 0.32 mm i.d. wall-coated open tubular (WCOT) CP-SIL5 CB (Chrompack, Antwerp, Belgium) capillary column (film thickness, 1.2 μm), preceded by a 1 m × 0.53 mm i.d. capillary column, coated with a thin film of methyl silicone phase (Hewlett Packard, Brussels, Belgium). The oven temperature was programmed to rise from 36 to 85 °C at 20 °C/min then to 145 °C at 1 °C/min and to 250 °C at 3 °C/min. The carrier gas was He at 1.5 ml min^{-1}. The injector temperature was maintained at 3 °C above the oven temperature. The detector temperature was 260 °C. The minimum peak area for data acquisition was set at 500 μV s. Retention indices were determined by interpolation of the retention times of a *n*-alkanes (C_6–C_{19}) mixture analysed under identical conditions. Two microliters of each extract were injected for analysis.

2.6. Quantification of volatile compounds by GC–FID

Concentration of compounds in the honey samples was calculated with respect to the external standard, according to the equation:

$$C_i = (P_i/P_e) \times (Q_e/Q_h) \times (1/K_i) \quad (1)$$

where the suffix *i*, e and h refer to the quantified compound, the external standard and the honey respectively; *P* refers to the peak area obtained in GC–FID; C refers to the concentration in the honey (ng g^{-1}); Q_e refers to the quantity of external standard in the dichloromethane extract (ng); Q_h refers to the quantity of honey used (g); K_i is the response factor at the FID detector of the compound *i* with regard to the external standard.

As previous analyses have shown that the Likens–Nickerson-derived method leads to recovery factors higher than 70% for most of the chemicals mentioned

Table 1
Volatile compounds in *Lavandula stoechas*, *Lavandula angustifolia* and *Lavandula angustifolia×latifolia* honeys[a]

Compounds	RI	*L. stoechas*			*L. angustifolia*			*L. angustifolia×latifolia*		
		Min	Max	Avg	Min	Max	Avg	Min	Max	Avg
Pyridine	712	8	1130	242	0	0	0	0	0	0
3-Methyl-2-buten-1-ol	749	24	129	80	146	207	179	64	238	147
n-Hexanal	774	7	32	18	613	1460	939	980	1845	1346
Octane	800	9	27	15	10	57	27	17	37	26
2-Furaldehyde	803	160	223	192	53	154	95	65	182	102
Furfurylalcohol	824	6	21	12	17	48	32	0	54	18
n-Hexanol	844	0	0	0	1630	4370	2729	1886	4930	3983
n-Heptanal	877	0	73	33	179	329	286	185	294	238
n-Nonane	900	0	0	0	2	11	8	0	10	3
5-Methylfurfural	929	0	27	17	32	124	71	54	96	78
Benzaldehyde	933	5	194	74	31	91	62	82	151	111
Hexanoic acid	946	7	4388	1766	0	235	80	0	30	7
n-Heptanol	947	4	33	13	521	754	566	416	884	715
n-Octanal	979	4	9	7	13	56	33	41	75	61
Benzylalcohol	1009	9	113	65	21	44	32	31	101	57
Phenylacetaldehyde	1013	74	1329	703	744	1303	964	1539	2969	2189
Heptanoic acid	1049	5	30	19	193	296	238	85	194	132
n-Nonanal	1081	255	988	577	1135	1648	1427	1508	2163	1787
2-Phenylethanol	1087	130	2010	1132	730	1172	904	728	1242	971
Coumarin	1397	0	0	0	101	253	193	62	292	201

[a] RI = retention index; Min, max, avg = minimal, maximal, average concentrations (ng g^{-1}) in the honeys analysed just after harvest.

(Bouseta & Collin, 1995), concentrations were calculated with an extraction recovery factor equal to 100%.

2.7. Gas chromatography–mass spectrometry (GC–MS) conditions

Chromatographic conditions were the same as those used for FID detection. The column was directly connected to an HP 5988 quadrupole mass spectrometer. Electron impact mass spectra were recorded at 70 eV (filament current: 300 mA; electron multiplier voltage: 2500; scan rate: 4 s^{-1}; m/z range: 40–250). Spectral recording throughout elution was automatic using HP59970C software. Identification was on the basis of peak enhancement by co-injection with authentic standard compounds and comparison with the NBS/EPA/NIH mass spectra library.

3. Results and discussion

3.1. Distinguishing Portuguese and French lavender honeys

After identification by GC–MS, 20 aroma compounds were quantified in *Lavandula* extracts by GC-FID (Table 1). Most of these peaks do not constitute reliable markers, due to their presence in honeys of other origins.

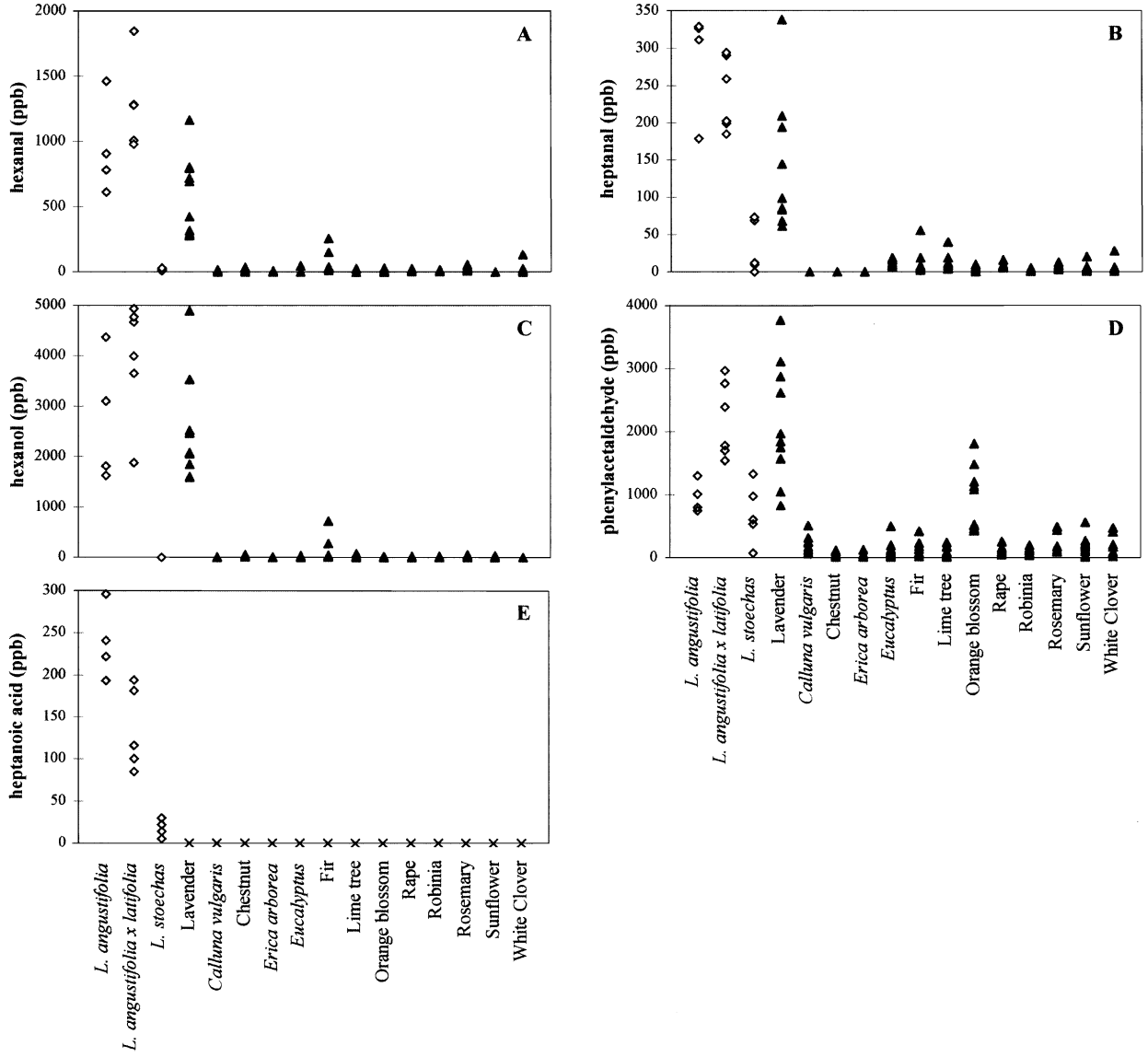

Fig. 1. Floral origin markers of French lavender honeys (*Lavandula angustifolia* and *Lavandula angustifolia*×*latifolia*): *n*-hexanal (A), *n*-heptanal (B), *n*-hexanol (C), phenylacetaldehyde (D) and heptanoic acid (E). Honey analysed just after harvest ◇; after storage at 4 °C for 1 year or more (Bouseta et al., 1996)* ▲; not quantified ×. * For the data derived from Bouseta et al. (1996), all French lavender honeys were analysed without defining the floral species.

The aromatic profiles of French (*L. angustifolia* and *L. angustifolia*×*latifolia*) and Portuguese (*L. stoechas*) lavender honey samples showed major qualitative and quantitative differences. No marker specific to *L. stoechas* honeys was found (Figs. 1A–E and 2A–B), but among honeys derived from flowers within the *Lavandula* genus, Portuguese lavender honeys show much lower concentrations of *n*-hexanal, *n*-heptanal, *n*-hexanol and heptanoic acid (below 32, 73, 0 and 30 ng g^{-1}, respectively) than French lavender honey samples. Compared to all other floral origins, these honeys are not easily identifiable. Their authentication could be based on the simultaneous absence of *n*-hexanol and *n*-nonane (Figs. 1C and 2A) and the presence of pyridine (Fig. 2B), though this last compound is almost negligible, except in one sample.

3.2. Authentifying the floral origin of L. angustifolia and L. angustifolia×latifolia honeys

For *L. angustifolia* and *L. angustifolia* × *latifolia* honeys, our findings for honeys analysed just after harvest confirm the finding of Bouseta et al. (1996) that *n*-hexanal, *n*-heptanal, hexanol, and phenylacetaldehyde are four typical markers of these lavender honeys (concentrations above 279, 61, 1594, and 744 ng g^{-1}, respectively) (Fig. 1A–D). From a qualitative point of view, the aromatic profiles of the *L. angustifolia* and *L. angustifolia*×*latifolia* honeys proved very similar. However, authentication between both types could be done on the basis of their phenylacetaldehyde and heptanoic acid contents (Figs. 1D and 1E): "lavandin" honeys exhibit higher phenylacetaldehyde and lower heptanoic acid concentrations (above 1539 and below 194 ng g^{-1}, respectively).

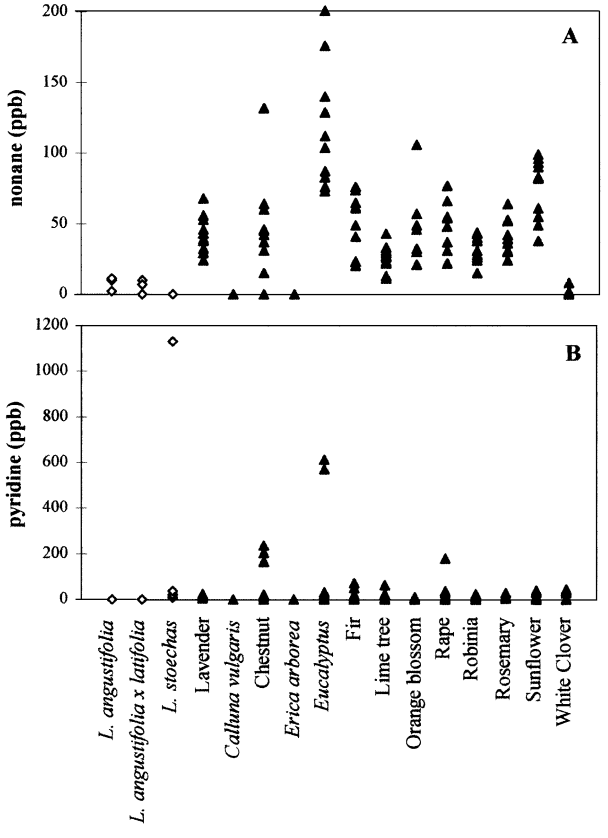

Fig. 2. Volatile compounds that distinguish *Lavandula stoechas* honeys from honeys of other floral origins: *n*-nonane (A) and pyridine (B), Honey analysed just after harvest ◇; after storage at 4 °C for 1 year or more (Bouseta et al., 1996)* ▲. * For the data derived from Bouseta et al. (1996), all French lavender honeys were analysed without defining the floral species.

3.3. Kinetics of coumarin release and determination of the coumarin potential

Results presented in Table 1 indicate lower coumarin concentrations in our French lavender honeys analysed just after harvest (from 62 to 292 ng g^{-1}) than in the 1 year-aged honey samples analysed by Bouseta et al. in 1996 (from 512 to 1720 ng g^{-1}, with an average equal to 954 ng g^{-1}). On this basis, we suggested progressive release of coumarin through storage, probably mainly at the beginning due to residual enzymatic activity. This phenomenon could be masked in longer storages due to

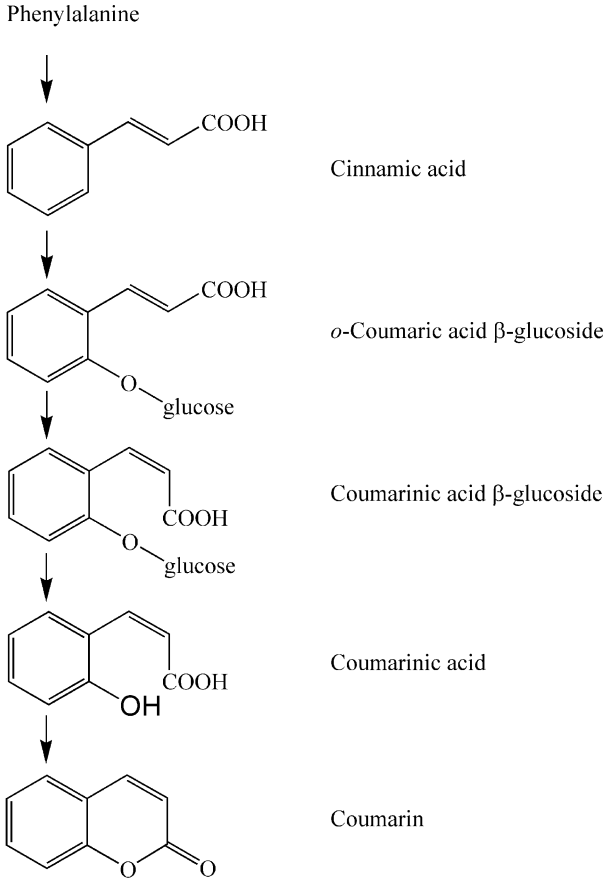

Fig. 3. Coumarin metabolic pathway (Herbert, 1989; Murray et al., 1982; Neish, 1965).

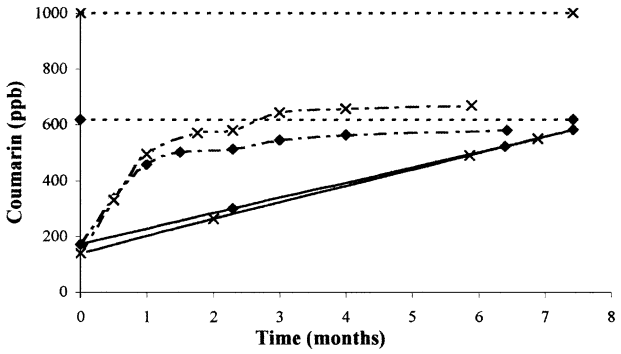

Fig. 4. Kinetics of coumarin release in "lavandin" honeys. ◆ Sample 1; × sample 2; — at 4 °C; -.-.-.- at 40 °C; · · · · total coumarin content determined after acid hydrolysis.

inevitable loss or hydrolysis of free coumarin, as suggested by the analyses of six 4 year-aged samples (coumarin concentration below 346 ng g^{-1} in all cases).

Coumarin is a shikimic pathway derivative resulting from phenylalanine metabolism (Fig. 3). Hydroxylation of cinnamic acid in the *ortho* position coupled with glucosylation is probably responsible for the formation of *o*-coumaric acid glucoside. This glucoside then undergoes a *trans*→*cis* conversion, possibly due to the action of sunlight, yielding coumarinic acid glucoside. Cleavage of the glycoside bond by a specific β-glucosidase yields unstable *cis*-coumarinic acid, which spontaneously lactonizes to coumarin (Herbert, 1989; Murray, Mendez, & Brown, 1982; Neish, 1965). Two lavandin honeys were used for this study. Each was divided into two parts, one being stored at 4 °C and the other at 40 °C for 7 months. The free and total coumarin contents are presented in Fig. 4.

Storage at 40 °C leads to rapid release of free coumarin, a maximum being already reached after 3 months. Release is as expected much slower at 4 °C, remaining significant even after 6 months. The free coumarin concentration reached the total coumarin level obtained by acid hydrolysis (free + bound to the glycoside form) in one honey sample, whatever the temperature.

These results suggest that coumarin can no longer be considered as a floral origin marker of French lavender honeys, although it could be very useful for checking the freshness of such samples.

4. Conclusion

In agreement with organoleptic observations (Gonnet & Vache, 1984), our preliminary results evidence that honeys derived from different lavender species (*L. stoechas*, *L. angustifolia* and *L. angustifolia*×*latifolia*) show specific volatile compounds profiles. French lavender honeys can be easily authenticated from *L. stoechas* samples or various other origins, thanks to their high content in linear aldehydes, linear alcohols, and phenylacetaldehyde. This last compound also emerges as a quantitative marker of *L. angustifolia* × *latifolia* honeys, whereas heptanoic acid preponderates in *L. angustifolia* honeys.

Coumarin, which seems to be released from glycosides through storage, could be used as a freshness marker of French lavender honeys.

Acknowledgements

This work was supported by a grant from the European Commission, project FAIR-CT985050. We are grateful to Paulo Nunes and Paulo Russo for providing us with the Portuguese *Lavandula stoechas* honey samples and to Bernard Oza (SCA Provence Miel) and Jean-Louis Lautard for supplying us with French *Lavandula angustifolia* and *Lavandula angustifolia*×*latifolia* honeys.

References

Abott, A. (1991). *The study of Shiraz grape berry composition in relation to the quality of table wine*. PhD thesis, Adelaide University.

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

Bouseta, A. (1994). *Caracterisation et voies de formation des flaveurs du miel* [Characterization and formation pathways of honey flavours]. PhD thesis, Catholic University of Louvain, Louvain-la-Neuve, Belgium.

Bouseta, A., & Collin, S. (1995). Optimized Likens-Nickerson methodology for quantifying honey flavors. *Journal of Agricultural and Food Chemistry*, *43*(7), 1890–1897.

Bouseta, A., Collin, S., & Dufour, J.-P. (1992). Characteristic aroma profiles of unifloral honeys obtained with a dynamic headspace GC–MS system. *Journal of Apicultural Research*, *31*(2), 96–109.

Bouseta, A., Scheirman, V., & Collin, S. (1996). Flavor and free amino acid composition of lavender and Eucalyptus honeys. *Journal of Food Science*, *61*(4), 683–687 694.

Gonnet, M. (1989). "Label Rouge" National pour un miel de crû. [Red label for a local honey]. *L'Abeille de France*, *741*, 389–391.

Gonnet, M., & Vache, M. (1984). *Le goût du miel. [Honey flavour]*. Paris, France: U.N.A.F. Editions.

Guyot, C., Scheirman, V., & Collin, S. (1999). Floral origin markers of heather honeys: *Calluna vulgaris* and *Erica arborea*. *Food Chemistry*, *64*(2), 3–11.

Herbert, R. B. (1989). *The biosynthesis of secondary metabolites*. London: Chapman and Hall.

Journal Officiel de la République Française. (1976). Decree n°76–717, 22 July, Annexe 2, 37–46.

Journal Officiel de la République Française. (1977). Decree n°77–79, 22 April, related to the official analysis methods of honey, 3485–3514.

Loublier, Y., Piana, M. L., Pham Delègue, M.-H., & Borneck, R. (1994). Caractérisation pollinique des miels français de lavande: premiers résultats. [Characterization of monofloral lavender honeys—pollen analysis of French lavender honeys—preliminary results]. *Grana*, *33*, 231–238.

Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of melissopalynology. *Bee World*, *59*, 139–157.

Murray, R. D. H., Mendez, J., & Brown, S. A. (1982). *The natural coumarins. Occurrence, chemistry and biochemistry*. Chichester: John Wiley & Sons.

Neish, A. C. (1965). Coumarins, phenylpropanes, and lignin. In J. Bonner, & J. E. Varner (Eds.), *Plant biochemistry* (pp. 581–617). New-York: Academic Press.

Pérez-Arquillué, C., Conchello, P., Ariño, A., Juan, T., & Herrera, A. (1995). Physicochemical attributes and pollen spectrum of some unifloral Spanish honeys. *Food Chemistry*, *54*, 167–172.

Pourtallier, J., Rognone, C. (1977). Méthode améliorée de dosage des sucres des miels par chromatographie en phase gazeuse [Improved method of sugars measurement in honeys by means of gas chromatography]. In *Proceedings of the Symposium International de Technologie Apicole* (pp. 73–83). Bucarest: Apimondia.

Serra Bonvehi, J., & Coll, F. V. (1993). Physico-chemical properties, composition and pollen spectrum of french lavender (*Lavandula stoechas* L.) honey produced in Spain. *Zum Lebensmittel Untersuchung und Forschung*, *196*, 511–517.